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Role of Baseline Human Immunodeficiency Virus Genotype as a Predictor of Viral Response to Tenofovir in Heavily Pretreated Patients

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Human immunodeficiency virus (HIV)-infected patients (n = 153) failing antiretroviral therapy after exposure to compounds from all three drug families were monitored for 6 months after beginning a rescue intervention program including tenofovir (TDF). At 3 months, levels of HIV RNA in plasma dropped by a mean of 0.9 $\log_{10}$ and the mean CD4 count increased by 52 cells/μl. At 6 months, HIV RNA levels had dropped by a mean of 1.06 $\log_{10}$ and the mean CD4 count had increased by 49 cells/μl. Only five (3.7%) patients discontinued TDF use due to adverse events. In the multivariate analysis, the presence of M41L and/or L210W at baseline was the only viral determinant of a lower response to TDF.

Tenofovir disoproxil fumarate (TDF), the first approved nucleotide analog, has shown a potent antiviral effect (mean reduction in levels of human immunodeficiency virus [HIV] RNA in plasma of 0.6 $\log_{10}$) in clinical trials (4). Moreover, side effects seem to be uncommon (4). However, preliminary evidence from clinical trials suggests that its potency may be compromised when thymidine-associated mutations (TAMs) are present, particularly M41L and/or L210W (2; M. Miller, N. Margot, and B. Lu, Program Abstr. 9th Conf. Retrovir. Opportunistic Infect., abstr. 43, 2002).

In order to assess the efficacy and toxicity of TDF outside clinical trials, all patients included in the drug expanded-access program in eight hospitals in Spain who had completed the first 24 weeks of follow-up were examined. As this study was not a clinical trial, it reflects the TDF efficacy in routine clinical practice. To make the study population more uniform, only those patients with prior exposure to antiretroviral compounds from all three drug families who began a rescue intervention which included TDF at 300 mg once daily were selected.

Baseline genotype, degree of compliance with the drug regimen, and use of concomitant drugs were entered into the univariate and multivariate logistic regression analyses. These variables were examined as potential determinants of viral response to TDF. Only variables with a P value below 0.2 in the univariate analysis were considered in the multivariate analysis. As virtual phenotype was available only for a small subset of patients, it was considered only in the univariate analysis. All statistical analyses were performed by using the SPSS software version 9.0.

A total of 153 patients were included in the study. Their mean age was 41 years, and 77% were male. They had acquired HIV infection through intravenous drug use (48%), homosexual contacts (33%), or heterosexual relationships (17%). Their mean time on antiretroviral therapy before beginning TDF was 86 months. Up to 63% of patients added a new concomitant drug(s) to the salvage regimen with TDF (Kaletra in 71% of cases and amprenavir in 5%). At baseline, the mean level of HIV RNA in plasma was 4 $\log_{10}$ and the mean CD4 count was 308 cells/μl.

Twenty-five patients (19%) did not complete 6 months of therapy. In five cases (3.7%), TDF use was discontinued due to adverse effects potentially attributed to the drug (gastrointestinal symptoms in three patients, fever in one, and rash in another). During the study period, no patients developed kidney laboratory abnormalities potentially linked to TDF use (hypophosphoremia, proteinuria, and elevated creatinine levels, etc.), which represent the main concern of using adefovir, a related nucleotide analog (1).

At 3 months, the mean drop in levels of HIV RNA in plasma was 0.9 $\log_{10}$, and the mean increase in the CD4 count was 52 cells/μl. At 6 months, the mean drop in HIV RNA levels was 1.06 $\log_{10}$ and the mean increase in CD4 count was 49 cells/μl. A significant viral response to TDF-based rescue therapy, defined as a drop in the level of HIV RNA in plasma of greater than 1 $\log_{10}$ and/or a decrease to below 50 copies/ml, was recorded at month 6 in 47.4% of patients in the "intent-to-treat" analysis (which assessed viral response in the whole study population that began treatment). This figure was 50.3% in the "on-treatment" analysis (which considered viral responses only in those who tolerated the drug and attended all visits). The increase in the CD4 count was significantly higher among viral responders (mean increase of 99 cells/μl).

The genotypic determinants of viral response to TDF could be assessed in 111 individuals from whom baseline HIV re-

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TABLE 1. Viral response to tenofovir at 6 months as a function of different variables (univariate analysis)

Variable	Variable setting	No. of patients with viral response/ total no. of patients	% with viral response[a] (HIV RNA drop of ≥1 $\log_{10}$ or decrease to <50 copies/ml)	*P*
No. of TAMs	≥3	21/50	42	0.21
	<3	33/61	54	
M41L	Present	20/53	38	0.03
	Absent	34/58	59	
L210W	Present	11/34	32	0.02
	Absent	43/77	56	
T215Y/F	Present	27/59	46	0.5
	Absent	27/52	52	
M184V	Present	23/50	46	0.7
	Absent	31/62	50	
TAM cluster				
41 ± 210	Present	22/56	39	
	Absent	32/55	58	
67 ± 70 ± 219	Present	22/53	42	0.15
	Absent	32/58	55	0.15
Loss of susceptibility to TDF	>2-fold	1/8	13	0.04
	≤2-fold	16/27	59	
Compliance with drug regimen	>95%	62/113	55	0.005
	≤95%	7/28	25	
Concomitant use of boosted PI	Yes	49/79	62	0.002
	No	23/64	36	0.002

