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Patterns of Resistance Mutations to Antiretroviral Drugs in Extensively Treated HIV-1–Infected Patients With Failure of Highly Active Antiretroviral Therapy

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Summary: Resistance-mutation patterns in the reverse transcriptase (RT) and protease genes of HIV-1 were analyzed in 22 patients who had been extensively pretreated and who failed to respond to highly active antiretroviral therapy (HAART). The number of mutations ranged from 8 to 19 (median, 13): 4 to 12 (median, 6) mutations in the RT gene, and 4 to 8 (median, 7) mutations in the protease gene. In the RT gene, the most frequent resistance mutations were found at codons 215 (100%), 41 (95%), 67 (91%), and 210 (77%). Multidrug-resistant mutation patterns including Q151M and insertion mutations at codon 69, which confer cross-resistance to the different nucleoside analogue RT inhibitors were detected in 1 and 3 patients, respectively; 1 patient with insertion mutation displayed a NGQCV sequence at codons 67 to 70. In the protease gene, the most frequent mutations were found at codons 63 (95%), 10 (86%), 90(86%), 71(77%), 46 (50%), 36 (45%), and 84 (45%). Genotypic resistance to zidovudine, saquinavir, and indinavir was found in 100% of the patients. All patients showed also resistance or possible resistance to stavudine, abacavir, ritonavir, and nelfinavir. Mutations conferring genotypic resistance to nonnucleoside analogue RT inhibitors (NNRTIs) were found in 12 (80%) of the NNRTI-experienced patients and 1 of 7 NNRTI-naive patients. Our results indicate that failure of HAART in the patients extensively pretreated results from the multiplicity of RT and protease mutations that confer genotypic resistance to almost all available antiretroviral drugs. In these patients, genotypic resistance tests confirm the lack of alternative salvage therapy strategies based on the currently available antiretroviral drugs. **Key Words:** Mutations—Genotypic resistance—Highly active antiretroviral therapy—Treatment failure.

Highly active antiretroviral therapy (HAART), based on the combination of three or more antiretroviral drugs including protease inhibitors (PIs), is the current standard for treatment of HIV-1–infected patients (1). In antiretroviral-naive patients, use of these potent drug regimens results in a dramatic decrease in virus replication, because a drop of HIV-1 RNA levels in plasma below the limit of detection is observed with a high frequency (2).

Nucleoside analogue reverse transcriptase inhibitor (NRTI)-based monotherapies or two-drug combination therapies that were administered before PI availability are not sufficiently potent to suppress HIV-1 replication (3,4). Given that viral replication in the presence of antiviral drugs leads to selection and development of HIV-1 variants that harbor resistance mutations, response to HAART is frequently impaired in patients pretreated with a drug regimen considered as suboptimal in

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terms of virologic efficacy, and HAART failure is more frequently observed in these patients (5,6).

To assess the extent of resistance to antiviral drugs in patients heavily pretreated who did not respond to HAART, we analyzed the sequence of RT and protease genes of HIV-1 in patients who had been treated for several years by successive antiretroviral therapies, including suboptimal regimens, and who thus presented with failure of HAART.

PATIENTS AND METHODS

Patients

The present analysis included 22 consecutive HIV-1–infected patients recruited between September 1998 and August 1999 who fulfilled the following criteria: 1) they have been extensively (>2 years) pretreated by antiretroviral drugs regimens including suboptimal treatments by NRTI monotherapy and/or dual combination therapy; 2) they presented after failure of at least two consecutive HAART regimens, and 3) they maintained a high level of viral replication defined by a plasma viral load >30,000 copies/ml. They comprised 18 males and 4 females. Their median age was 42 years (range, 11.0–67.5 years). The median viral load as determined by the Amplicor HIV-1 Monitor assay version 1.5 (Roche Diagnostics Systems, Meylan, France) was 430,564 HIV-1 RNA copies/ml (range, 37,866–1,515,146 HIV-1 RNA copies/ml) and the median CD4 T cell count was 84 cells/mm^3 (range, 2–782 cells/mm^3).The median cumulative duration of antiretroviral treatment was 196 weeks (range, 131–442 weeks). Of these patients, 16 had been initially treated by NRTI monotherapy (zidovudine [ZDV] or didanosine [ddI]) for a median duration of 54 weeks (range, 12–244 weeks); all patients underwent a dual NRTI combination regimen for a median duration of 49 weeks (range, 1–131 weeks). Patients have been treated by HAART for a median duration of 115 weeks (range, 69–186 weeks). Drugs administered, cumulative duration of treatment with each drug, and time since discontinuation of these drugs are detailed in Table 1. All patients have received therapy with at least four NRTIs (median, 5; range, 4–6) and two PIs (median, 4; range: 2–5); 15 of these patients have experienced one (n = 8) or two (n = 7) non-NRTIs (NNRTI).

