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Short communication

Genotypic resistance profile in treatment-experienced HIV-infected individuals after abacavir and efavirenz salvage regimen

Alejandro Vallejo*, Mercedes Olivera, Amalia Rubio, Armando Sánchez-Quijano, Eduardo Lissen, Manuel Leal

Grupo de Estudio Hepatitis Viricas y SIDA, Edificio de Laboratorios, 6 Planta, Hospital Universitario Virgen del Rocio, Manuel Siurot s/n, 41013 Seville, Spain

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Abstract

Once highly active antiretroviral therapy (HAART) fails to suppress HIV replication and resistant viruses emerge, it is difficult to find a salvage regimen since cross-resistance is high among the available classes of antiretroviral drugs. In this retrospective analysis, genotypic resistance profiles were analysed in 24 patients who switched treatment to abacavir (ABV), efavirenz (EFV), and either a NRTI or a PI at baseline and after 24 weeks of treatment. At baseline, 71% of patients harboured at least one resistance mutation in the protease gene. In the RT gene, 87.5% of the patients showed nucleoside analogue resistance mutations, and an equal 87.5% showed resistance mutations to non-nucleoside analogues. After 24 weeks of treatment, only mutations to nucleoside analogues raised in 95.8% of the patients, while resistance mutations to the other drug classes remained constant. Substitutions conferring cross-resistance within each drug family were very common among this treatment-experienced population. These data also indicate that salvage therapy is likely to remain one of the most important issues in the treatment of HIV infections.

Keywords: HIV; HAART; Resistance; Virological failure

The risk of failure of highly active antiretroviral therapy (HAART) is particularly high in patients who have been pre-treated with nucleoside reverse transcriptase inhibitors (NRTIs) as mono-therapy or as part of a bi-therapy in the early era of antiretroviral therapy. Therapy failure may be defined as an insufficient decrease, increase or rebound of viral load and/or a decrease of CD4 cell counts and/or an HIV-associated disease occurring during HAART (Ledergerber et al., 1999). A probability of up to 40% has been reported for viral rebound in the first 2 years in pre-treated patients. Specific risk factors such as a low CD4 cell counts and high viral loads could also contribute to virological failure (Van Vaerenbergh et al., 2002). Unfortunately, the potential for cross-resistance to other protease inhibitors (PIs) and NRTIs is high in these patients, which makes it difficult to find an effective alternative. Although the use of resistance testing is recommended in the setting of antiretroviral failure, it is not clear how to use genotype or phenotype to select a regimen for treatment-experienced patients (Falloon et al., 2002).

We identified 24 heavily pre-treated patients with HAART failure who switched to a combination of abacavir (ABV), efavirenz (EFV), and either a NRTI (stavudine (d4T), lamivudine (3TC) or zidovudine (ZDV)) or a PI (nelfinavir (NFV), ritonavir (RTV) or indinavir (IDV)) in a retrospective analysis. All patients were naïve to abacavir and efavirenz and had been heavily pre-treated with a variety of antiretroviral drugs, including PIs, NRTIs and NNRTIs (Table 1). It is not clear whether abacavir, a NRTI, maintains its activity against HIV strains resistant to AZT, 3TC, ddI, d4T and a number of NNRTIs (Rakik et al., 1999; McMahon et al., 2001; Wasmuth et al., 2002; Khanna et al., 2000). There are two important multinucleoside resistance patterns: one is associated with the mutation Q151M, and the other is associated with multiple amino acid insertions at position 68 or 69 in the RT gene of HIV-1 (Miller et al., 2000). Furthermore, a combination of the mutations K65R, L74V, Y115F and M184V is necessary to confer significant resistance to abacavir in vitro (Van Laethem et al., 2000; Tenorio et al., 2000). In the context of efavirenz, mutations

* Corresponding author. Tel.: +34-955-013-294; fax: +34-955-013-292.
E-mail address: avallejo@cica.es (A. Vallejo).