[a] On-treatment analysis.

verse transcriptase sequences could be obtained. Table 1 records the viral response as a function of distinct HIV genotypes in the univariate analysis. Only changes previously reported to influence the response to TDF were considered in this analysis (2, 3; Miller et al., Program Abstr. 9th Conf. Retrovir. Opportunistic Infect.). In contrast with the findings of previous reports, the presence of three or more TAMs or the presence of the T215Y/F mutation did not significantly compromise the viral response to TDF in our study. Similarly, subjects having viruses with M184V did not have a better viral response to TDF, despite this mutation's having been shown to confer increased susceptibility to adefovir and TDF in vitro (3). Nevertheless, our results are in agreement with those of a recent report which highlighted the role of M41L and/or L210W in the loss of antiviral activity of TDF (Miller et al., Program Abstr. 9th Conf. Retrovir. Opportunistic Infect.). Results of virtual phenotype were available for only 35 patients; a significantly lower viral response was noted for individuals carrying viruses with a loss of susceptibility to TDF above twofold.

Table 2 records the significance of those variables associated with viral response to TDF at 6 months in both univariate and multivariate analyses. Only the lack of the M41L and/or L210W mutations, a compliance with the medication regimen above 95%, and the concomitant use of ritonavir-boosted protease inhibitors (PI) were significantly associated with the attainment of viral response to TDF in the multivariate analysis. The concomitant use of a new ritonavir-boosted PI by a large number of our patients most likely explains the mean HIV RNA drop of 1.06 $\log_{10}$ seen in this study, slightly greater than that reported in an older intensification trial with TDF (4). In fact, levels of HIV RNA in plasma fell by 1.45 $\log_{10}$ in our trial when a new ritonavir-boosted PI was added to TDF and by only 0.61 $\log_{10}$ otherwise ($P = 0.0001$). Overall, the proportion of subjects with viral response in the presence of M41L and/or L210W was 39% but increased to 58% when these mutations were absent.

In order to examine in more detail the independent contribution of the number of TAMs and of specific TAMs to the viral response to TDF, we analyzed in conjunction the impacts of these two determinant viral variables. In patients with viruses harboring fewer than three TAMs, the viral response occurred in 50 to 56% of cases and was not significantly influenced by M41L or L210W mutations. Conversely, in subjects with viruses having three or more TAMs, the viral response was seen in 67% (8 of 12) in the absence of M41L and/or L210W but in only 34% (13 of 38) in the presence of these mutations ($P = 0.047$). Thus, these results confirm the greater impact of M41L and/or L210W over that of the simple TAM score number on compromising TDF activity. However, the negative influence of these mutations seems to be exerted particularly in viruses with additional TAMs.

In summary, TDF-based salvage regimens may provide a significant viral response in heavily pretreated HIV-infected patients, particularly in subjects lacking the M41L and/or L210W mutations. This finding may have implications for drug

TABLE 2. Determinants of viral response to tenofovir at 6 months (logistic regression)

Variable	Univariate analysis		Multivariate analysis	
	OR (95% CI)[a]	*P*	OR (95% CI)	*P*
<3 TAMs	1.6 (0.8 to 3.5)	0.2		
Absence of M41L	2.3 (1.1 to 5)	0.03	2.6 (1.1 to 6.3)	0.03
Absence of L210W	2.6 (1.1 to 6.3)	0.02	3 (1.2 to 7.7)	0.02
Absence of T215Y/F	1.3 (0.6 to 2.7)	0.5		
Presence of M184V	0.9 (0.4 to 1.8)	0.7		
Absence of TAM clusters				
41 ± 210	2.1 (1 to 4.6)	0.05	2.3 (1 to 5.3)	0.06
67 ± 70 ± 219	1.7 (0.8 to 3.7)	0.15		
Loss of susceptibility to TDF of ≤2-fold	10 (1.1 to 100)	0.04		
Compliance with drug regimen of ≥95%	3.7 (1.4 to 9.3)	0.005	4.8 (1.5 to 15.3)	0.007
Concomitant use of ritonavir-boosted PI	2.9 (1.5 to 5.8)	0.002	4.2 (1.7 to 9.9)	0.001

[a] OR, odds ratio; CI, confidence interval.

sequencing, given that zidovudine seems to select for M41L more frequently than other nucleoside analogs (D. Kuritzkes, R. Bassett, and R. Young, abstract from the XI International HIV Drug Resistance Workshop, Antivir. Ther. **7**[Suppl.]**:**S41, 2002).

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