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Virological Response to Highly Active Antiretroviral Therapy in Patients Infected with Human Immunodeficiency Virus Type 2 (HIV-2) and in Patients Dually Infected with HIV-1 and HIV-2 in The Gambia and Emergence of Drug-Resistant Variants▽

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Drug design, antiretroviral therapy (ART), and drug resistance studies have focused almost exclusively on human immunodeficiency virus type 1 (HIV-1), resulting in limited information for patients infected with HIV-2 and for those dually infected with HIV-1 and HIV-2. In this study, 20 patients, 12 infected with HIV-2 and 8 dually infected with HIV-1 and HIV-2, all treated with zidovudine (ZDV), lamivudine (3TC), and lopinavir-ritonavir (LPV/r), were followed up longitudinally for about 3 years. For 19/20 patients, viral loads were reduced to undetectable levels; the patient whose viral load remained detectable reported adverse effects associated with LPV/r that had caused him to stop taking all the drugs. HIV-2 strains containing mutations in both the protease and the reverse transcriptase gene that may confer drug resistance were observed in two patients with viral rebound, as early as 130 days (4.3 months) after the initiation of therapy. We conclude that the combination of ZDV, 3TC, and LPV/r is able to provide efficient and durable suppression of HIV-1 and HIV-2 for as long as 3 years in HIV-2-infected and dually infected patients. However, the emergence of HIV-1 and HIV-2 strains containing drug-resistant mutations can compromise the efficacy of this highly active ART.

Human immunodeficiency virus type 2 (HIV-2) is the second human immunodeficiency virus known to cause AIDS (8). However, this retrovirus, although very similar to HIV-1, usually presents an attenuated HIV infection with lower plasma viral loads, a slower decline in the number of $CD4^+$ T cells, a longer asymptomatic phase, and much lower transmission rates (2–5, 20, 29, 34, 40, 47, 51).

Highly active antiretroviral therapy (HAART) has changed the face of the AIDS epidemic from a death sentence to a treatable, chronic infectious disease (46) and has had a dramatic effect on mortality, slowing down disease progression and increasing the quality of life for infected individuals with access to treatment (16, 17, 44). However, the high mutation rate of HIV and incomplete viral suppression due to suboptimal therapy inevitably result in the emergence of drug-resistant viruses. Suboptimal therapy is associated with low levels of drugs in the blood due either to lack of adherence to toxic and complex regimens or to problems with drug absorption or metabolism (33, 46). The development of drug resistance has posed a major obstacle to the effective treatment of HIV, limiting both the magnitude and the duration of the response to treatment as well as reducing the number of active antiretroviral (ARV) drugs available for HAART (33).

Drug development, susceptibility tests, and drug resistance studies have focused almost exclusively on HIV-1; limited work has been done on HIV-2. This is due mainly to the lower prevalence of HIV-2 than of HIV-1 and the restriction of the HIV-2 epidemic mainly to West Africa, where access to treatment has been limited. The drugs that are currently approved were designed for HIV-1 subtype B, but due to the highly conserved nature of the critical HIV-1 and HIV-2 enzymes protease and reverse transcriptase (RT), especially around the active sites, it was assumed that these drugs would be active against both types of HIV infections. The nonnucleoside RT inhibitors (NNRTIs), however, target allosteric sites of the RT enzyme, and it was later discovered that HIV-1 group O strains and HIV-2 are naturally resistant to these drugs (14, 22, 42, 48). In addition, HIV-2 has been found to be naturally resistant to the entry inhibitor T-20, and it may have reduced susceptibility to some protease inhibitors (PIs) (19, 45, 50). Therefore, drugs used for the treatment of HIV-2 should be carefully selected to allow optimal and durable viral suppression. Dual infection with HIV-1 and HIV-2 occurs mainly in West Africa, where the two viruses cocirculate. Several papers have indicated that dually infected patients have a disease course similar to that of patients infected with HIV-1 alone (21, 52). The presence of HIV-2 in individuals dually infected with HIV-1 and HIV-2 complicates the treatment of these patients (53). Therefore, the optimal regimen for the treatment of dually

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TABLE 1. Epidemiological, immunological, and virological data of the patients

Patient no.	Reference no.	Sex[a]	Age (yr)	HIV type(s)	Date[b] on which ART was:		CD4 cells[c]		VL[c,d] (RNA copies/ml of plasma)	
					Approved	Started	% of total lymphocytes	Abs[e] count (cells/μl)	HIV-1	HIV-2
1	20041287	M	44	2	3/5/2005	20/5/2005	10	300		315,118
2	20020797	M	54	2	31/5/2005	13/6/2005	5	90		108,650
3	19921115	M	42	2	19/4/2005	5/5/2005	16	180		
4	20047429	F	30	2	28/6/2005	22/7/2005	4	130		50,950
5	20053368	M	48	2	16/8/2005	17/8/2005	10	130		
6	2003A182	F	43	2	16/8/2005	1/9/2005	5	70		38,645
7	20051154	F	31	2	25/10/2005	17/11/2005	12	180		54,324
8	20052184	M	31	2	25/10/2005	17/11/2005	7	50		120,695
9	20054262	F	46	2	15/11/2005	28/11/2005	8	230		311,055
10	2001A731	F	50	2	15/11/2005	28/11/2005	16	130		3,162
11	20009737	F	31	2	30/11/2004	10/3/2005	4	10		484,047
12	19934174	F	37	2	6/9/2005	19/12/2005	19	210		5,656
13	19813502	F	46	1 and 2	25/1/2005	14/3/2005	11	220	111,867	144,691
14	19927800	F	40	1 and 2	7/12/2004	25/1/2005	7	40	380,315	167
15	19972375	F	28	1 and 2	19/4/2005	5/5/2005	5	160	78,487	5,735
16	20018919	M	49	1 and 2	1/3/2005	4/4/2005	3	40	10,570	41,506
17	20034661	F	38	1 and 2	28/6/2005	22/7/2005	4	210	1,000,000	7,941
18	19965521	F	36	1 and 2	15/11/2005	28/11/2005	12	300	42,462	100
19	20056249	M	30	1 and 2	7/2/2006	2/3/2006	7	60	951,770	100
20	19936825	F	51	1 and 2	16/8/2005	2/3/2006	14	180	1,000,000	37,334

