MAJOR ARTICLE

Genotypic and Phenotypic Predictors of the Magnitude of Response to Tenofovir Disoproxil Fumarate Treatment in Antiretroviral-Experienced Patients

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Results from 2 placebo-controlled intensification trials of tenofovir disoproxil fumarate (DF) in treatment-experienced human immunodeficiency type 1 (HIV-1)–infected patients ($n = 332$) were integrated to determine the effects of resistance at baseline on HIV-1 RNA response. In these trials, there was a high prevalence of HIV-1 resistance mutations, with 94% of patients having nucleoside-associated mutations and 71% having thymidine analogue–associated mutations (TAMs). Statistically significant HIV-1 RNA reductions associated with tenofovir DF treatment, relative to placebo ($P < .001$), were observed for patients without TAMs ($n = 97$) or for patients with 1–2 ($n = 88$) or $\geqslant 3$ TAMs ($n = 147$). Response to tenofovir DF was reduced among patients with HIV-1 with $\geqslant 3$ TAMs inclusive of either the M41L or L210W mutation ($n = 86$) or patients who had a preexisting K65R mutation ($n = 6$). Slightly increased treatment responses were observed when the M184V mutation was present. Phenotypic cutoffs were established at 1.4-fold and 4-fold, respectively, for the beginning of reduced response to tenofovir DF and for a strongly reduced response. The results from these controlled clinical trials provide guidance for the use of tenofovir DF for treatment-experienced patients.

Two mechanisms of resistance to nucleoside reverse-transcriptase inhibitors (NRTIs) have been defined. The first mechanism involves a mutation directly interfering with the binding or incorporation of the NRTI, as has been observed for lamivudine (3TC) and its signature reverse-transcriptase (RT) mutation M184V [1]. The second mechanism involves enhanced excision of the newly incorporated NRTI in a reaction that is the reverse of the incorporation reaction [2]. The resistance mutations that are known as "thymidine analogue mutations" (TAMs; i.e., M41L, D67N, K70R, L210W, T215Y/F, and K219Q/E/N/R) and can result from exposure to zidovudine (AZT) or stavudine (d4T) mediate resistance via this second mechanism [3]. The incidence of TAMs among treatment-experienced patients currently ranges from 30% to 40% [4, 5]. In addition to their effects on susceptibility to AZT and d4T, TAMs can mediate cross-resistance to all other NRTIs, including 3TC [6–11].

Tenofovir is a nucleotide analogue that is unique among the NRTIs in that it is an acyclic nucleoside phosphonate, analogous to the monophosphate form of the other NRTIs [12]. Tenofovir disoproxil fumarate (tenofovir DF) is an oral prodrug of tenofovir that is rapidly converted to tenofovir on absorption [13, 14]. On the basis of results of in vitro analyses, tenofovir appears to be active against a wide variety of NRTI-resistant strains, including viruses with TAMs, L74V/I or T69D [15, 16]. Susceptibility to tenofovir is enhanced with the presence of the M184V mutation [15, 17]. Tenofovir retains activity against the Q151M complex of mutations, whereas T69SS insertion mutations show high-level resistance to tenofovir and all other NRTIs [18]. Tenofovir can select for the K65R mutation in vitro and in vivo, as can zalcitabine, didanosine, d4T, and abacavir, and the K65R mutation results in a 3–4-fold decreased susceptibility to tenofovir in laboratory

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strains [9, 19–21]. However, the prevalence of K65R is low (2%–4%) among antiretroviral-experienced patients [4, 5].

Among treatment-experienced patients, treatment with tenofovir DF resulted in a decrease of ~0.6 $\log_{10}$ HIV-1 RNA copies/mL by week 24; this decrease was sustained through week 48 [22]. However, in short-term monotherapy studies, reductions of 1.5–1.6 $\log_{10}$ HIV-1 RNA copies/mL were observed [23, 24]. The reduced response among treatment-experienced patients suggested some level of cross-resistance. Analyses of a phase 2 study of tenofovir DF demonstrated statistically significant treatment responses among patients with TAMs [25]. The present study integrates the results from the phase 2 study with those from a larger phase 3 study of similar design. Both genotypic and phenotypic predictors of treatment response are analyzed.

SUBJECTS AND METHODS

Study population and design. GS-99-907 (study 907) was a randomized, double-blind, 48-week, placebo-controlled, multicenter clinical trial of tenofovir DF in HIV-1–infected patients with plasma HIV-1 RNA levels ≥400 copies/mL and ≤10,000 copies/mL. Patients receiving failing antiretroviral therapy for ≥8 weeks were assigned 2:1 to add either 300 mg of tenofovir DF or placebo to their existing regimens [26]. GS-98-902 (study 902) was a phase 2 dose-ranging study of similar design. However, in study 902, the upper limit of HIV-1 RNA was 100,000 copies/mL. Patients were randomly assigned 2:2:2:1 to receive either tenofovir DF in 1 of 3 doses (75 mg, 150 mg, or 300 mg) or placebo [22]. In both studies, at 24 weeks after randomization, patients who were initially assigned to receive placebo were crossed over to receive treatment with 300 mg of tenofovir DF.