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Table 1
Patient characteristics at baseline and after 24 weeks of salvage regimen

Patient	Therapy history	Salvage regimen (ABV, EFV plus)	Baseline		Week 24	
			Viral load RNA copies/ml	CD4 cell/μl	Viral load RNA copies/ml (log increment)	CD4 cell/μl (increment)
46	ZDV, ddI, d4T, 3TC, SQV, NVP, NFV	NFV	4.560	236	3.220 (−0.15)	253 (stable)
68	ZDV, ddI, d4T, 3TC, SQV, NVP, RTV	RTV	1.520	300	2.030 (0.12)	553 (>30%)
250	d4T, 3TC, SQV, NVP, NFV	NFV	2.050	241	2.660 (0.11)	295 (stable)
72	ZDV, ddI, d4T, 3TC, SQV, NVP, NFV	NFV	11.000	261	892 (−1.09)	439 (>30%)
23	ZDV, ddI, ddC, SQV, d4T, IDV, 3TC, NVP, NFV	NFV	12.100	72	71.300 (0.77)	121 (>30%)
163	ZDV, ddI, d4T, 3TC, SQV, NVP, NFV	NFV	53.300	598	65.000 (0.09)	613 (stable)
323	ZDV, d4T, 3TC, SQV, NVP, NFV, IDV	NFV	90.800	410	210.000 (0.37)	261 (<30%)
70	ZDV, ddI, d4T, 3TC, SQV, NVP, NFV, IDV	NFV	138.000	395	2.740 (−1.70)	260 (<30%)
164	ZDV, ddI, d4T, 3TC, SQV, NVP, NFV	NFV	124.000	76	80.400 (−0.19)	32 (<30%)
147	ZDV, ddI, d4T, 3TC, SQV, NVP, NFV, IDV	IDV	126.000	169	188.000 (0.17)	123 (stable)
44	ZDV, ddI, ddC, d4T, SQV, 3TC, IDV, NVP, NFV	d4T	6.660	44	559 (−1.08)	92 (>30%)
83	ZDV, ddI, d4T, ddC, 3TC, SQV, NVP, NFV, IDV	3TC	5.850	223	15.500 (0.43)	144 (<30%)
241	ZDV, ddI, d4T, 3TC, SQV, NVP, NFV	d4T	21.500	438	644 (−1.53)	634 (>30%)
71	ZDV, ddI, d4T, 3TC, SQV, NVP, NFV, IDV	d4T	19.500	239	526 (−1.57)	368 (>30%)
9	ZDV, ddI, 3TC, SQV, NVP, NFV, RTV, IDV	d4T	16.300	489	9.750 (−0.23)	428 (stable)
279	d4T, 3TC, SQV, RTV, NVP, NFV	d4T	14.300	179	411.000 (1.46)	46 (<30%)
109	ZDV, ddI, d4T, 3TC, SQV, NVP, NFV	d4T	20.600	34	13.000 (−0.23)	25 (stable)
95	ZDV, ddI, d4T, 3TC, SQV, NVP, NFV, RTV, IDV	d4T	40.100	33	425.000 (1.02)	34 (stable)
199	ZDV, ddC, d4T, 3TC, IDV, NVP, NFV	d4T	43.900	157	297 (−217)	220 (>30%)
187	ZDV, ddI, d4T, 3TC, SQV, NVP, NFV	d4T	73.200	248	38.000 (−0.29)	322 (stable)
223	ZDV, ddI, d4T, 3TC, SQV, NVP, NFV	d4T	59.700	76	25.900 (−0.36)	154 (>30%)
169	ZDV, ddI, d4T, 3TC, NVP, NFV, IDV	d4T	41.600	276	906.000 (1.34)	343 (stable)
194	ZDV, ddC, d4T, 3TC, SQV, NVP, NFV, IDV	ZDV	75.000	14	137.000 (0.26)	9 (<30%)
69	ZDV, ddI, d4T, 3TC, SQV, NVP, IDV	d4T	453.000	54	436.000 (−0.02)	46 (stable)

L100I, K103N, Y188L and G190S confer high-level resistance, while mutations V106A, G190A/E, and M230L afford only intermediate resistance when present as a single mutation.

All patients were monitored for 24 weeks and analysed for CD4 cell counts, HIV RNA levels, and RT and protease resistance profiles. Most of them (83.3%) had been pre-treated with a variety of antiretroviral drugs for more than 8 years with a median number of therapy changes of 4 (range: 3–7).