[a] M, male; F, female.
[b] Given as day/month/year.
[c] At the start of therapy.
[d] VL, viral load.
[e] Abs, absolute.

infected patients should include drugs with simultaneous activity against both HIV-1 and HIV-2.

In the developed world, as well as in a few countries in Africa, a few HIV-2-infected individuals have been treated with ARV drugs and protocols developed for HIV-1 (1, 6, 9–12, 23, 49). However, the increasing accessibility of these drugs in Africa means that a substantial number of HIV-2-infected individuals will be treated, making the study of HIV-2 response to HAART and the development of resistance to ARV drugs a priority.

We have monitored the response to treatment, and the emergence of HIV-1 and HIV-2 strains containing potential drug-resistant mutations, for patients treated with a combination of two nucleoside RT inhibitors (NRTIs), zidovudine (ZDV) and lamivudine (3TC), plus a PI, lopinavir-ritonavir (LPV/r), for as long as 3 years. The presence of HIV strains containing drug-resistant mutations was analyzed for patients with viral rebound.

MATERIALS AND METHODS

Study subjects. The Genitourinary Medicine clinic at the Medical Research Council Laboratories in The Gambia follows a cohort of HIV-2 patients, who are routinely monitored for CD4 T-cell counts, plasma viral loads, and clinical signs and symptoms. ARVs became available in The Gambia in 2004, and patients meeting the criterion of a CD4 T-cell count of ≤350 cells/ml are now treated according to the national guidelines. HIV-2-infected patients and patients dually infected with HIV-1 and HIV-2 are put on a fixed regimen of ZDV, 3TC, and LPV/r as the first-line regimen. Study subject details are shown in Table 1.

The Joint Gambian government–Medical Research Council Ethics Committee approved this study.

CD4 T-cell count and viral load. CD4 T-cell measurements were carried out using flow cytometry (FACSCalibur; Becton Dickinson) (35, 36). The FACSCalibur determines both the absolute CD4 cell count (in cells per microliter) and the CD4 percentage (CD4 T cells as a proportion of total lymphocytes). The CD4 percentage rather than the absolute CD4 cell count was used to monitor immunological response, because the former is more stable than the latter, which is influenced by a number of factors, such as time of day and other concurrent infections (18, 25). The viral load, expressed as the number of HIV-2 RNA copies per milliliter of plasma, was quantified using an in-house viral load assay (4), which has a limit of detection of 100 RNA copies/ml of plasma. This assay is a quantitative reverse transcription-PCR of the long terminal repeat sequence of HIV-2, in which the test samples are quantified by comparison with a standard curve. Test and control PCR products are detected and quantified in an enzyme-linked oligonucleotide assay (4). This assay was modified to include an internal molecular control for extraction and amplification efficiency (30).

Nucleic acid extraction, PCR amplification, and sequence analysis. HIV-2 RNA was extracted from 140 μl of EDTA-plasma using the QIAamp viral RNA kit (Qiagen, Venlo, The Netherlands) and was eluted into 50 μl nuclease-free water. The entire HIV-2 protease and RT were amplified using a single-tube reverse transcription-PCR method (Titan one-tube RT-PCR; Roche Applied Science, Lewes, United Kingdom) starting from 3 μl of RNA, followed by a nested PCR with Expand High-Fidelity DNA polymerase and 0.5 to 1 μl of the first-round product as previously described (24). Purified PCR products were directly sequenced on both strands and were analyzed with DNAStar software (Lasergene; DNAStar, Madison, WI) (24). Sequencing was performed only when there was detectable viral load during treatment. For one dually infected patient, HIV-1 became detectable while HIV-2 remained undetectable; therefore, genotyping was done for only HIV-1. The inner primers SJH11A (forward; 5′-AAAAGGGCTGTTGGAAATGTGG-3′; positions 2018 to 2139) and SJH12A (reverse; 5′-CCTAATGCATACTGTGAGTCTG-3′; positions 4060 to 4039) and the outer primers SJH13A (forward; 5′-GAGAGACAGGCTAATTTTTTAGGG; positions 2071 to 2094) and SJH14 (reverse; 5′-CCTATTAGCTGCCCCATCTACATA-3′; positions 3893 to 3870) were used to amplify the entire HIV-1 protease and RT. These primer positions refer to HIV-1 HXB2 (GenBank accession no. K03455). The PCR conditions used for HIV-2 were also used for HIV-1 except for the annealing temperatures: 56°C was used for the first round, and 57°C was used for the nested PCR. Three sequencing primers were used to generate the HIV-1 protease and RT: SHJ13A, SJH14, and SJH15 (forward; 5′-GATGTGGGGGAYGCATATTTTTCAG-3′; positions 2877 to 2901). The viral subtype was determined using an online NCBI program (http://www.ncbi.nih.gov/projects/genotyping/). All HIV-2 strains belonged to subtype A, and the

