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Comparison of the Dynamics of Resistance-Associated Mutations to Nucleoside Reverse Transcriptase Inhibitors, Nonnucleoside Reverse Transcriptase Inhibitors, and Protease Inhibitors after Cessation of Antiretroviral Combination Therapy

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The dynamics of mutations associated with resistance to antiretroviral drugs were analyzed after cessation of therapy. The results showed that the kinetics of the shift to wild-type amino acid residues were significantly faster for protease inhibitors, intermediate for nonnucleoside reverse transcriptase inhibitors, and slower for nucleoside reverse transcriptase inhibitors.

Structured treatment interruptions (STIs) have been proposed as a strategy for minimizing the cost and toxicity of long-term highly active antiretroviral therapy while also providing a mechanism for enhancing human immunodeficiency virus type 1 (HIV-1)-specific immunity (1, 9, 11, 12). This strategic treatment interruption is also proposed for individuals whose virus has become resistant to treatment, to induce reversion of resistance to the wild type and therefore to improve the success of subsequent salvage (6–8, 10). However, prospective randomized studies have reported conflicting results regarding the latter strategy. The CPCRA 064 study (J. Lawrence, D. Mayers, K. Huppler Hullsiek, G. Collins, D. Abrams, R. Reisler, L. Crane, B. Schmetter, T. Dionne, J. Saldanha, M. Jones, and J. Baxter, Abstr. 10th Conf. Retrovir. Opportunistic Infect., abstr. 67, 2003) showed no benefits of STI before changing therapy in patients with a multidrug-resistant HIV infection, while the ANRS GIGHAART study (C. Katlama, S. Dominguez, C. Duvivier, C. Delaugerre, G. Peytavin, M. Legrand, V. Calvez, K. Gourlain, and D. Costagliola, Abstr. 10th Conf. Retrovir. Opportunistic Infect., abstr. 68, 2003) described virologic and immunologic benefits after 8 weeks of treatment interruption and a subsequent salvage regimen including more than six drugs. Deeks et al. (4) also described a durable viral suppression with a subsequent therapy containing only one fully active agent. Therefore, further studies are required to specify the optimal conditions for conducting an STI strategy with success, especially as concerns the baseline level of resistance, the duration of the interruption, and the drugs included in the salvage therapy. Furthermore, the expected shift to wild-type amino acid residues which is associated with a virologic response is not systematically observed during the time of treatment discontinuation. Theoretically, the longer the duration of the interruption, the more reversion is observed (6, 7), but the dynamics of reversion seem to differ among the patients and among the resistance-associated mutations.

Devereux et al. (7) compared the proportions of primary and secondary mutations detectable on and off therapy. They reported a significant decline in primary and secondary mutations whether samples were tested a median of 6.4 and 12.9 weeks after therapy discontinuation, respectively. Birk et al. (2) described a significant difference between the outcome of primary and secondary protease inhibitor (PI) mutations and between primary PI versus primary reverse transcriptase mutations. Moreover, Deeks et al. (5) observed that phenotypic resistance seemed to wane simultaneously for nonnucleoside reverse transcriptase inhibitors (NNRTIs) and PIs but could be delayed for nucleoside reverse transcriptase inhibitors (NRTIs). So far, no study has clearly compared the dynamics of disappearance among the following three mutation groups: NRTIs, NNRTIs, and PIs. To know whether, during an STI, an antiretroviral class could recover a favorable genetic background faster than another, we compared these dynamics in patients harboring multiresistant viruses and in treatment interruption.

This study was conducted in 19 HIV-1-infected patients who were enrolled in a prospective STI study to obtain a reversion of resistance in plasma. Salvage therapy was resumed depending on clinical events, the patient's wish, or predetermined criteria of reversion to recover plasmatic viruses with no mutations for at least two classes of antiretrovirals. The patients were monitored at day 0 and weeks 2, 4, 8, 14, 20, and 26 with a clinical examination, biochemical tests, measurement of the HIV-1 RNA load in plasma (Roche Amplicor HIV-1 Monitor assay 1.5) and the CD4 cell count (flow cytometric analysis [Coulter]), and (except at weeks 2 and 4) genotypic HIV-1 drug resistance testing (automated, population-based full-sequence analysis [ABI system]).