At baseline the mean viral load was 4.78 ± 3.97 (mean ± S.D.) $\log_{10}$ HIV RNA copies/ml, and a mean of 5.05 ± 5.32 $\log_{10}$ HIV RNA was observed after 24 weeks of treatment (increment with no statistical significance). Of 13 patients with a decrease in viral load, 6 (25%) decreased by more than 0.5 $\log_{10}$, and 5 (20.8%) reached a viral load of <1000 RNA copies/ml (Table 1), while 11 patients had an increased viral load (4 with an increase of >0.5 $\log_{10}$).

The mean CD4 cell count at baseline was 219 ± 159 (mean ± S.D.) cells/μl, including eight patients with less than 100 CD4 cells/μl. The mean CD4 cell count raised to 242 ± 190 (mean ± S.D.) cells/μl after 24 weeks of salvage regime (increment with statistical significance, $P < 0.001$) in eight patients, including three patients with <100 cells/μl at baseline CD4 cell counts increased by more than 30%. Ten patients showed a stable CD4 cell count (−30% to +30%) while in six the CD4 cell count decreased by greater than 30% (two of them had <100 CD4 cells/μl at baseline) (Table 1). The increase in CD4 cell counts was also observed in some patients who showed no beneficial virological response, which agrees with several recent studies that indicate that virological and immunological responses do not always correlate. Overall, salvage therapy stabilised or enhanced the immunological response in 75% of the patients, and decreased viral load in 54% of patients after 24 weeks of treatment.

Since all patients were heavily pre-treated, it is not surprising that many resistance-associated mutations were found at baseline. High-level resistance mutations in the protease gene at positions 30, 82, 84, and 90 were found in 12, 20.8, 12, and 58% of the patients, respectively (Table 2). Thirteen patients (54%) demonstrated at least two mutations at positions 30, 46, 48, 82, 84, 88, and 90 in the protease gene. A similar proportion of resistance mutations were found after 24 weeks. Seven patients (29%) showed no mutations in the protease gene after the salvage regimen.

In the RT gene, the Q151M mutation, which confers high-level resistance to abacavir and other NRTIs, was found in two patients (8.3%; patients 72 and 71), and the T69SSG insertion was found in one patient (4.16%) at baseline. Other resistance-associated mutations were also found at positions 65, 74, 184, and 215. The most common high-level resistance mutations for NNRTIs were K103N, found in 9 patients (37.5%) at baseline and in 11 patients after 24 weeks (45.8%), and Y181C, found in 18 patients (75%) at baseline and in 15 patients (62.5%) after 24 weeks. The G190A/S mutation, which confers high-level resistance to efavirenz