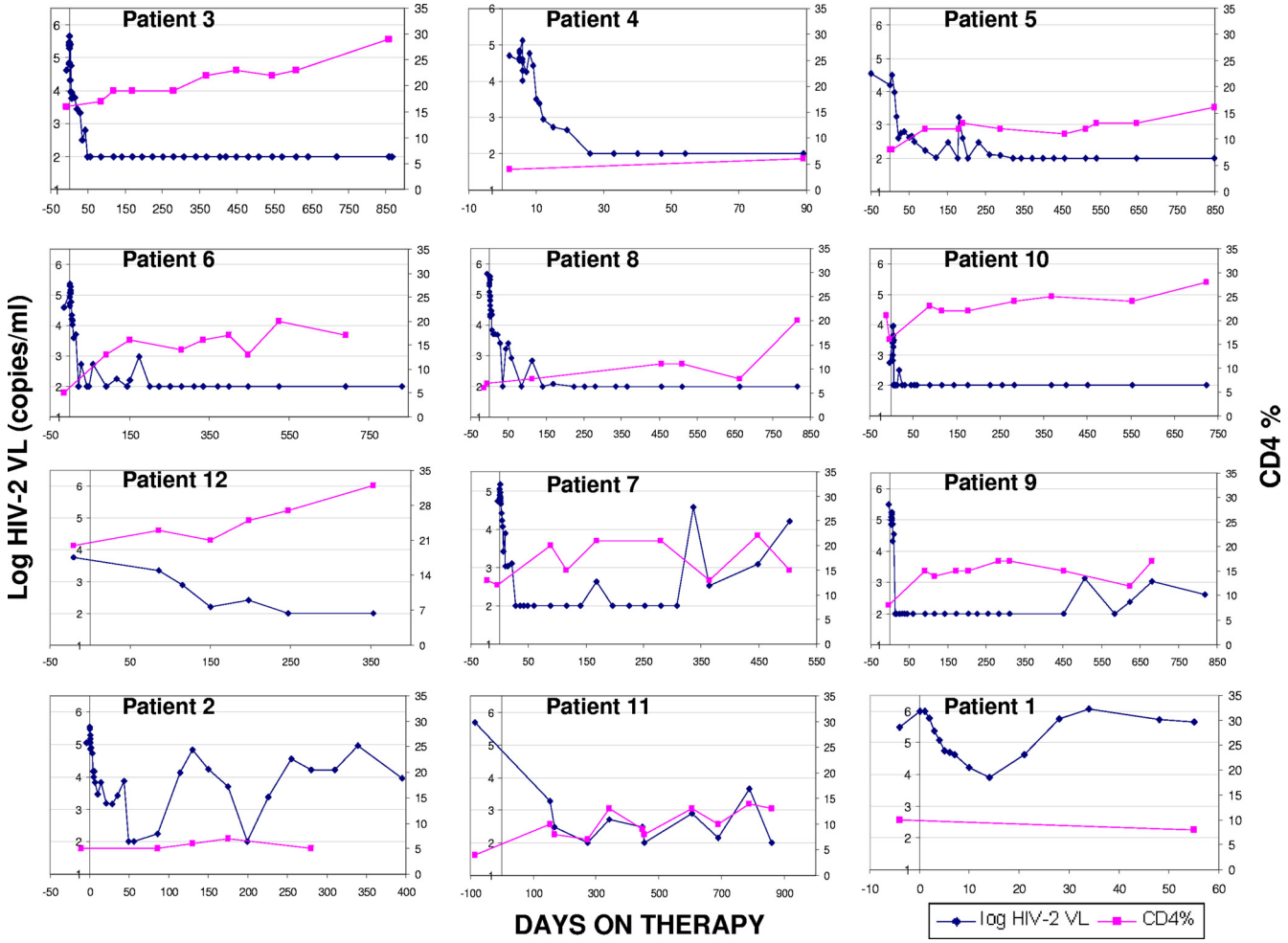

FIG. 1. Virological and immunological responses of HIV-2-infected patients treated with ZDV, 3TC, and LPV/r. Graphs depict the changes in log HIV-2 RNA copies per milliliter of plasma (blue lines) and in the percentages of $CD4^+$ T cells (pink lines) over time. The limit of detection of the viral load (VL) assay is log 2 or 100 copies/ml.

HIV-1 strain in the dually infected patients belonged to the CRF-02_AG subtype.

Nucleotide sequence accession numbers. The sequences generated were assigned EMBL accession numbers AM408175 to AM408184 and FM877562 to FM877574.