Prospectively designed virology substudies included all patients who received ≥1 dose of study drug in study 902 ($n = 186$; known as the "intent-to-treat" [ITT] population), as well as 50% of patients who were randomly assigned, at study entry, to a virology substudy of study 907 ($n = 274$). The HIV-1 RT and protease genes from banked plasma samples were genotypically analyzed, in a treatment-blinded fashion, at baseline, at weeks 24 and 48, or on early termination of the study. Phenotypic analyses were performed at baseline and either at week 48 or on early termination of the study, for all patients assigned to the treatment arms in which 300 mg of tenofovir DF was administered.

All biological specimens from patients were obtained with the informed consent of the patients and in accordance with the human experimentation guidelines of the US Department of Health and Human Services.

Genotypic analyses. For study 902, HIV-1 RT nucleotides 1–750 and all of the protease gene were sequenced (TruGene assay; Applied Sciences). For study 907, HIV-1 RT nucleotides 1–1200 and all of the protease gene were sequenced (Virco). The HIV-1 RT mutations associated with NRTI resistance were defined as M41L, A62V, K65R, D67N, T69D/N, K70R, L74V/I, V75T, F77L, Y115F, F116Y, Q151M, M184V, L210W, T215Y/F, and K219Q/E/N/R [27]. In addition, RT mutations T39A, K43E/N, E44D, V118I, H208Y, and L228H/R were included in the group of HIV-1 RT mutations that were associated with NRTI resistance.

Phenotypic analyses. Data on the susceptibility of HIV-1 to tenofovir and all other approved antiretroviral agents were generated using the Antivirogram assay (Virco). For these phenotypic analyses, plasma samples with >500 HIV-1 RNA copies/mL were available at baseline for each of 222 patients (53 samples were available from patients in study 902, and 169 samples were available from patients in study 907). The 53 patient isolates from study 902 have been described elsewhere [25]. A secondary set of phenotypic analyses was performed at baseline, by use of the PhenoSense HIV-1 assay (ViroLogic), for tenofovir DF–treated patients who were not originally assigned to the virology substudy of study 907.

HIV-1 RNA quantitation. HIV-1 RNA concentrations in plasma samples were determined using the standard Roche Amplicor HIV-1 Monitor assay or the Roche Ultrasensitive HIV-1 Monitor assay (lower limit of quantitation, 50 HIV-1 RNA copies/mL), to quantify <400 HIV-1 RNA copies/mL.

Primary efficacy end point. For both studies, the primary efficacy end point was the mean change in the HIV-1 RNA level from baseline to week 24 (known as "$DAVG_{24}$"). "$DAVG_{48}$" was defined as the mean change in the HIV-1 RNA level from baseline to week 48. $DAVG_{24}$ was calculated for the ITT population and, also, for an "as-treated" (AT) population. The AT population included all patients, but it excluded all HIV-1 RNA data after permanent discontinuation of the assigned study medication or addition of other antiretroviral medication. If HIV-1 RNA data were missing, the DAVG was calculated with a wider time interval, to account for the missing observation. All DAVG data presented reflect the arithmetic mean of the individual DAVG values for all subjects in the analyzed group.

Statistical analyses. All statistical analyses of HIV-1 RNA were performed using SAS software (version 8.1; SAS Institute). Correlation analyses were conducted using the Spearman rank-order method. Multivariate linear regression analyses were performed to evaluate the effect of different mutations, along with the effects of other baseline parameters, on HIV-1 RNA response. A stepwise method was applied, with a significance level of $P = .15$ used for entry and for staying in the model. P values were not adjusted for multiple comparisons.

Statistical analyses to determine phenotypic cutoffs were performed using Splus (version 6.0; Insightful). The classification and regression tree method used binary recursive partitioning

whereby the data were successively split along the predictor (susceptibility to tenofovir at baseline) to maximally distinguish virologic response in the left and right branches. The resultant tree was pruned back to the optimal split number through 10-fold cross-validation with the cv.tree function. In this cross-validation, 10% of the data was left out, and 90% was used as the learning sample to produce a complete tree. For a given value of the complexity parameter, the 10% of the data that was left out was used as a test sample to estimate the cost. This process was repeated 10 times, and an average cross-validated cost was calculated. The tree size corresponding to the subtree with the smallest cross-validated cost was defined as the optimal size. The final cutoff values from the optimal cross-validated tree are reported.

RESULTS

Genotypic analysis at baseline. HIV-1 genotypic data at baseline were obtained from 184 of 186 patients in study 902 and from 253 of 274 patients in the virology substudy of study 907. The remaining patients had insufficient amounts of HIV-1 RNA available for analysis. Consistent with the extensive treatment experience of the patients in these trials (mean treatment duration, 4.6 years [in study 902] and 5.4 years [in study 907]), 94% of patients from both trials had plasma HIV-1 that expressed ⩾1 primary NRTI-associated resistance mutation in RT, according to HIV-1 genotypic data at baseline. In both studies, a similar percentage of patients had HIV-1 that expressed various patterns of NRTI-associated mutations (figure 1); slightly more patients in study 907, compared with patients in study 902, had nonnucleoside reverse-transcriptase inhibitor–associated mutations. Most patients (71%) had HIV-1 with typical TAMs at codons 41, 67, 70, 210, 215, or 219 (mean, 2.8 mutations), and 67% had HIV-1 with M184V/I mutations. At study entry, few patients (6 patients [1.4%]) had HIV-1 that expressed the K65R mutation.