Table 2
Resistance-associated mutations in RT and protease genes

Patient	Protease	Reverse transcriptase NRTI	Reverse transcriptase NNRTI
Baseline			
46	G73S, L90M	M41L, E44D, D67N, L210W, T215Y	A98G, K101E, Y181C
68	G73S, I84V, L90M	M41L, E44D, D67N, M184V, L210W, T215Y	K103N, V179I, Y181C
250	M46I, G48V, I54T, V82A, L90M	T69SSG, T215S, K219N	K103N, Y181C
72	I84V, L90M	M41L, Q151M, T215Y	K103N, Y181C
23	G48V, I54A, V82A	T69N, L74I, M184V, T215F	K103N, Y181C
163	L90M	M41L, T215Y	Y181C
323	D30N, F53L, L90M	M41L, L74V	K101E, Y181C
70	I84V, L90M	D67N, K70R, K219Q	K103N, Y181C
164	None found	None found	None found
147	None found	L74V, K219E	Y181C
44	D30N, N88D, L90M	M41L, T215Y	L100M, K103N
83	None found	M184V	K103N
241	G73S, L90M	None found	None found
71	M46I, L90M	V75I, Q151M	V108I, Y181C, G190D
9	M46I, L90M	M41L, T215Y	None found
279	V32I, M46I, F53L, V82A	M184V	K103N, V179I
109	None found	None found	Y181C, G190A
95	None found	M41L, E44D, T69D, L210W, T215Y, K219E	A98G, V179I, Y181C, G190S
199	D30N, M46L, N88D	T215S	L100R, K103N, Y181C
187	I54L, G73S, L90M	D67N, M184I, L210W, T215C, K219E	Y181C, G190A
223	I54V, G73S, V82A, L90M	M41L, L210W, T215Y, K219E	Y181C
169	None found	D67N, K70R, M184I, L210W, T215Y, K219E	K101H, V106A, Y181C, G190A
194	M46l, I54V, G73S, V82A, L90M	M41L, E44D, L210W, T215C, K219N	K101E, Y181C, G190A
69	None found	M41L, E44A, T69D, V75M, M184V, L210W, T215Y	A98G, K101E, Y181C, G190A
After 24 weeks			
46	G73S, L90M	M41L, E44D, L210W, T215Y	A98G, K101E, Y181C, G190A
68	I54V, I84V, L90M	M41L, E44D, L210W, T215Y	K103N, V179I, G190A
250	M46I, G48V, I54T, V82A	M41L, D67G, T69SSG	L74I, K101R, K103N, Y181C
72	D30N, L90M	M41L, L210W, T215Y	K101E, Y181C, G190A
23	G48V, I54V, V82A	D67N, T69N, L74I, M184V, T215F, K219E	K103N, Y181C, G190A
163	M46I, L90M	M41L, T215Y	K103N, Y181C, G190A
323	D30N, N88D, L90M	M41L, D67N, L74V, L210W, T215Y, K219N	K101E, K103N, Y181C, G190A
70	I84V,	D67N, K70R, M184I, K219Q	K103N, Y181C
164	None found	None found	None found
147	G73S, L90M	K70R, L74V, M184V, K219E	V108I, Y181C, G190A
44	D30N, N88D, L90M	M41L, D67N, L210W, T215Y	G190A
83	None found	M184V	K103N, P225H
241	None found	K70R	K101Q, V108V, Y181C, G190S
71	L90M	V75I, Q151M	None found
9	M46I, L90M	M41L, T215Y	None found
279	None found	D67G	K103N, V108I, V179I
109	L90M	L74V	K103N, Y181C, G190A
95	None found	M41L, E44D, T69D, M184I, L210W	A98G, Y181C, G190A
199	None found	L210W, T215S	None found
187	I54L, G73S, L90M	M41L, D67N, M184V, L210W, T215C/S, K219E	V108I, Y181C
223	I54V, G73S, V82A, L90M	M41L, K70R, T215Y	K103N, Y181C, G190A
169	None found	None found	K103N
194	M46l, I54V, G73S, V82A, L90M	M41L, E44A, L210W, T215Y, K219N	K101E, Y181C, G190S
69	G73S	M41L, E44A, D67N, T69D, V75M, L210W, T215Y	A98G, K101E, Y181C, G190S

and nevirapine, was found in 6 patients (25%) at baseline and in 14 patients (58.3%) after treatment, which suggests that this mutation was generated during the treatment period. After treatment, no NRTI-associated mutations were found in two patients, no NNRTI-associated mutations were found in four patients, and no PI mutations were found in seven patients. It is noteworthy that one patient (no. 164) had no mutations in any of the known resistance positions at baseline and after 24 weeks of salvage therapy in spite of, or owing to, strict adherence to the treatment regimen.

Since all patients were naïve to abacavir and efavirenz, we assumed that this therapy might have been more successful, especially as abacavir-resistant virus has been reported to be only partially cross-resistant to other NRTIs. Our results suggest limited antiviral activity of abacavir and efavirenz

in pre-treated patients. However, this treatment regimen succeeded in immunologically stabilising more than 70% of the patients, which is of importance when newer compounds are not yet available or when other regimens are not applicable because of poor adherence or serious side effects. These results also show that drug resistance mutations are very prevalent among heavily pre-treated patients who have failed several treatment regimens. Lower levels of plasma HIV RNA seems to have had no effect on the evolution of mutations in these patients. Salvage therapy continues to be one of the most important issues in the treatment of HIV infection.

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