RESULTS

The response to therapy for each patient was computed with respect to the reduction in the viral load during the first few months of therapy. Among the HIV-2-infected patients, viral loads were initially reduced to undetectable levels (i.e., <100 copies/ml, the limit of detection) in all but one patient (patient 1), accompanied by an increase in the CD4 T-cell count over pretherapy levels. The viral load in patient 1 was reduced by 2 log units during the first 2 weeks of therapy but rapidly rebounded to pretreatment levels. For seven patients (patients 3, 4, 5, 6, 8, 10, and 12), the viral load is still undetectable after 3 to 28 months on therapy. However, for patient 12, the viral load dropped slowly and became undetectable only after 5 months. For the rest of the patients (patients 2, 7, 9, and 11), the viral load was undetectable for 3 to 15 months, but viral rebound was observed afterwards (Fig. 1). In patients 2 and 11, the viral load rebounded and returned to undetectable levels several times, indicating possible problems with adherence.

Among the dually infected patients (patients 13 to 20) (Table 1), a very good virological response to therapy was observed for both HIV-1 and HIV-2, as shown by the decline of both HIV-1 and HIV-2 loads to undetectable levels (Fig. 2). In seven out of eight patients, viral loads are still undetectable after 18 to 36 months on therapy. For patient 19, the HIV-1 load increased by 2 log units while the HIV-2 load remained undetectable.

The reduction of the viral load to undetectable levels was accompanied by an increase in the percentage of CD4 T cells for all patients taking this treatment.

The proteases of HIV-1 and HIV-2 have an amino acid sequence similarity of about 50% (55). Analysis of the HIV-2 protease gene from pretherapy samples showed the presence of several mutations that are associated with drug resistance when found in HIV-1. Nine minor HIV-1 mutations, L10V, G16E, K20R, L33V, M36I, I62V, A71V, G73A, and I93L, and three major mutations, V32I, M46I, and I47V, were present

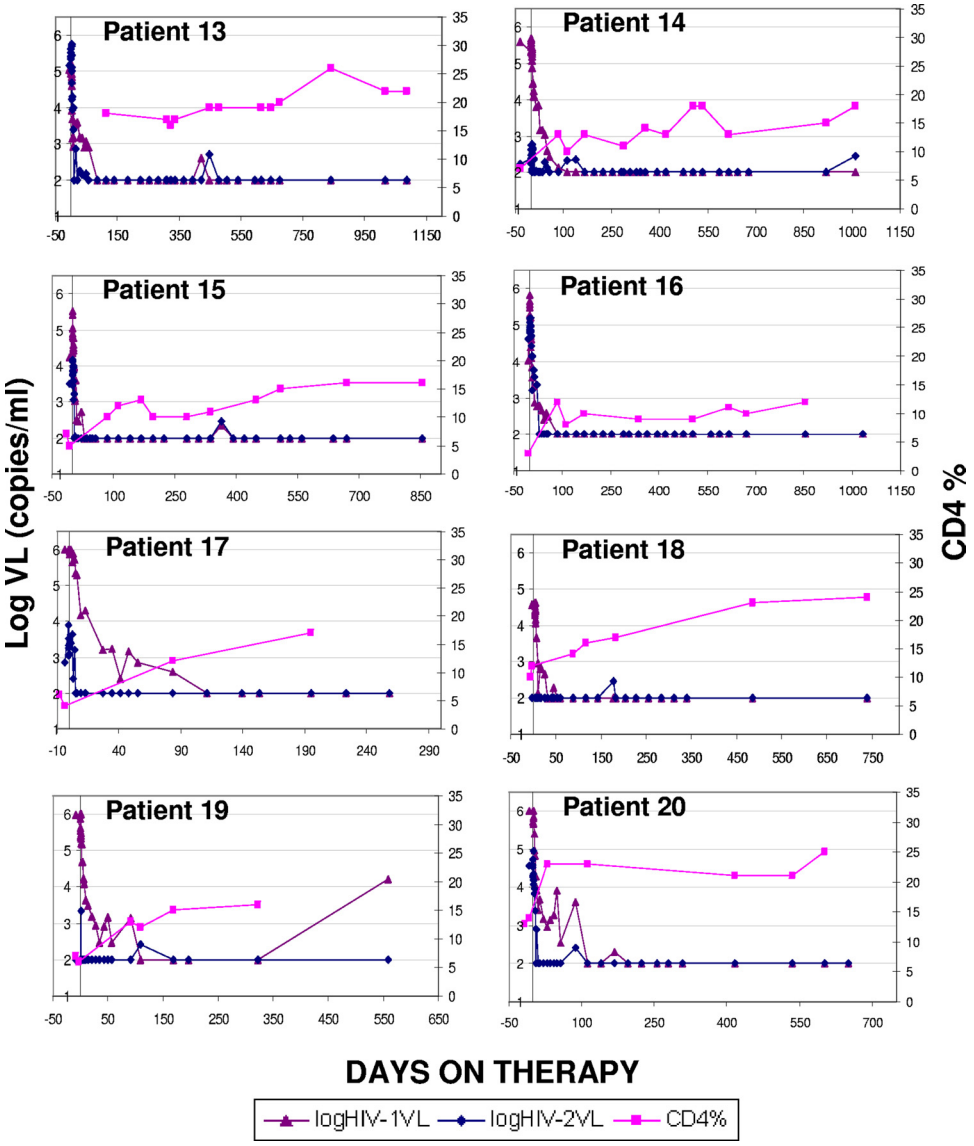

FIG. 2. Virological and immunological responses of patients dually infected with HIV-1 and HIV-2 and treated with ZDV, 3TC, and LPV/r. Graphs depict changes in log HIV-2 RNA copies per milliliter of plasma (blue lines), in log HIV-1 RNA copies per milliliter of plasma (purple lines), and in the percentage of $CD4^+$ T cells (pink lines) over time. The limit of detection of the viral load (VL) assay is $\log_2$, or 100 copies/ml.