Genotype at baseline and HIV-1 RNA response. Despite the extensive presence of RT resistance mutations at baseline, patients who added 300 mg of tenofovir DF to their existing failing regimen demonstrated a statistically significant decrease in plasma HIV-1 RNA, as evidenced by the primary efficacy end point of the $DAVG_{24}$ (-0.58 $\log_{10}$ HIV-1 RNA copies/mL in study 902 [$P < .001$ vs. placebo; ITT population] [22] and -0.59 $\log_{10}$ HIV-1 RNA copies/mL in study 907 [$P < .001$ vs. placebo; ITT population] [26]). Given the similar study populations and the nearly identical treatment responses observed, additional analyses combined data for patients in the treatment arms receiving 300 mg of tenofovir DF ($n = 222$) with data for patients in the placebo arms ($n = 110$) from each study. In comparison with the results of the ITT analyses, the results of an AT analysis that included all patients but excluded HIV-1 RNA data after a treatment change showed a nearly identical HIV-1 RNA response to 300 mg of tenofovir DF ($DAVG_{24}$, -0.58 $\log_{10}$ HIV-1 RNA copies/mL [for AT analysis] vs. -0.59 $\log_{10}$ HIV-1 RNA copies/mL [for ITT analysis]; $n = 222$). Of the total group of 332 patients, 80 patients from study 902 have been described elsewhere [25]. Efficacy results for the remaining 104 patients from study 902 who had genotypic data were not combined, because these patients were treated with lower doses of tenofovir DF.

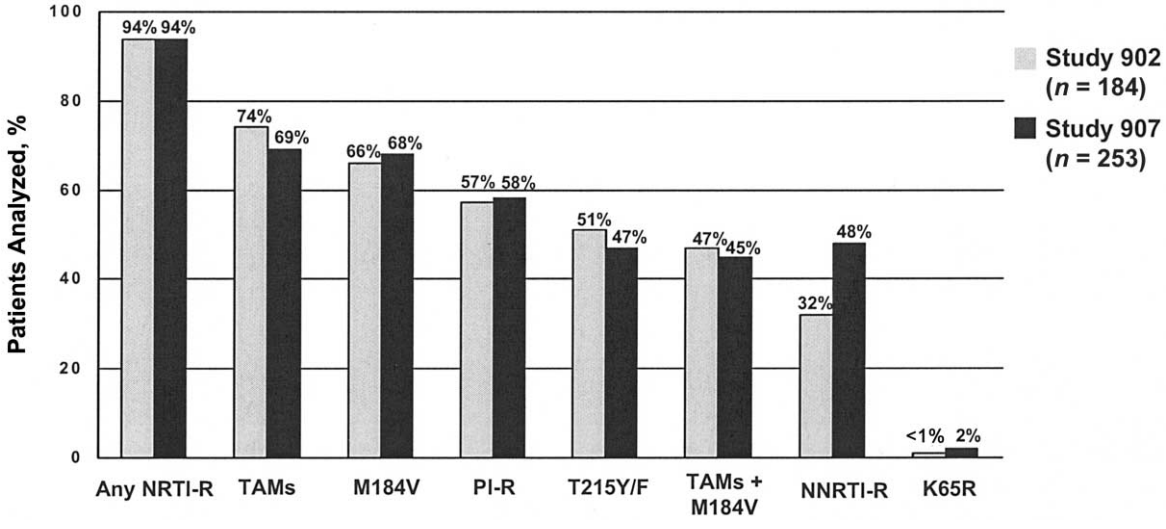

Figure 1. Percentage of patients with HIV-1 expressing antiretroviral-associated resistance mutations at baseline in studies GS-98-902 and GS-99-907. Shown are nucleoside reverse-transcriptase inhibitor–associated mutations (NRTI-R), thymidine analogue–associated mutations (TAMs), protease inhibitor–associated mutations (PI-R), nonnucleoside reverse-transcriptase inhibitor–associated mutations (NNRTI-R), and specific reverse-transcriptase point mutations M184V, K65R, and T215Y/F.