Reverse Transcriptase and Protease Gene Sequencing

HIV-1 RNA was extracted from plasma using the QIAamp viral RNA kit (Qiagen, Courtaboeuf, France) and reverse-transcribed using the Expand RT (Roche Diagnostic Systems, Meylan, France). A 2219-bp DNA fragment encompassing the RT and protease genes was amplified by seminested polymerase chain reaction (PCR). The primers G25Rev (5′-GCAAGAGTTTTGGCTGAAGCAATGAG) and In3 (5′-TCTATBCCATCTAAAAATAGTACTTTCCTGATTCC) were used for the first round of amplification and the primers AV150 (5′-GTGGAAAGGAAGGACACCAAATGAAAG) and In3 were used for the second round. Seven overlapping fragments were sequenced using the primers AV150, In5 (5′-AATTTTCCCATTAGTCCTATTGANACTGTACCAG), PolMO (5′-TCCCTCAGATCACTCTTTGGCA), PolM1 (5′-GTTAAACAATGGCCATTGACAGA), Pol M2 (5′-GATTTGTATGTAGGATCTGA) for the positive strand and the primers Pol M4 (5′-CTATTAGCTGCCCCATCTACATA) and PolM6 (5′-CTTTGATAAAACCTCCAATT) for the negative strand. Sequences were analyzed with the ABI prism 377 automatic sequencing system (Applied Biosystems, des Upis, France) and the amino acid sequences were compared with the HIV-1_{LAI} RT and protease sequences. By sequencing these overlapping fragments, each nucleotide position in the RT and protease genes was analyzed by two to four independent sequence reactions. DNA sequences have been submitted to GenBank under the accession numbers AJ405947 to AJ405968.

Sequence Interpretation

Interpretation of genotype in terms of drug-resistance was based on an algorithm established by the "Resistance" group of the French National Agency for AIDS Research (ANRS) (7). Mutations indexed in the ANRS report are: M41L, A62V, K65R, D67N/E/S, T69D, insertion at codon 69, K70R, L74V, V75M/S/A/T/I, F77L, Y115F, F116Y, Q151M, M184I/V, L210W, T215Y/F, and K219Q/E for NRTIs ; A98G, L100I, K101E, K103N, V106A, V108I, Y181C/I, Y188L/C, G190A/S, P225H, and P236L for NNRTIs, and L10I/R/V, K20R/M, D30N, M36I, M46I/L, I47V, G48V, I50V, I54V/L, L63P, A71V/T, G73S, V77I, V82A/F/T/S, I84V, N88S/D, and L90M for PIs. Taking into account the major or minor character of the different mutations in term of resistance, the algorithm provided in the ANRS report distinguished patterns of mutations conferring "resistance" from patterns conferring "possible resistance" to a given antiretroviral drug (see Appendix).

RESULTS

RT and Protease Gene Mutations

Resistance mutations in the RT and protease genes were detected in all patients. As shown in Table 2, the number of resistance mutations detected in individual patients ranged from 8 to 19 (median, 13). The number of resistance mutations in the RT gene ranged from 4 to 12 (median, 6): all patients showed mutations (3–11) confering resistance to NRTIs and 13 of them showed mutations (1–4) conferring resistance to NNRTIs. The number of resistance mutations on the protease gene ranged from 4 to 8 (median, 7).

The mutations most frequently detected in the RT gene were T215Y/F (100%), M41L (95%), D67N (91%), and L210W (77%). Prevalences of the zalcitabine (ddC)-specific mutation T69D, lamivudine (3TC)-specific mutation M184V, stavudine (d4T)-specific mutation V75M//T, and didanosine (ddI)-specific mutation M74V were 32%, 27%, 9%, and 5%, respectively. One patient presented the multidrug resistant (MDR) mutation pattern A62V + V75I + F77L + F116Y + Q151M. Insertion between codons 69 and 70 was observed in 3 patients: one SS insertion (patient no. 15), one SV insertion (patient no. 16), and a NGQCV sequence instead of the DSTK sequence at codons 67 to 70.