naturally in all the HIV-2 samples sequenced (Table 2). Also, polymorphisms were found at positions L76V (M in HIV-2) and V82A/T/F (I in HIV-2), which are known to harbor major HIV-1 PI mutations, and at positions of minor mutations, E34Q (A in HIV-2), D60E (K in HIV-2), and L63P (E in HIV-2) (10, 11, 49).

The RTs of HIV-1 and HIV-2 have a higher sequence homology (~60%) than the proteases of these viruses (54). As expected, analysis of the HIV-2 RT gene from pretherapy samples revealed only a few mutations associated with HIV-1 drug resistance. Two RT mutations—V75I, associated with multi-NRTI resistance in HIV-1, and K219Q/E, associated with ZDV resistance in HIV-1—were found to occur naturally in all HIV-2 samples (Table 3). Polymorphisms were also found at positions T69D (N in HIV-2), L210W (N in HIV-2), T215Y/F (S in HIV-2), and G333D/E (Q in HIV-2), known to harbor HIV-1 RT mutations (Table 3).

Sequence analysis for the presence of drug resistance mutations was performed for 10 patients (9 infected with HIV-2 and 1 dually infected) with detectable viral loads despite therapy. The protease and RT regions of the sequences were aligned and analyzed for drug resistance mutations. The alignment of the patient samples for different time points, before and after therapy, shows mutations that may have arisen as a result of drug pressure (Tables 2 and 3). A major HIV-2 PI mutation, V47A (38), and a primary HIV-1 NRTI mutation, M184V/I, were observed in patients 2 and 11. Patient 2 had the M184I mutation after 4 months on ART and the V47A and M184V mutations after 8.5 months on ZDV, 3TC, and LPV/r (Tables 2 and 3). In addition, the F53V mutation, at a position known