In prespecified protocol analyses, patients with HIV-1 that contained TAMs or M184V at baseline demonstrated statistically significant reductions in HIV-1 RNA after they received tenofovir DF, compared with patients who received placebo (table 1). The HIV-1 RNA response in patients with TAMs ($DAVG_{24}$, -0.50 $\log_{10}$ HIV-1 RNA copies/mL) was notable, because such patients had a mean of 2.8 TAMs. However, patients without TAMs had an HIV-1 RNA response (-0.80 $\log_{10}$ HIV-1 RNA copies/mL) that was significantly stronger than that shown by patients with TAMs. Patients with HIV-1 with the M184V mutation in the absence of TAMs had the strongest HIV-1 RNA response (-0.96 $\log_{10}$ HIV-1 RNA copies/mL), which was significantly superior to that of patients without M184V. Among patients with TAMs, there was a slightly improved HIV-1 RNA response when M184V was present versus not present (-0.52 vs. -0.45 $\log_{10}$ HIV-1 RNA copies/mL, respectively), although this difference was not significant in univariate analyses ($P = .44$). Patients who entered these trials with a K65R mutation at baseline did not show a treatment response to tenofovir DF (-0.01 $\log_{10}$ HIV-1 RNA copies/mL).

In non–protocol-defined analyses, the effects of specific TAMs on the response to treatment with tenofovir DF were further explored (table 1). Significantly reduced responses were observed for patients with ⩾3 TAMs, relative to patients without TAMs. Relative to the response observed when placebo was given, however, this response was still significant. Two distinct patterns of TAMs were observed. There were highly significant positive correlations among the M41L, L210W, and T215Y mutations, with all 3 pair-wise correlation coefficients ⩾0.57 (table 2). Another set of positive correlations was observed for the D67N, K70R, and K219Q/E/N/R mutations, with all 3 pair-wise correlation coefficients ⩾0.62. The T215F mutation was significantly associated with these 3 latter mutations as well ($r \geqslant 0.27$). Strongly negative correlations were observed for the M41L-K70R, K70R-L210W, and K70R-T215Y mutation pairs ($r \leqslant -0.24$).

Table 1. HIV-1 RNA response to tenofovir disoproxil fumarate (tenofovir DF), by genotype at baseline.

	Mean HIV-1 RNA response						
	Subjects given tenofovir DF			Subjects given placebo			
Genotype at baseline[a]	*n*	$DAVG_{24}$[b]	$DAVG_{48}$[b]	*n*	$DAVG_{24}$[b]	P[c]	P[d]
All	222	−0.59	−0.57	110	−0.03	<.001	…
No M184V	73	−0.42	−0.43	40	0.08	<.001	…
M184V	149	−0.67	−0.64	70	−0.09	<.001	.003
M184V and no TAMs	51	−0.96	−0.88	20	−0.12	<.001	<.001
No TAMs	68	−0.80	−0.74	29	−0.11	<.001	…
TAMs	154	−0.50	−0.50	81	0.00	<.001	<.001
And no M184V	56	−0.45	−0.46	31	0.13	<.001	.001
And M184V	98	−0.52	−0.52	50	−0.08	<.001	.002
1 or 2	55	−0.66	−0.63	33	−0.04	<.001	.11
⩾3	99	−0.40	−0.43	48	0.03	<.001	<.001
With M41L or L210W	57	−0.21	−0.24	29	0.01	.013	<.001
Without M41L or L210W	42	−0.67	−0.67	19	0.07	<.001	.15
D67N	79	−0.53	−0.58	43	−0.03	<.001	.004
K70R	67	−0.71	−0.70	40	−0.03	<.001	.17
K219Q/E/N/R	57	−0.60	−0.59	27	0.11	<.001	.03
T215Y/F	106	−0.35	−0.37	53	0.03	<.001	<.001
M41L	81	−0.26	−0.29	40	0.06	<.001	<.001
L210W	46	−0.17	−0.21	22	0.06	.025	<.001
T215Y/F without M41L or L210W	25	−0.70	−0.66	13	−0.01	.012	.32

NOTE. $DAVG_{24}$, mean change in the HIV-1 RNA level from baseline to week 24; $DAVG_{48}$, mean change in the HIV-1 RNA level from baseline to week 48; TAMs, thymidine analogue–associated mutations (i.e., M41L, D67N, K70R, L210W, T215Y/F, or K219Q/E/N/R).

[a] Unless specifically excluded, mutations other than those listed may also be present.

[b] Expressed as $\log_{10}$ HIV-1 RNA copies per milliliter; intent-to-treat population.

[c] Wilcoxon rank sum test comparing tenofovir DF and placebo arms.

[d] Wilcoxon rank sum test comparing mutation category and corresponding category without mutations (e.g., no M184V mutation or no TAMs).

Table 2. Correlations among thymidine analogue–associated mutations (TAMs).

TAMs	r[a]	P
With positive correlations		
41-210-215Y Pattern		
M41L-T215Y	0.72	<.001
M41L-L210W	0.57	<.001
L210W-T215Y	0.58	<.001
67-70-219-215F Pattern		
D67N-K70R	0.62	<.001
D67N-K219Q/E/N/R	0.69	<.001
D67N-T215F	0.27	<.001
K70R-K219Q/E/N/R	0.68	<.001
K70R-T215F	0.27	<.001
T215F-K219Q/E/N/R	0.35	<.001
With negative correlations		
M41L-K70R	−0.28	<.001
K70R-T215Y	−0.29	<.001
K70R-L210W	−0.24	<.001
L210W-T215F	−0.13	.015
T215Y-K219Q/E/N/R	−0.14	.010

[a] Spearman rank correlation coefficients *(r)* for all pairwise combinations of M41L, D67N, K70R, L210W, T215Y, T215F, and K219Q/E/N/R were determined for all HIV-1 samples with TAMs at baseline ($n = 235$). All correlation coefficients for which $P < .05$, according to Student's t test, are indicated.