Among the RT gene mutations conferring resistance to NNRTIs, Y181C (27%), K103N (23%), and G190A (18%) were the most frequently detected.

TABLE 1. *Drugs administered to 22 extensively treated HIV-1–infected patients*

Patient	Current treatment		Previous treatment		Other drugs experienced [cumulative duration of administration (weeks)/time since discontinuation (wk)]
	Drugs[a]	Duration (wk)	Drugs[a]	Duration (wk)	
1	3TC (9), RTV (43), NFV (24)	6	3TC, d4T (34), RTV, SQV (38)	3	ZDV (160/50), ddI (62/11), IDV (54/65)
2	d4T (75), NVP (9), NFV (25)	9	d4T, 3TC (144), NFV	16	ZDV (180/75), ddI (46/166), ddC (21/153), SQV (75/66), IDV (50/25)
3	d4T (67), NVP (22), NFV (22)	10	ZDV (257), 3TC (40), NVP, NFV	4	ddI (107/67), SQV (36/31), IDV (21/67), RTV (36/31)
4	ZDV (150), 3TC (76), ABC (4)	4	ddI (132), d4T (80), SQV (89), NFV (30)	36	ddC (23/113), IDV (5/42), RTV (45/36)
5	ddI (174), d4T (90), SQV (67), NFV (35)	19	ZDV (143), 3TC (41), SQV, NFV	16	ddC (60/62), IDV (53/62), RTV (28/34)
6	ddI (136), d4T (50), NVP (18), NFV (22)	18	ZDV (114), ddI, 3TC (66), NFV	4	ABC (2/22), SQV (52/87), IDV (3/114), RTV (3/44)
7	ddI (88), d4T (62), 3TC (62), RTV (50)	25	ZDV (131), ddI, IDV (9)	9	ddC (13/124), SQV (61/59)
8	ddI (61), SQV (42), NFV (52)	8	ddI, EFV (5), NFV	5	ZDV (41/107), d4T (49/42), 3TC (19/89), ABC (2/26), NVP (4/23), IDV (18/80), RTV (12/68)
9	ddI (137), ABC (18), EFV (18), IDV (61)	5	ABC, EFV, NFV (23)	13	ZDV (134/27), ddC (5/110), d4T (38/50), 3TC (115/27), NVP (18/18), SQV (53/18), RTV (31/27)
10	ZDV (167), ddI (116), SQV (66), NFV (42)	11	ZDV, ABC (2), SQV, NFV	2	d4T (13/128), 3TC (73/53), IDV (48/70), RTV (16/53)
11	d4T (105), EFV (32), NFV (75), SQV (71)	32	d4T, NVP (5), NFV	5	ZDV (2/188), ddI (65/53), 3TC (36/53), IDV (19/80)
12	d4T (117), SQV (74), RTV (74)	74	d4T, 3TC (43), IDV (36)	32	ZDV (155/121), ddI (20/165)
13	d4T (63), 3TC (128), SQV (79), NFV (29)	2	d4T, NVP (21), SQV, NFV	5	ZDV (203/75), ddI (155/159), ddC (10/170), IDV (43/75), RTV (44/30)
14	3TC (24), ABC (34), SQV (56), NFV (45)	4	ABC, EFV (8), SQV, NFV	8	ZDV (73/34), ddI (104/12), ddC (4/114), d4T (19/94), NVP (7/43) IDV (25/55), RTV (62/34)
15	ABC (24), EFV (24), IDV (41), RTV (23)	8	ABC, NFV (76), EFV	16	ZDV (89/61), ddI (30/153), ddC (30/123), d4T (62/24), 3TC (60/39), NVP (8/31), SQV, (60/24)
16	ABC (61), NFV (56)	26	ABC, EFV (28), NFV	18	ZDV (210/46), ddI (23/69), ddC (27/161), d4T (55/69), 3TC (84/46), NVP (1/60), SQV (57/33), IDV (19/49), RTV (23/69)
17	ABC (40), EFV (40), NFV (40)	40	3TC (60), SQV (52), RTV (40)	32	ZDV (29/78), ddI (32/106), d4T (54/55), IDV (35/87)
18	d4T (108), SQV (69), NFV (13)	13	ddI (56), d4T, SQV (69)	56	ZDV (35/108), ddC (35/108), 3TC (39/69)
19	ddI (107), d4T (72), SQV (59), RTV (46)	34	ABC (18), NVP (12), SQV, RTV	12	ZDV (86/77), 3TC (51/52), IDV (13/77), NFV (25/52)
20	ddI (118), NVP (23), SQV (30), RTV (30)	14	ZDV (254), 3TC (162), NVP, SQV, RTV	9	ddC (14/153), d4T (51/50), IDV (12/153), NFV (28/90)
21	ABC (53), NVP (20), SQV (61), RTV (61)	20	ABC, ddI (65), SQV, RTV	33	ZDV (66/93), ddC (61/136), d4T (78/53), 3TC (51/61), IDV (37/61)
22	ddI (99), ABC (25), EFV (5), SQV (72), NFV (38)	5	ddI, d4T (92), APV (25)	25	ZDV (48/73), ddC (51/134), 3TC (30/73), NVP (66/43), IDV (8/104), RTV (61/35)