In our study, the genotypes allowed a comparison of the

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TABLE 1. Resistance-associated mutations at baseline and at the end of the STI period

Patient and time point	Mutation(s) associated with resistance to:		
	NRTIs	NNRTIs	PIs
1			
Baseline	M41L, D67N, T69D, MI84V, L210W, T215Y, K219N	K101E, Y181C, G190A	M46L, I54V, V82A
Wk 8	M41L/M, D67N/D, L210W/L, T215Y/S	K101R	None
2			
Baseline	M41L, D67N, V75S, MI84V, L210W, T215Y, K219N	V108I, Y181C, G190A	M46I, I54V, I84V, L90M
Wk 26	M41L/M	None	None
3			
Baseline	M41L, D67N, V75S, MI84V, T215F, K219W	K101E, V108I, Y181C, G190A	M46I, I54V, I84V, L90M
Wk 26	T215F/S, K219Q	None	I84V/I, L90M/L
4			
Baseline	M41L, D67N, L74V, MI84V, L210W, T215Y, K219N	K101E, V108I, Y181C, G190A	M46I, I54M, I84V, L90M
Wk 26	M41L, D67N, L74V, MI84V, L210W, T215Y, K219N	K101E/K, V108I/V, Y181C, G190A	I84V, L90M
5			
Baseline	D67N, K70R, MI84V, T215F, K219E	K101E, V108I, Y181C, G190A	G48M, I54M, V82A, L90M
Wk 8	None	None	None
6			
Baseline	M41L, D67N, T69N, K70R, T215F, K219Q	None	V82A, I84V, L90M
Wk 14	M41L, T215F	None	None
7			
Baseline	M41L, D67N, T69D, K70R, MI84V, T215F, K219Q	K103N, V108I, Y181C, G190A	M46I, I54S, I84V, L90M
Wk 26	D67N, T69D, K70R, T215F, K219Q	None	None
8			
Baseline	M41L, D67N, K70R, MI84V, T215Y, K219Q	None	M46I, I54V, V82A, L90M
Wk 26	D67N/D, K70R/G, T215F/S, K219Q/K	None	None
9			
Baseline	D67N, T69N, K70R, T215F, K219Q	K101E, K103R, G190A	I54M, I84V, L90M
Wk 8	None	None	None
10			
Baseline	M41L, L74V, Y115F, MI84V, L210W, T215Y, K219Q	Y188L	I50V, I54V, V82A, L90M
Wk 26	M41L, L74V, MI84V, L210W/L, T215Y	Y188L	I50V, I54V, V82A, L90M
11			
Baseline	M41L, D67N, L74V, V75T, MI84V, L210W, T215Y, K219N	Y181C, G190S	G48V, I54M, L90M
Wk 14	M41L/M, T215Y/S	None	None
12			
Baseline	M41L, D67N, MI84V, L210W, T215Y	Y188L	M46L, G48V, I50V, I54V, V82A
Wk 26	None	None	None
13			
Baseline	M41L, D67N, T69D, V75M, M184I, L210W, T215Y, K219R	K101E, Y188L	M46L, I54V, I84V, L90M
Wk 20	M41L, D67N, T69D, V75M, M184I, L210W, T215Y, K219R	K101E, Y188L	M46L, I54V, I84V, L90M
14			
Baseline	M41L, D67N, MI84V, L210W, T215Y	Y188L	M46I, I54M, V82A, I84V, L90M
Wk 8	M41L, L210W, T215S	None	None
15			
Baseline	M41L, D67N, T69D, Q151M, L210W, T215Y	Y181C, Y188L, G190A	M46I, I54V, V82A, L90M
Wk 26	M41L/M, D67N/D, T69D, Q151M/L	None	I54M, V82A/V, L90M/L
16			
Baseline	M41L, D67N, L74V, V75M, MI84V, L210W, T215Y, K219N	Y181C, G190C	I50V, I54V, V82A, L90M
Wk 20	None	None	None
17			
Baseline	M41L, D67N, T69N, K70R, V75T, MI84V, T215Y, K219Q	G190A	M46I, I54V, I84V, L90M
Wk 26	None	G190A/G	None
18			
Baseline	M41L, D67N, T69N, K70R, L74V, L210W, T215Y, K219Q	G190A	M46I, I54V, I84V, L90M
Wk 14	D67N/D, K70R/K, L210F, K219Q/K	None	None
19			
Baseline	M41L, D67N, V75M, MI84V, L210W, T215F, K219Q	K103N	M46I, I84V, L90M
Wk 20	None	None	None