Response to treatment differed markedly among patients, depending on which pattern of TAMs was present. In the absence of M41L and L210W mutations, patients with ⩾3 TAMs (e.g., D67N, K70R, K219Q/E/N/R, and +/−T215F) had an HIV-1 RNA response of −0.67 $\log_{10}$ HIV-1 RNA copies/mL, compared with −0.21 $\log_{10}$ HIV-1 RNA copies/mL in the presence of M41L or L210W. The M41L and L210W mutations appeared to be the best predictor of reduced response, because, in the absence of these mutations, patients with the T215Y/F mutation in HIV-1 showed an HIV-1 RNA response of −0.70 $\log_{10}$ HIV-1 RNA copies/mL. All of these responses to treatment with tenofovir DF were sustained through week 48 (table 1).

Among patients with HIV-1 with ⩾3 TAMs that included either M41L or L210W, there was a wide range of treatment responses (range, −0.82 to +0.50 $\log_{10}$ HIV-1 RNA copies/mL; $n = 57$). The upper quartile of responses in this group showed a decrease of >0.42 $\log_{10}$ HIV-1 RNA copies/mL. Analysis of HIV-1 RT genotypes by quartile revealed a higher prevalence of L210W in the lowest quartile (93%) versus that in the upper quartile (64%). Other mutations, including the M184V mutation, were similarly distributed among quartiles.

Multivariate response analyses. Multivariate linear regression analyses were performed to determine the predictors of change in HIV-1 RNA levels. Duration of prior antiretroviral therapy, the number of antiretroviral drugs received concurrently, age, and sex were not significantly associated with $DAVG_{24}$. In addition to treatment with tenofovir DF, the significant predictors of HIV-1 RNA response were the HIV-1 RNA level at baseline, the CD4 cell count at baseline, the number of TAMs, and the presence of the M184V mutation (table 3). Treatment with tenofovir DF had the greatest effect on HIV-1 RNA (−0.59 $\log_{10}$ HIV-1 RNA copies/mL). Increasing numbers of TAMs were a significant predictor of a weaker response (+0.08 $\log_{10}$ HIV-1 RNA copies/mL per TAM). The M184V mutation was associated with a modest, but statistically significant, improvement in response (−0.12 $\log_{10}$ HIV-1 RNA copies/mL).

Additional models were created to examine the effects of specific patterns of TAMs. With adjustment for the other significant parameters (the HIV-1 RNA level at baseline, the CD4 cell count at baseline, and the presence of the M184V mutation), these models confirmed the strong negative effects of the 41-210-215Y and 41-67-210-215Y mutational patterns (table 3). In contrast, mutations of the 67-70-219 and 67-70-215F-219 patterns were not associated with a significant alteration in the HIV-1 RNA response ($P > .28$).

A final set of analyses was performed to determine whether

Table 3. Multivariate linear regression models of HIV-1 RNA response to tenofovir disoproxil fumarate (tenofovir DF).

Model,[a] parameter	Parameter estimate, $\log_{10}$ HIV-1 RNA copies/mL	P[b]
1		
Tenofovir DF treatment	−0.59	<.001
HIV-1 RNA level at baseline	−0.18[c]	.001
CD4 cell count at baseline	−0.06[d]	<.001
No. of TAMS[e]	+0.08[f]	<.001
M184V	−0.12	.04
2, M41L, L210W, and T215Y	+0.41	<.001
3, M41L, D67N, L210W, and T215Y	+0.32	.003
4, M41L, D67N, and T215Y	+0.24	.01
5, D67N, K70R, T215F, and K219Q/E/N/R	+0.14	.28
6, D67N, K70R, and K219Q/E/N/R	−0.03	.70

NOTE. TAMs, thymidine analogue–associated mutations.

[a] Models 2–6 also included treatment with tenofovir DF (−0.59 to −0.60 $\log_{10}$ HIV-1 RNA copies/mL), HIV-1 RNA level at baseline (−0.14 to −0.16 $\log_{10}$ HIV-1 RNA copies/mL), CD4 cell count at baseline (all −0.06 $\log_{10}$ HIV-1 RNA copies/mL), and the presence of M184V (−0.15 to −0.18 $\log_{10}$ HIV-1 RNA copies/mL). All of these parameters maintained significance ($P < .01$), and parameter estimates varied minimally, as indicated in parentheses.

[b] By Student's t test.

[c] Per log increase.

[d] Per 100-cell increase.

[e] One to 6 mutations.