[a] Cumulative duration of treatment (weeks) with each drug is indicated within parentheses.

ZDV, zidovudine; ddI, didanosine; ddC, zalcitabine; d4T, stavudine; 3TC, lamivudine; ABC, abacavir; NVP, nevirapine; DLV, delavirdine; EFV, efavirenz; SQV, saquinavir; IDV, indinavir; RTV, ritonavir; NFV, nelfinavir; APV, amprenavir; ABT, ABT-378.

TABLE 2. *Viral load, reverse transcriptase and protease mutations, and genotypic resistance to antiretroviral drugs in individual patients*

Subject	HIV-1–RNA level (copies/ml)	Mutations		Genotypic resistance[a]		
		Reverse transcriptase	Protease	NRTI	NNRTI	PI
1	823,986	41L, 67N, 184V, 210W, 215Y	10I, 36I, 63P, 71V, 73S, 77I, 84V, 90M	**ZDV, 3TC, ABC,** ***d4T***	—	**SQV, IDV, RTV, NFV, APV, ABT**
2	306,902	41L, 67N, 75M, 106A, 210W, 215Y	10I, 20R, 36I, 48V, 63P, 71V, 82A	**ZDV, d4T,** ***ABC***	**NVP, DLV**	**SQV, IDV, RTV,** ***NFV***
3	795,852	41L, 67N, 69D, 188L, 210W, 215Y	10I, 46I, 63P, 77I, 90M	**ZDV, ddC, d4T,** ***ABC***	**NVP**	**SQV, IDV, NFV,** ***APV, RTV***
4	690,854	41L, 67N, 98G, 103N, 184V, 210W, 215Y	10I, 20R, 36I, 54V, 63P, 71T, 84V, 90M	**ZDV, 3TC, ABC,** ***d4T***	**NVP, DLV, EFV**	**SQV, IDV, RTV, NFV, APV, ABT**
5	172,487	41L, 67N, 69D, 210W, 215Y	10R, 46I, 63P, 77I, 84V, 90M	**ZDV, ddC, d4T,** ***ABC***	—	**SQV, IDV, RTV, NFV, APV, ABT**
6	1,515,146	41L, 67N, 210W, 215Y	46I, 63P, 71T, 90M	**ZDV,** ***d4T, ABC***	—	**SQV, IDV, NFV,** ***RTV, APV***
7	281,124	41L, 67N, 69D, 184V, 210W, 215Y	10I, 54V, 63P, 71V, 73S, 77I, 82A, 90M	**ZDV, ddC, 3TC, d4T, ABC**	—	**SQV, IDV, RTV, NFV,** ***APV***
8	451,096	41L, 67N, 69D, 181C, 210W, 215Y	10I, 46I, 48V, 63P, 71V, 77I, 82A	**ZDV, ddC, d4T,** ***ABC***	**NVP, DLV**	**SQV, IDV, RTV,** ***NFV, APV***
9	37,866	62V, 67N, 69D, 70R, 75I, 77L, 103N, 116Y, 151M, 184V, 215F, 219Q	10I, 20M, 36I, 54V, 63P, 73S, 90M	**ZDV, ddI, ddC, 3TC, d4T, ABC**	**NVP, DLV, EFV**	**SQV, IDV, NFV,** ***RTV, APV***
10	612,030	41L, 67N, 184V, 210W, 215Y	10I, 36I, 46I, 63P, 71V, 84V, 90M	**ZDV, 3TC, ABC,** ***d4T***	—	**SQV, IDV, RTV, NFV, APV, ABT**
11	252,804	41L, 67N, 190A, 210W, 215Y	36I, 63P, 71T, 73S, 90M	**ZDV,** ***d4T, ABC***	**NVP, EFV**	**SQV, IDV, NFV,** ***RTV***
12	221,023	41L, 67N, 210W, 215Y	10I, 36I, 63P, 84V, 90M	**ZDV,** ***d4T, ABC***	—	**SQV, IDV, RTV, NFV, APV, ABT**