TABLE 2. Drug resistance mutations emerging as a result of PI exposure

Sample[a]	Accession no.	Amino acid[b] associated with mutation common in HIV-1 patients exposed to PIs																										
		L10F/I/V	G16E	K20M/R/V	L24I	D30N	V32I	L33F/I/V	E34Q	M36I/L/V	M46I/L	I47A/V	G48V	I50V	F53Y/L	I54V/A/M	D60E	I62V	L63P	I64L/M/V	A71V/T	G73A/S	T74P	L76V	V82A/T/F	I84V	N88S	L90M	I93L/M
WT HIV-1	K03455	L	G	K	L	D	V	L	E	M	M	I	G	I	F	I	D	I	L	I	A	G	T	L	V	I	N	L	I
Patient 19																													
0	FM877573	**I**	**E**	**R**	L	D	V	L	E	I	M	I	G	I	F	I	D	I	P	I	A	G	T	L	V	I	N	L	I
559	FM877574	**I**	**E**	I	L	D	V	L	E	I	M	I	G	I	F	I	D	I	P	I	A	G	T	L	V	I	N	L	I
WT HIV-2	M15390	**V**	**E**	**V**	L	D	**I**	**V**	A	**I**	**I**	**V**	G	I	F	I	K	**V**	E	I	**V**	**A**	T	M	I	I	N	L	**L**
Patient 1																													
0	AM408175	**V**	**E**	**V**	L	D	**I**	**V**	A	**I**	**I**	**V**	G	I	F	I	K	**V**	E	I	**V**	**A**	T	M	I	I	N	L	**L**
55	AM408176	**V**	**E**	**V**	L	D	**I**	**V**	A	**I**	**I**	**V**	G	I	F	I	K	**V**	E	I	**V**	**A**	T	M	I	I	N	L	**L**
Patient 2																													
0	AM408177	**V**	**E**	**V**	L	D	**I**	**V**	A	**I**	**I**	**V**	G	I	F	I	K	**V**	E	I	**V**	**A**	T	M	I	I	N	L	**L**
130	AM408178	**V**	**E**	**V**	L	D	**I**	**V**	A	**I**	**I**	**V**	G	I	**V**	I	K	**V**	E	I	**V**	**A**	T	M	I	I	N	L	**L**
255	AM408179	**V**	**E**	**V**	L	D	**I**	**V**	A	**I**	**I**	**A**	G	I	F	I	K	**V**	E	I	**V**	**A**	T	M	I	I	N	L	**L**
310	FM877562	**V**	**E**	**V**	L	D	**I**	**V**	A	**I**	**I**	**A**	G	I	F	I	K	**V**	E	I	**V**	**A**	T	M	I	I	N	L	**L**
395	FM877563	**V**	**E**	**V**	L	D	**I**	**V**	A	**I**	**I**	**A**	G	I	F	I	K	**V**	E	I	**V**	**A**	T	M	I	I	N	L	**L**
511	FM877564	**V**	**E**	**V**	L	D	**I**	**V**	A	**I**	**I**	**A**	G	I	F	I	K	**V**	E	I	**V**	**A**	T	M	I	I	N	L	**L**
Patient 5 (0)	FM877565	**V**	**E**	**V**	L	D	**I**	**V**	A	**I**	**I**	**V**	G	I	F	I	K	**V**	E	I	I	**A**	T	M	I	I	N	L	**L**
Patient 6																													
0	AM408180	**V**	**E**	**V**	L	D	**I**	**V**	A	**I**	**I**	**V**	G	I	F	I	K	**V**	E	I	**V**	**A**	T	M	I	I	N	L	**L**
174	AM408181	**V**	**E**	**V**	L	D	**I**	**V**	A	**I**	**I**	**V**	G	I	F	I	K	**V**	E	I	**V**	**A**	T	M	I	I	N	L	**L**
Patient 7																													
0	FM877566	**V**	**E**	**V**	L	D	**I**	**V**	A	**I**	**I**	**V**	G	I	F	I	K	**V**	E	I	**V**	**A**	T	M	I	I	N	L	**L**
504	FM877567	**V**	**E**	**V**	L	D	**I**	**V**	A	**I**	**I**	**V**	G	I	F	I	K	**V**	E	I	**V**	**A**	T	M	I	I	N	L	**L**
Patient 8 (0)	AM408182	**V**	**E**	**V**	L	D	**I**	**V**	A	**I**	**I**	**V**	G	I	F	I	K	**V**	E	I	**V**	**A**	T	M	I	I	N	L	**L**
Patient 9																													
0	FM877568	**V**	**E**	**V**	L	D	**I**	**V**	A	**I**	**I**	**V**	G	I	F	I	R	**V**	E	I	I	**A**	T	M	I	I	N	L	**L**
816	FM877569	**V**	**E**	**V**	L	D	**I**	**V**	A	**I**	**I**	**V**	G	I	F	I	K	**V**	E	I	**V**	**A**	T	M	I	I	N	L	**L**
Patient 11																													
0	FM877570	**V**	**E**	**V**	L	D	**I**	**V**	A	**I**	**I**	**V**	G	I	F	I	K	**V**	E	I	**V**	**A**	T	M	I	I	N	L	**L**
775	FM877571	**V**	**E**	**V**	L	D	**I**	**I**	A	**I**	**I**	**A**	G	I	F	I	K	**V**	E	I	**V**	**A**	T	M	I	I	N	L	**L**
945	FM877572	**V**	**E**	**V**	L	D	**I**	I	A	**I**	**I**	A	G	I	F	I	K	**V**	E	I	**V**	**A**	T	M	I	I	N	L	**L**
Patient 12																													
0	AM408183	**V**	**E**	**V**	L	D	**I**	**V**	A	**I**	**I**	**V**	G	I	F	I	K	**V**	E	I	**V**	**A**	T	M	I	I	N	L	**L**
107	AM408184	**V**	**E**	**V**	L	D	**I**	**V**	A	**I**	**I**	**V**	G	I	F	I	K	**V**	E	I	**V**	**A**	T	M	I	I	N	L	**L**

[a] Given as wild-type (WT) HIV-1 or HIV-2 or as the patient number and the length of therapy (in days).

[b] Underlined boldface letters represent emerging drug resistance mutations. Boldface letters that are not underlined represent HIV-1 drug resistance mutations found as natural polymorphisms in our samples. Underlined lightface letters represent HIV-2 polymorphisms that could affect drug susceptibility.