[f] Per TAM.

other RT mutations might be associated with an altered response to tenofovir DF. In this final set of analyses, 22 RT mutations that were observed in these patients at baseline and that were known to be associated with NRTI resistance were evaluated as univariate predictors of HIV-1 RNA response (see the "Genotypic analysis" subsection of the Subjects and Methods section for a list of these mutations). Any mutation shown to be significant in these univariate analyses ($P < .05$) was retained in the multivariate analysis. Of the TAMs, only the mutations at codons 41, 67, 210, and 215 were found to be significant in univariate analyses. Additional mutations were added to the model in a stepwise fashion, on the basis of their degree of significance in the univariate models and the overall model improvement tested by Student's *t* test. In addition to the TAMs at codons 41, 67, 210, and 215, the mutations at RT codons 39, 43, 65, 74, 184, and 208 were retained in the final model. With the exception of the mutation at codon 184, all mutations were associated with a weaker HIV-1 RNA response. The negative effects of the K65R mutation at baseline were also an independent predictor of poor treatment response ($P = .0015$).

Phenotype at baseline and HIV-1 RNA response. By use of the Antivirogram assay, phenotypic data were obtained, at baseline, for 204 of the 222 patients treated with tenofovir DF in the present analysis. The $DAVG_{24}$ for these patients was -0.65 $\log_{10}$ HIV-1 RNA copies/mL. In multivariate linear regression analyses, there was a significant association of susceptibility to tenofovir at baseline with response to treatment with tenofovir DF (parameter estimate, .653 $\log_{10}$ HIV-1 RNA copies/mL; $P = .0014$). Figure 2 shows the HIV-1 RNA responses, according to 5 strata of susceptibility to tenofovir at baseline. The best HIV-1 RNA responses were observed among patients with <1-fold change in susceptibility to tenofovir at baseline (i.e., patients who were slightly hypersusceptible to tenofovir). The response diminished in the higher strata, with patients who had >4-fold reduced susceptibility to tenofovir showing the most limited treatment response (-0.22 $\log_{10}$ HIV-1 RNA copies/mL; $n = 19$).

A recursive partitioning analysis was used to approximate phenotypic cutoffs for response to treatment with tenofovir DF. For these analyses, a "responder" was defined as a patient who had a decrease of >0.5-$\log_{10}$ HIV-1 RNA copies/mL from baseline to the week 24 nadir. The cross-validated recursive partitioning tree defined 2 cutoffs in response to tenofovir DF: one at a susceptibility of 1.4-fold at baseline and the other at 3.8-fold. Bootstrap replications of these analyses confirmed the values of both cutoffs and allowed an estimate of their precision. For the first split at 1.4-fold, the 90% confidence interval (CI) was 0.75–2.25; for the second split at 3.8-fold, the 90% CI was 2.45–5.4. The 2 HIV-1 RNA response cutoffs of 1.4-fold and 3.8-fold divide the patient population into 3 groups (table 4). The first cutoff of 1.4-fold defines the beginning of a reduced response to tenofovir DF, whereas the second cutoff of 3.8-fold defines a stronger cutoff for reduced response or no response.

An identical analysis approach was used for data on susceptibilities to tenofovir at baseline that were obtained using the PhenoSense HIV-1 assay. Phenotypes were obtained, at baseline, for 112 patients from study 907 who were treated with tenofovir DF. By use of the categorical response variable of a decrease of >0.5 $\log_{10}$ HIV-1 RNA copies/mL from baseline to the week 24

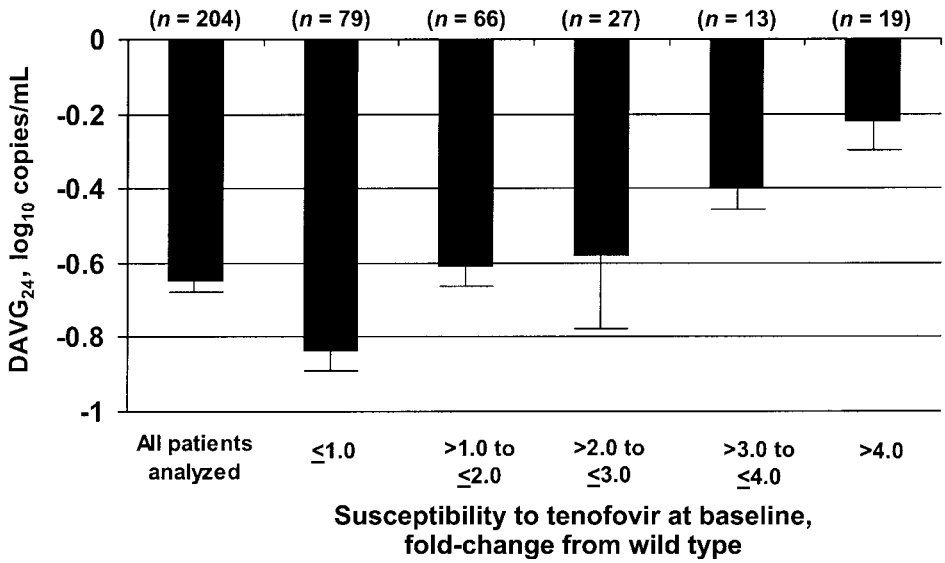

Figure 2. HIV-1 RNA response to treatment with tenofovir disoproxil fumarate, according to susceptibility to tenofovir at baseline. Phenotypic susceptibility to tenofovir at baseline was determined using the Antivirogram assay (Virco). The mean change in the HIV-1 RNA level from baseline to week 24 ($DAVG_{24}$), with the standard error, is shown for patients grouped according to their fold-change from wild-type susceptibility.