13	817,881	41L, 62V, 67N, 69insertion, 181 C, 190A, 210W, 215Y	10V, 36I, 46I, 63P, 71T, 88S, 90M	**ZDV, ddI, ddC, 3TC, d4T, ABC**	**NVP, DLV, EFV**	**SQV, IDV, NFV,** ***RTV, APV***
14	446,957	41L, 67N, 70R, 210W, 215Y, 219Q	10I, 36I, 46I, 54V, 63P, 73S, 84V, 90M	**ZDV,** ***d4T, ABC***	—	**SQV, IDV, RTV, NFV, APV, ABT**
15	532,637	41L, 69insertion, 98G, 103N, 181C, 190A, 215Y	10I, 46I, 63P, 71V, 73S, 77I, 90M	**ZDV, ddI, ddC, 3TC, d4T, ABC**	**NVP, DLV, EFV**	**SQV, IDV, NFV,** ***RTV, APV***
16	100,000	41L, 62V, 69insertion, 188L, 215Y	63P, 71V, 77I, 84V, 90M	**ZDV, ddI, ddC, 3TC, d4T, ABC**	**NVP, DLV, EFV**	**SQV, IDV, RTV, NFV, APV, ABT**
17	414,170	41L, 67N, 69D, 100I, 103N, 210W, 215Y	10I, 48V, 54V, 71V, 77I, 82A	**ZDV, ddC, d4T,** ***ABC***	**NVP, DLV, EFV**	**SQV, IDV, RTV,** ***NFV, APV***
18	154,641	41L, 67N, 210W, 215Y	10I, 20R, 36I, 63P, 71T, 73S, 84V, 90M	**ZDV,** ***d4T, ABC***	—	**SQV, IDV, RTV, NFV, APV, ABT**
19	638,883	41L, 67N, 69D, 98G, 181C, 210W, 215Y	10I, 46I, 63P, 71V, 73S, 77I, 84V, 90M	**ZDV, ddC, d4T,** ***ABC***	**NVP, DLV**	**SQV, IDV, RTV, NFV, APV, ABT**
20	690,449	41L, 67S, 70R, 184V, 215F, 219E	10I, 54V, 63P, 71V, 82A, 90M	**ZDV, 3TC, ABC,** ***d4T***	—	**SQV, IDV, RTV, NFV,** ***APV***
21	153,000	41L, 67N, 74V, 103N, 181C, 210W, 215Y	10V, 46I, 54V, 63P, 71V, 82A, 90M	**ZDV, ddI, 3TC,** ***d4T, ABC***	**NVP, DLV, EFV**	**SQV, IDV, RTV, NFV,** ***APV***
22	1,482,050	41L, 67N, 70R, 75T, 101E, 181C, 190A, 215Y, 219Q	10I, 46I, 63P, 71V, 73S, 82A, 84V, 90M	**ZDV, d4T,** ***ABC***	**NVP, DLV, EFV**	**SQV, IDV, RTV, NFV, APV, ABT**

[a] Genotypic resistances are indicated **in bold,** possible resistances are indicated *in italics.*

NRTI, nucleoside reverse transcriptase inhibitors; NNRTI, nonnucleoside reverse transcriptase inhibitors; *PI,* protease inhibitors; ZDV, zidovudine; ddI, didanosine; ddC, zalcitabine; 3TC, lamivudine; d4T, stavudine; ABC, abacavir; NVP, nevirapine; DLV, delavirdine; EFV, efavirenz; SQV, saquinavir; IDV, indinavir; RTV, ritonavir; NFV, nelfinavir; APV, amprenavir; ABT, ABT-378.

In the protease gene, the most prevalent mutations were L63P (95%), L10I/R/V (86%), L90M (86%), A71V/T (77%), M46I (50%), M36I (45%), and I84V (45%).