TABLE 3. Drug resistance mutations emerging as a result of NRTI exposure

Sample[a]	Accession no.	Amino acid[b] associated with mutation common in HIV-1 patients exposed to NRTIs															
		M41L	K65R	D67N	T69D	K70R	L74V	V75I	F77L	Y115F	F116Y	Q151M/L	M184V	L210W	T215Y/F	K219Q/E	G333D/E
WT HIV-1	K03455	M	K	D	T	K	L	V	F	Y	F	Q	M	L	T	K	G
Patient 19																	
0	FM877573	M	K	D	T	K	L	V	F	Y	F	Q	M	L	T	K	G
559	FM877574	M	K	D	T	K	L	V	F	Y	F	Q	M	L	T	K	G
WT HIV-2	M15390	M	K	D	N	K	L	I	F	Y	F	Q	M	N	S	**E**	Q
Patient 1																	
0	AM408175	M	K	D	N	K	L	**I**	F	Y	F	Q	M	N	S	**E**	Q
55	AM408176	M	K	D	N	K	L	**I**	F	Y	F	Q	M	N	S	**E**	Q
Patient 2																	
0	AM408177	M	K	D	N	K	L	**I**	F	Y	F	Q	M	N	S	**E**	Q
130	AM408178	M	K	D	N	K	L	**I**	F	Y	F	Q	**I**	N	S	**E**	Q
255	AM408179	M	K	D	N	K	L	**I**	F	Y	F	Q	**V**	N	S	**E**	Q
310	FM877562	M	K	D	N	K	L	**I**	F	Y	F	Q	**V**	N	S	**E**	Q
395	FM877563	M	K	D	N	K	L	**I**	F	Y	F	Q	**V**	N	S	**E**	Q
511	FM877564	M	K	D	N	K	L	**I**	F	Y	F	Q	M	N	S	**E**	Q
Patient 5 (0)	FM877565	M	K	D	N	K	L	**I**	F	Y	F	Q	M	N	S	**E**	C
Patient 6																	
0	AM408180	M	K	D	N	K	L	**I**	F	Y	F	Q	M	N	S	**E**	Q
174	AM408181	M	K	D	N	K	L	**I**	F	Y	F	Q	M	N	S	**E**	Q
Patient 7																	
0	FM877566	M	K	D	N	K	L	**I**	F	Y	F	Q	M	N	S	**E**	Q
504	FM877567	M	K	D	N	K	L	**I**	F	Y	F	Q	M	N	S	**E**	Q
Patient 8 (0)	AM408182	M	K	D	N	K	L	**I**	F	Y	F	Q	M	N	S	**E**	Q
Patient 9																	
0	FM877568	M	K	D	N	K	L	**I**	F	Y	F	Q	M	N	S	**E**	Q
816	FM877569	M	K	D	N	K	L	**I**	F	Y	F	Q	M	N	S	**E**	Q
Patient 11																	
0	FM877570	M	K	D	N	K	L	**I**	F	Y	F	Q	M	N	S	**E**	Q
775	FM877571	M	K	D	N	K	L	**I**	F	Y	F	Q	**V**	N	S	**E**	Q
945	FM877572	M	K	D	N	K	L	**I**	F	Y	F	Q	**V**	N	S	**E**	Q
Patient 12																	
0	AM408183	M	K	D	N	K	L	**I**	F	Y	F	Q	M	N	S	**E**	Q
107	AM408184	M	K	D	N	K	L	**I**	F	Y	F	Q	M	N	S	**E**	Q

[a] Given as wild-type (WT) HIV-1 or HIV-2 or as the patient number and the length of therapy (in days).
[b] Underlined boldface letters represent emerging drug resistance mutations. Boldface letters that are not underlined represent HIV-1 drug resistance mutations found as natural polymorphisms in our samples. Underlined lightface letters represent HIV-2 polymorphisms that could affect drug susceptibility.

to harbor PI resistance in HIV-1 (F53Y/L), emerged due to drug pressure in patient 2 (27). Patient 11 had two PI mutations, V33I and V47A, and the 3TC mutation M184V at the time of genotyping.

In addition to the known HIV-1 mutations, several other mutations not previously detected in HIV-1 were observed. In the protease sequences, the K45N (patient 1), H14R, and M95I (patient 11) mutations seemed to emerge as a result of LPV/r pressure, and in the RT sequences, the L260P (patient 1) and V371I (patient 2) mutations seemed to emerge as a result of ZDV and/or 3TC pressure.

DISCUSSION

Currently approved ARV drugs were designed and optimized for the treatment of HIV-1 subtype B infections. These drugs were expected to have similar efficacy for HIV-2 patients as for HIV-1 patients, due to the high structural and enzymatic function similarities between the protease and RT genes and proteins of these two viruses (42). However, it was discovered that HIV-2 is naturally resistant to the NNRTIs and the entry inhibitor T-20 and that it may have reduced susceptibility to some PIs (42, 49). These resistance properties restrict the treatment of HIV-2-infected and dually infected patients with HAART by limiting the drugs available for the second and subsequent regimens. The use of a potent and effective first-line regimen to which HIV-1 is sufficiently sensitive can result in full viral suppression for more than 7 years (13). Our study reports durable and efficient suppression of both HIV-1 and HIV-2 in 19/20 treated patients. Patient 1, whose viral load remained detectable, had stopped using this treatment due to adverse effects. Even though previous studies have reported that various HAART regimens suppress HIV-2 replication by only 0.4 to 2 log units and usually fail to achieve undetectable viral loads (1, 41), viral suppression to undetectable levels was achieved for all patients taking the drugs and was maintained for as long as 3 years (range, 3 to 36 months). The reduction in the viral load was accompanied by a general increase in the percentage of CD4 cells over pretherapy levels, indicating that these patients were benefiting immunologically from this treatment.

These findings indicate that the combination of ZDV, 3TC, and LPV/r is effective at achieving efficient and durable viral suppression in HIV-2-infected and dually infected patients.

The responses to therapy indicate that dually infected patients seem to do better than singly HIV-2 infected patients.

During natural HIV infection, the virus is under pressure from the immune system, and in the case of dual infection, competition between the two viruses might increase the pressure on each of the viruses. When effective treatment is used, viruses have only a small window of opportunity to acquire resistance during periods of suboptimal drug levels, unless the mutant virus is already archived. Therefore, the reduced pressure on the singly infecting virus (monoinfection) might enable it to acquire resistance more easily than the viruses in a dually infected patient.