TABLE 2. Prior antiretroviral treatment of the patients in this study

Patient	No. of prior antiretroviral regimens	No. of years on antiretroviral regimens	Antiretroviral compound(s) not received in prior regimens		
			NRTIs[a]	NNRTIs[b]	PIs[c]
1	8	10	None	Efavirenz	Amprenavir
2	17	6	Zalcitadine	Nevirapine	None
3	21	10	None	None	None
4	14	8	None	None	Indinavir
5	11	7	None	None	Lopinavir
6	13	7	Zalcitadine	Nevirapine, efavirenz	Lopinavir
7	14	11	Abacavir	Efavirenz	Amprenavir
8	9	7	None	Nevirapine, efavirenz	Nelfinavir
9	11	7	Zalcitadine	None	Indinavir
10	23	6	None	None	None
11	12	12	None	None	Nelfinavir
12	16	8	Zalcitadine	None	None
13	36	9	None	None	None
14	8	4	Zalcitadine	Nevirapine	Saquinavir, nelfinavir
15	10	8	None	Efavirenz	Saquinavir, lopinavir
16	4	7	None	None	Indinavir, nelfinavir
17	17	6	Zalcitadine	None	None
18	13	6	Abacavir	None	Amprenavir
19	19	8	None	Nevirapine	None

[a] Zidovudine, didanosine, zalcitadine, lamivudine, stavudine, and abacavir.
[b] Nevirapine and efavirenz.
[c] Saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, and lopinavir/ritonavir.

dynamics of disappearance for the three mutation groups. For each antiretroviral class, we took into account the time point at which the genotype harbored a shift of all of the baseline resistance mutations reported in Table 1 to wild-type amino acid residues. These dynamics were compared by using the nonparametric log rank (Mantel-Cox) test.

The median baseline HIV-1 RNA level was 5.06 log copies/ml (range, 4.51 to 5.69 log copies/ml), and the median CD4 cell count was 61.5/mm^3 (range, 4 to 361/mm^3). Genotypic HIV-1 drug resistance testing revealed a median of seven (range, five to eight) mutations associated with resistance to NRTIs, two (range, zero to four) mutations associated with resistance to NNRTIs, and four (range, three to five) major mutations associated with resistance to PIs (Table 1). The prior antiretroviral therapies experienced by the patients are summarized in Table 2. As illustrated in Fig. 1 (actuarial cumulative hazard curves), after therapy withdrawal, the shift of PI and, to a lesser extent, NNRTI mutations was on the increase during the period of treatment interruption while NRTI mutations were more stable. Finally, the data showed that the dynamics of the three mutation groups differed significantly according to the nonparametric log rank (Mantel-Cox) test ($P < 0.05$) and that the kinetics of the shift to wild-type amino acid residues was faster for the PIs, intermediate for the NNRTIs, and slower for the NRTIs.

These results describe the kinetics of antiretroviral resistance mutations in patients with extensive treatment experience who harbored virologic failures and stopped their treatment. In our study, we observed that the mutations associated with resistance to PIs and NNRTIs disappeared faster than those selected by NRTIs. The actuarial curves presented in Fig. 1 show that if the goal is to obtain maximum disappearance of resistance-associated mutations, it could be interesting to increase the duration of treatment interruption for PIs and NNRTIs. However, for NRTIs, some mutations seem to be very stable (M41L, T215Y/F, K219Q/E/N) and increasing the duration of the treatment interruption does not rapidly enhance the probability of their disappearance. This is probably linked to the fact that these mutations do not impact the fitness of the viruses. Further studies can be useful to confirm whether these kinetic results could be observed with other resistance profiles selected by different therapeutic histories.