Genotypic Resistance

The patterns of genotypic resistances presented by individual patients are given in Table 2. All patients showed genotypic resistance to ZDV, saquinavir (SQV), and indinavir (IDV); they also showed resistance or possible resistance to d4T, abacavir (ABC), ritonavir (RTV), and nelfinavir (NFV); 91% of them showed resistance or possible resistance to amprenavir (APV) (Fig. 1). Genotypic resistance to ddI, ddC, 3TC, and ABT-378 (ABT) were less frequently found. Among the 15 patients experienced with NNRTIs, 12 (80%) showed resistance to this class of compounds. Genotypic resistance to NNRTIs was also found in one NNRTI-naive patient (#4). In the same way, we observed that 4 of 9 (44%) patients naive to ddC had the T69D ddC-specific mutation (patients #3, #8, #17, and #19).

For 13 (59%) patients, we detected mutations conferring resistance or possible resistance to all drugs administered at the time of testing. The other patients showed absence of genotypic resistance to only one (ddI, 6 cases; 3TC, 1 case) or two (ddI + NVP, 2 cases), of the drugs administered. We did not observe a significant difference in HIV-1 RNA levels between patients showing genotypic resistance to all the drugs administered and patients in whom one or more of the drugs administered were not concerned by genotypic resistance ($p = .89$ with Mann-Whitney [Wilcoxon] W test).

DISCUSSION

In this study, we analyzed mutation patterns of the RT and protease genes of HIV-1 in patients extensively pretreated by successive antiretroviral therapies who failed to respond to HAART. Multiple mutations conferring resistance to RT and protease inhibitors were identified in all patients. In nearly 60% of these patients, the mutations patterns indicated genotypic resistance or pos-