The presence of several HIV-1 PI mutations as natural polymorphisms in HIV-2 has resulted in reduced susceptibility to some PIs (42, 49). A recent study of the activities of currently approved PIs against the HIV-2 protease has shown that lopinavir, saquinavir, tipranavir, and darunavir exhibit the highest potencies (in that order) and that atazanavir, nelfinavir, and amprenavir show the lowest potencies (in that order) (7). Sequence analysis of the protease region during this study has revealed the natural presence of nine minor HIV-1 PI mutations, L10V, G16E, K20R, L33V, M36I, I62V, A71V, G73A, and I93L, and three major mutations, V32I, M46I, and I47V, which may explain the reduced susceptibility of wild-type HIV-2 strains to several PIs (26).

Drug resistance mutations are selected less frequently in patients taking a ritonavir-boosted PI than in those taking a nonboosted PI. However, it has been reported that while the same mutations usually emerge with boosted and nonboosted PIs, the relative frequency of mutations may differ (26), and that an accumulation of several mutations is often necessary to cause significant resistance to boosted PIs (26). In HIV-1, LPV/r resistance is usually associated with the accumulation of specific resistance mutations in the HIV-1 protease gene (L10F/I/R/V, K20M/R, L24I, M46I/L, F53L, I54L/T/V, L63P, A71I/L/T/V, V82A/F/T, I84V, and L90M) (31, 37); however, resistance is rare in patients whose first PI is LPV/r, with some exceptions (26). A few specific mutations, most notably I47A and V32I, are associated with high-level resistance to LPV/r even for patients whose first PI is LPV/r (15, 28, 39). A recent report on the phenotypic susceptibility of HIV-2 to LPV/r has shown that the presence of the V47A mutation resulted in a substantial reduction in susceptibility to lopinavir (38). In our study, the protease sequences, after viral rebound, showed the V47A (patients 2 and 11), V33I (patient 11), and F53V (patient 2) mutations emerging as a result of LPV/r pressure in a background where the L10V, K20R, V32I, L33V, M36I, M46I, I62V, A71V, G73A, and I93L mutations were present as natural polymorphisms. Therefore, the virological failure observed for patients 2 and 11 could be attributed partly to high-level drug resistance to LPV/r.

Analysis of the RT sequences in patients with viral rebound revealed first the M184I (patient 2) and later the M184V (patients 2 and 11) mutation emerging due to drug pressure. These mutations are associated with 1,000-fold phenotypic resistance to 3TC (24, 27). The Q151M mutation, frequently found in HIV-2 patients failing an NRTI-containing regimen (42), was absent in these patients. Apart from the ZDV-associated secondary mutations V75I and K219Q/E, present as natural polymorphisms, other ZDV mutations have not been found in any of the patients. As previously observed, accumulation of the six thymidine analogue resistance mutations, M41L, D67N, K70R, L210W, T215Y, and K219Q/E (32), marking classic ZDV resistance in HIV-1 (1, 6, 9, 23, 49), was also absent in the HIV-2 sequences. Therefore, we conclude that high-level resistance to 3TC also contributed to the virological failure observed for patients 2 and 11 and that the viruses in these patients either are sensitive to ZDV or have as yet unidentified ZDV-associated mutations.

The presence of these high-level PI and RT inhibitor resistance mutations in patient 2 (after 5 months on HAART) and patient 11 explains the observed virological failures. Although it has been reported that drug resistance is rare in patients taking LPV/r as part of the first-line therapy (15, 28, 39), the presence of background mutations may shorten the time to development of resistance in HIV-2 relative to that for HIV-1 (43), as observed in this study. In HIV-1, the emergence of the LPV/r mutation I47A is a two-step process, going from I to V to A, while in HIV-2, it occurs in a single step from V to A, making the emergence of this mutation easier and faster in HIV-2 (38). However, HIV-2 V47A mutants were found to retain susceptibility to other PIs and seemed to be hypersusceptible to atazanavir and saquinavir (38). This finding makes these drugs useful as second-line PIs for our HIV-2 patients, especially saquinavir, which has been reported to have potent activity against HIV-2 (7).

Generally, fewer mutations accumulated in these HIV-2 patients with viral rebound than in those from previous studies (1, 6, 9, 23), and the Q151M mutation was absent. This finding indicates that efficient therapy results in fewer mutations and therefore less cross-resistance to other drugs. This allows the viruses to retain susceptibility to other drugs that can be used in subsequent treatment regimens.

Analysis of sequences from other patients with viral rebound did not indicate the presence of known drug resistance mutations. However, in subsequent samples, the viral load either returned to undetectable levels or was substantially reduced, indicating that the rebound was in fact only a viral blip (a temporarily elevated viral load) or a consequence of poor adherence.

We report the response of 20 HIV-2-infected and dually infected patients to a HAART regimen and the finding that the combination of ZDV, 3TC, and LPV/r is effective at achieving efficient and durable viral suppression in both HIV-2-infected and dually infected patients. After more than 3 years, only three patients need second-line therapy, one due to adverse reactions to LPV/r and two due to high-level resistance to LPV/r and 3TC. New potential drug resistance mutations, not previously observed in HIV-1, were observed in this study. These mutations could be primary determinants of drug resistance in HIV-2, so there is a need to develop phenotypic resistance assays for HIV-2 to determine their clinical relevance. In conclusion, the response of HIV-2 to HAART can be similar to that of HIV-1, provided that an optimal drug regimen is chosen. This further highlights the importance of choosing PIs and RT inhibitors that are active against HIV-2.

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