Table 4. HIV-1 RNA responses, by recursive partitioning susceptibility cutoffs.

Susceptibility to tenofovir at baseline[a]	No. (%) of patients (n = 204)	$DAVG_{24}$, log_{10} HIV-1 RNA copies/mL
<1.4	102 (50)	−0.77
⩾1.4 and <3.8	80 (39)	−0.47
⩾3.8	22 (11)	−0.24

NOTE. $DAVG_{24}$, mean change in the HIV-1 RNA level from baseline to week 24.

[a] Fold-change from the wild type, as determined by the Antivirogram assay (Virco).

nadir, bootstrap replications (n = 3000) of the recursive partitioning analysis revealed 2 splits, one at 1.4-fold and the other at 4-fold. Identical cutoffs were obtained using other definitions of treatment response (decreases in $DAVG_{24}$ of >0.3, >0.4, and >0.5 log_{10} HIV-1 RNA copies/mL, for the AT population). These results also define 3 patient groups: a 1.4-fold change denotes the beginning of a reduced response, and a 4-fold change denotes a strongly reduced response or no response. By use of these cutoffs, patients with ⩽1.4-fold, >1.4–4-fold, and >4-fold changes in susceptibility to tenofovir had a $DAVG_{24}$ of −0.78 (n = 78; 70%), −0.35 (n = 26; 23%), and −0.15 (n = 8; 7%) log_{10} HIV-1 RNA copies/mL, respectively.

Genotypic and phenotypic correlations. Genotypic and phenotypic correlations could be assessed for the 204 patients for whom both a genotype and a phenotype were obtained at baseline (Antivirogram assay). There was a trend toward decreased susceptibility to tenofovir, according to the number of TAMs in a patient's HIV-1 (table 5). Patients with viruses with ⩾3 TAMs, including M41L or L210W, had a mean 2.9-fold reduction in susceptibility to tenofovir versus a mean 1.7-fold reduction for patients with viruses with ⩾3 TAMs but without M41L or L210W (P = .003). For patients with a ⩾3.8-fold change in susceptibility to tenofovir at baseline (n = 22), the vast majority of patients had multiple TAMs that included M41L or L210W (n = 20) and, also, a large number of other mutations found either exclusively or predominantly among NRTI-experienced patients. Many of these mutations were defined in the multivariate statistical analyses described in the "Multivariate response analyses" subsection of the Results section (e.g., mutations at positions 39, 43, and 208). The remaining patients had either a K65R mutation (n = 1) or a T69 insertion mutation (n = 1).

DISCUSSION

Studies 902 and 907 demonstrated that tenofovir DF is an effective treatment option for treatment-experienced patients who have HIV-1 with a broad range of resistance mutations. However, in comparison with the reductions of 1.5 log_{10} HIV-1 RNA copies/mL observed in short-term monotherapy studies of treatment-naive patients, the HIV-1 RNA response among treatment-experienced patients was lower (mean reduction, 0.6 log_{10} HIV-1 RNA copies/mL). This comparison suggests some degree of cross-resistance with preexisting resistance mutations. Given the design of these treatment-intensification studies, the specific activity of tenofovir DF against different types of resistance mutations was discernable.

In vitro studies have previously shown that the K65R mutation can be selected by tenofovir and that it results in decreased susceptibility to tenofovir. Consistent with its low frequency in other studies [4, 5], the K65R mutation was found, at baseline, in only 6 (1.8%) of 333 patients who entered these clinical studies. The HIV-1 RNA response among these patients was poor; however, definitive conclusions are difficult to make because of the low number of patients with this mutation and because of other possible confounding factors (e.g., treatment compliance). Nevertheless, it is unlikely that patients who already have the K65R mutation will benefit significantly from initiation of tenofovir DF therapy. In contrast, patients who developed the K65R mutation during therapy may continue to benefit from tenofovir DF therapy because of maintenance of a less-fit mutant virus and/or partial drug activity. This possibility is based on other studies of the continued benefit of the regimen for patients with resistant HIV-1 and the reduced capacity for in vitro replication of the K65R mutant HIV-1 [28, 29].

In vitro studies have shown some degree of cross-resistance to tenofovir, for TAMs [8]. In the present study of tenofovir DF, >70% of patients had TAMs in their HIV-1 at baseline. Although this group of patients, as a whole, has a significant response to tenofovir DF therapy (with a 0.50-log_{10} decrease in HIV-1 RNA copies/mL), a subgroup of patients with multiple TAMs (i.e., ⩾3), including either the M41L or L210W muta-

Table 5. Thymidine analogue–associated mutations (TAMs) and susceptibility to tenofovir, at baseline, among 204 study subjects.