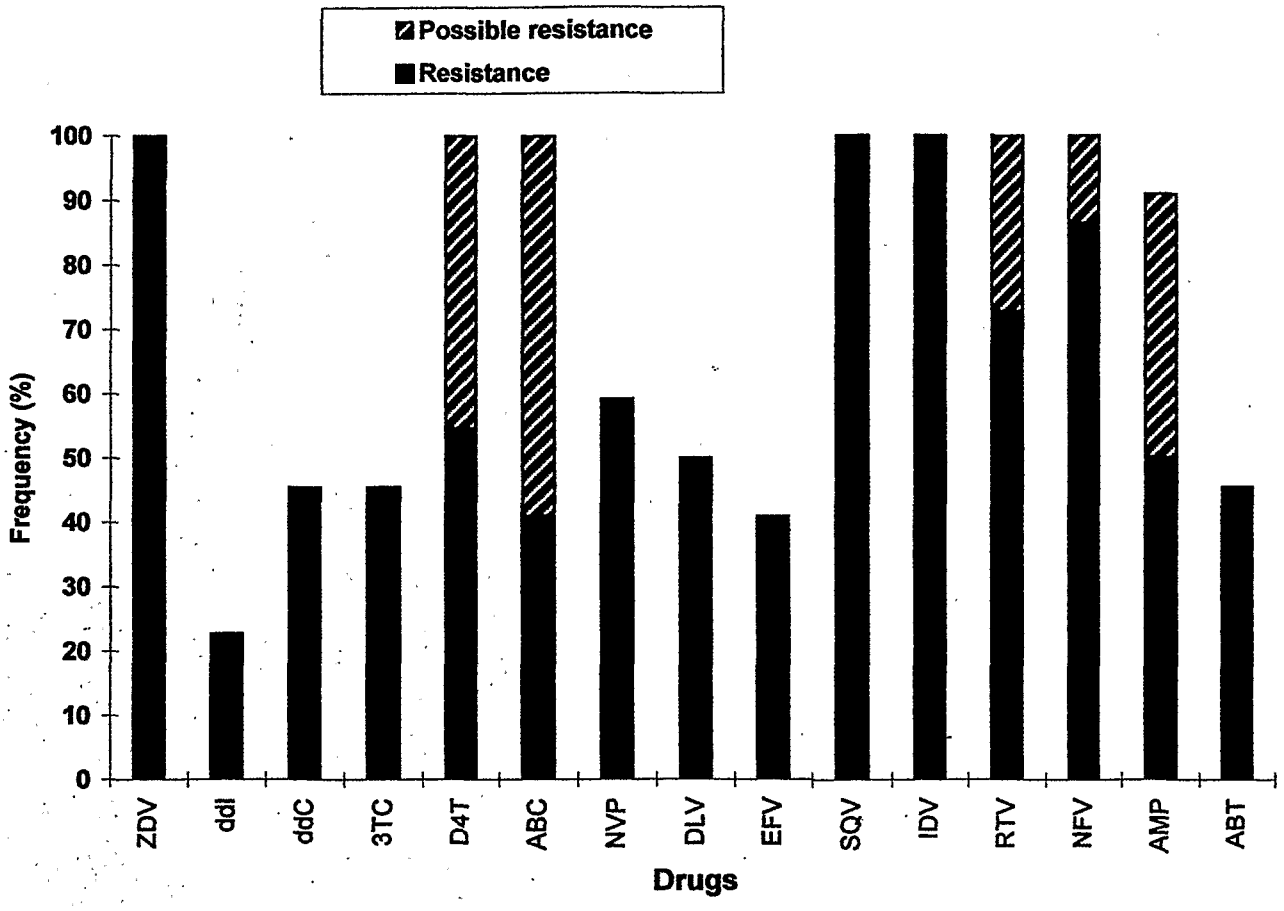

FIG. 1. Frequency of genotypic resistances to antiretroviral drugs.

sible resistance to every drug included in HAART, whereas in the remainder of these patients, the number of drugs not concerned by genotypic resistance remained very limited. It has been previously reported that failure of HAART is predicted by the number of resistance mutations in RT and protease genes (8,9). Results obtained in our patients strongly suggest that HAART failure resulted from the resistance of HIV-1 to the drugs administered and not from other causes such as inadequate drug exposure due to pharmacologic factors or suboptimal patient adherence to treatment (10).

All patients had genotypic resistance to ZDV and also demonstrated resistance or possible genotypic resistance to d4T and ABC because it was considered that the presence of three or more ZDV-specific mutations including T215Y/F confers possible resistance to d4T and ABC (4,7,11–13). We also observed a high frequency of genotypic resistance to PIs. Resistance concerned not only the more widely used PIs such as SQV, IDV, RTV, and NFV but also APV and ABT. Indeed, all patients but one were naive to APV and 91% of them had genotypic resistance or possible resistance to APV. In the same way, all patients were naive to ABT and 45% of them had genotypic resistance to ABT. The presence of secondary mutations that confer cross resistance to different PIs accounted for this resistance. In particular, the mutation I84V is known to confer a cross-resistance to various PIs, including APV and ABT (14,15). Therefore, our results are in agreement with previous studies indicating that the multiplicity of RT and protease mutations confers cross-resistance to almost all available antiretroviral drugs, including compounds in clinical development (16,17).

All patients were experienced with ddI but only 5 showed genotypic resistance to this compound. Genotypic resistance to ddI involved a L74V mutation (1 patient), an insertion at codon 69 (3 patients) and a MDR pattern involving codons 62, 75, 77, 116, and 151 (1 patient). The low prevalence of the ddI-specific resistance mutation L74V in patients with combination therapy has been previously observed (18,19). However, it has been shown that ddI monotherapy can promote development of ZDV-specific resistance mutations (20,21) and that the antiviral effect of NRTI combinations containing ddI is diminished in patients infected with HIV-1 strains harboring ZDV-specific resistance mutations (4,11). Therefore, despite the low frequency of genotypic resistance to ddI in heavily treated patients, further investigation is needed to assess the efficacy of this drug in patients infected with HIV-1 variants harboring multiple resistance mutations in the RT gene.

All these patients were experienced with 3TC but only 27% of them had the 3TC-specific mutation M184V. The relatively low proportion of patients harboring the M184V mutation can be explained because 77% of these patients had discontinued 3TC at the time of testing. Indeed, the M184V mutation impairs viral fitness and the M184V variants are rapidly replaced by a HIV-1 population with wild-type sequence at codon 184 after stopping therapy with 3TC (22,23).

The mutation T69D, considered as ddC-specific, was observed in 32% of patients. This relatively low frequency can be explained because only 59% of patients were experienced with ddC and that all had discontinued ddC treatment for 26 to 170 weeks. Interestingly, we also detected the T69D mutation in 44% of the ddC-naive patients. This observation suggests that cross resistance to ddC conferred by mutation at codon 69 can be promoted by protracted administration of other NRTIs. In the same way, we observed genotypic resistance to NNRTIs in one of the seven NNRTI-naive patients. To our knowledge, cross-resistance between NRTIs and NNRTIs has not been reported. Therefore, further investigation at a larger scale is needed to study genotypic resistance to NNRTIs in NNRTI-naive patients extensively treated by NRTIs.