The higher number of NRTI mutations cannot explain these results. For example, the median number of NNRTI mutations was only two, but these mutations are not the first ones to disappear over the time. In all likelihood, as illustrated by four examples in Table 3, most of the resistance mutations are linked in different ways and do not disappear simply one after the other. The difference in kinetics observed between PI and NRTI resistance mutations could probably be explained by the

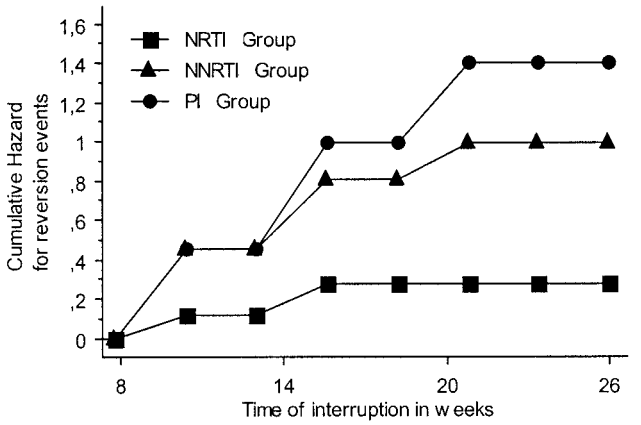

FIG. 1. Actuarial cumulative hazard curves of the three mutation groups for time spent in treatment interruption ($P < 0.05$).

TABLE 3. Examples of genotypic reversions during the STI

Patient and time point	Viral load (no. of copies/ml)	Mutation(s) associated with resistance to:		
		NRTIs	NNRTIs	PIs
2				
Baseline	111,000	M41L, D67N, V75S, MI84V, L210W, T215Y, K219N	V108I, Y181C, G190A	M46I, I54V, I84V, L90M
Wk 8	110,000	M41L, D67N, V75S, MI84V, L210W, T215Y, K219N	V108I, Y181C, G190A	M46I, I54V, I84V, L90M
Wk 14	128,000	M41L, D67N, V75S, MI84V, L210W, T215Y, K219N	V108I, Y181C, G190A	None
Wk 20	1,290,000	M41L, T215Y	None	None
Wk 26	722,000	M41L/M	None	None
3				
Baseline	32,100	M41L, D67N, V75S, MI84V, T215F, K219W	K101E, V108I, Y181C, G190A	M46I, I54V, I84V, L90M
Wk 8	171,000	T215F/S, K219Q	None	I84V/I, L90M
Wk 14	108,000	T215F/S, K219Q	None	I84V/I, L90M
Wk 20	303,000	T215F/S, K219Q	None	I84V/I, L90M
Wk 26	583,000	T215F/S, K219Q	None	I84V/I, L90M/L
5				
Baseline	143,000	D67N, K70R, MI84V, T215F, K219E	K101E, V108I, Y181C, G190A	G48M, I54M, V82A, L90M
Wk 8	836,000	None	None	None
11				
Baseline	144,000	M41L, D67N, L74V, V75T, MI84V, L210W, T215Y, K219N	Y181C, G190S	G48V, I54M, L90M
Wk 8	700,000	M41L, D67N/D, L74V, V75T, L210W, T215Y	None	None
Wk 14	1,700,000	M41L/M, T215Y/S	None	None

large impairment of fitness described as associated with PI mutations (3).

These data are of some importance for strategies based on resistance reversions, such as those observed during the ANRS GIGHAART trial, and it would be interesting to use multiple PIs in a salvage regimen if it is confirmed that the mutations associated with resistance to this antiretroviral class disappeared significantly faster than those associated with resistance to NRTIs. Other results are that it is difficult to achieve reversion of some NRTI resistance-associated mutations (M41L, T215Y/F, K219Q/E/N) and that the time needed to do so is probably longer. Thus, the benefit/risk ratio for this class has to be discussed carefully for trials based on resistance mutation reversion during treatment interruption.

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