Patients with mutations at baseline	Mean susceptibility to tenofovir at baseline[a]	n
All	1.8	204
Without TAMs[b]	1.0	60
With TAMs		
1 or 2	1.4	52
⩾3	2.4	92
With M41L or L210W	2.9	55
Without M41L or L210W	1.7	37

[a] Fold-change from the wild type, as determined by the Antivirogram assay (Virco).

[b] TAMs M41L, D67N, K70R, L210W, T215Y/F, or K219Q/E/N/R; other mutations may be present.

tion, or both mutations, had a change of only -0.22 $\log_{10}$ HIV-1 RNA copies/mL. Although this response was significantly superior to the response with placebo, it was statistically inferior to that among patients without TAMs. In contrast, patients with 1 or 2 TAMs had a similar HIV-1 RNA response, relative to patients without TAMs, as did patients with ⩾3 TAMs that did not include M41L or L210W. This latter group of patients includes patients with a multiple-TAM pattern that appears to be distinct from the 41-210-215Y pattern, as has been observed elsewhere [30–32]. The data presented in the current study demonstrate a significant difference in treatment response among patients with these different patterns of TAMs.

Within the group of patients with ⩾3 TAMs, including M41L or L210W, there was a wide range of treatment responses. The upper quartile of responses within this group showed reductions of -0.42 to -0.82 $\log_{10}$ HIV-1 RNA copies/mL. Attempts were made to further determine the genotypic basis for this variation. Additional mutations that appear to contribute to cross-resistance were identified at RT positions 39, 43, 44, 74, 118, and 208. These mutations are generally present along with multiple TAMs and appear to represent additional resistance mutations related to prior NRTI therapy [33]. However, because of the limited sample size and the overall genetic complexity of HIV-1 in these patients, more-precise genotypic predictors of reduced response were not obtained. It appears that the genotypic rule of the presence of ⩾3 TAMs, including M41L or L210W, is the best predictor of the risk of a significantly reduced response to tenofovir DF, but it does not precisely define a population of nonresponders.

The M184V mutation has shown increased susceptibility to tenofovir in vitro and can also result in resensitization of the negative effects of TAMs and K65R on susceptibility to tenofovir in vitro [15, 17, 34]. In the multivariate statistical analyses presented in the current study, M184V was associated with a modest, but statistically significant, improvement in HIV-1 RNA response (-0.12 $\log_{10}$ HIV-1 RNA copies/mL; $P = .04$). It is important to note that, in these studies, patients maintained stable background therapy, which included 3TC for 70% of patients. Thus, M184V was largely maintained among these patients. Loss of M184V may be associated with increased replication capacity, which may result in clinical or virologic consequences that were not measured in these studies.

The HIV-1 RNA response to tenofovir DF appeared to have a linear and continuous relationship with susceptibility to tenofovir at baseline. Nevertheless, phenotypic cutoffs are useful numbers to provide some interpretive context for phenotypic values. Phenotypic cutoffs for tenofovir DF were obtained for the 2 widely available phenotypic assays (the Antivirogram assay and the PhenoSense HIV-1 assay). Although these assays are technically different, results from both assays yielded nearly identical cutoffs. In both assays, phenotypic susceptibility changes of >1.4-fold were associated with the beginning of a reduced response to tenofovir DF. Phenotypic susceptibility changes >3.8-fold (as determined by the Antivirogram assay) or >4-fold (as determined by the PhenoSense HIV-1 assay) were associated with a strongly reduced response or no response. Given the low numbers of patients with changes >4-fold, there is poor precision for this upper-level estimate.

A limitation of these analyses was the cohort of patients studied. The majority of patients were patients from study 907 who had a fairly low virus load at study entry (mean, 2340 HIV-1 RNA copies/mL). Nevertheless, there were significant numbers of patients with TAMs and M184V to characterize the effects of these mutations on response to therapy. More-advanced cohorts of patients may have greater numbers of NRTI-associated mutations that may further influence response to treatment with tenofovir DF. Moreover, there was an insufficient number of patients in this trial to make definitive conclusions regarding the efficacy of tenofovir DF against the less-frequent HIV-1 RT resistance patterns, including K65R, T69 insertions, and the Q151M multinucleoside resistance complex.

In summary, the genotypic and phenotypic analyses performed in conjunction with the clinical development of tenofovir DF have provided a context for interpretation of resistance information with respect to tenofovir DF activity. Partially resistant and fully resistant forms of HIV-1 were defined phenotypically at 1.4-fold and 4-fold, respectively. A specific genotypic pattern of ⩾3 TAMs, including the M41L or L210W mutations, corresponded to a reduced response to tenofovir DF, but no genotypic pattern of full resistance was discerned. Of interest, although tenofovir DF can be affected by the presence of TAMs, it does not appear to select for TAMs in either treatment-experienced or treatment-naive patients [25, 35, 36]. Results from other analyses of response to tenofovir DF have shown good agreement with the genotypic rules presented in the current study, and they have confirmed the importance of specific TAMs in potentially reducing treatment response [37].

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