An insertion between codons 69 and 70 was detected for 3 (14%) patients. Insertions in this region—located in the "fingers" domain involved in the nucleoside triphosphate binding site of the enzyme—are known to confer multinucleoside analogue resistance (24,25). In 1 such patient, we identified a novel sequence characterized by a single amino acid insertion associated with a set of other substitutions between codons 67 and 70, giving a NGQCV sequence instead of the DSTK consensus sequence. A novel genotype characterized by a single amino acid insertion at codon 69, associated with five other substitutions located between codons 64 and 74 has been recently described and recognized as conferring multiple resistance to NRTIs (26). We therefore considered that the NGQCV sequence at codons 67 to 70 conferred multinucleoside analogue resistance, but this remains to be confirmed by phenotypic analysis.

It has been shown that in patients with antiretroviral therapy failure, genotypic-resistance testing is of interest for choosing a therapeutic alternative (18). However, results obtained in the present study indicate that in patients extensively pretreated, failure of HAART results from resistance to almost all available antiretroviral drugs. Therefore, genotypic resistance tests seem to lack usefulness in these patients to propose a salvage therapy based on the currently available antiretroviral drugs. These tests, however, remain probably useful to identify patients who could benefit from experimental treatments by new classes of compounds such as fusion inhibitors.

APPENDIX

Interpretation of HIV-1 reverse-transcriptase and protease mutations according to the algorithm established by the Resistance group of the ANRS (1999 report [7]).

Genotypic resistance to nucleoside analogue reverse-transcriptase inhibitors

Drugs	Mutations conferring	
	Genotypic resistance	Possible genotypic resistance
ZDV	T215Y/F Q151M ± A62V ± V75I ± F77L ± F116Y 69 Insertion ± D67E/S	≥3 mutations among M41L, D67N, K70R, L210W, K219Q/E
ddI	L74V ± K65R ± M184V/I ± V75T Q151M ± A62V ± V75I ± F77L ± F116Y 69 Insertion ± D67E/S	K65R ± V75T ± M184V/I
ddC	T69D Q151M ± A62V ± V75I ± F77L ± F116Y 69 Insertion ± D67E/S	K65R ± L74V ±V75T ± M184V/I
3TC	M184V/I 69 Insertion ± D67E/S	Q151M ± A62V ± V75I ± F77L ± F116Y
d4T	V75M/S/A/T M41L + D67N + L210W + 215Y/F + T69D/N Q151M ± A62V ± V75I ± F77L ± F116Y 69 Insertion ± D67E/S	≥3 mutations ZDV including T215Y/F
ABC	T215Y/F + M41L + M184IV Q151M ± A62V ± V75I ± F77L ± F116Y 69 Insertion ± D67E/S	≥3 mutations ZDV including T215Y/F M184V/I + L74V M184V/I + K65R Isolated mutations: K65R, L74V, Y115F, M184V/I

Genotypic resistance to nonnucleoside analogue reverse-transcriptase inhibitors

Mutations	Genotypic resistance
L100I	NVP, DLV, EFV
K101E	NVP, DLV, EFV
K103N	NVP, DLV, EFV
V106A	NVP, DLV
Y181C	NVP, DLV
Y188C	NVP
Y188L	NVP, DLV, EFV
G190A/S	NVP, EFV
P225H	EFV
P236L	DLV

Genotypic resistance to protease inhibitors

Drugs	Mutations conferring	
	Genotypic resistance	Possible genotypic resistance
SQV	G48V I84V L90M	V82/A/F/S/T + ≥2 minor mutations other than L10I and L63P
IDV	M46I/L V82/A/F/S/T I84V L90M + ≥2 minor mutations other than L10I and L63P	L90M
RTV	V82/A/F/S/T I84V	L90M + I54V/L or A71T or M46I/L or G48V
NFV	D30N I84V N88S/D L90M	V82/A/F/S/T + ≥2 mutations among M36I, M46I/L, A71V/T, V77I
APV	I50V I84V	M46I + I47V I54V/L
ABT-378	I84V	

ZDV, zidovudine; ddI, didanosine; ddC, zalcitabine; 3TC, lamivudine; d4T, stavudine; ABC, abacavir; NVP, nevirapine; DLV, delavirdine; EFV, efavirenz; SQV, saquinavir; IDV, indinavir; RTV, ritonavir; NFV, nelfinavir; APV, amprenavir.

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