

Tenofovir Disoproxil Fumarate in Nucleoside-Resistant HIV-1 Infection

A Randomized Trial

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Background: Resistance to antiretroviral agents remains a leading cause of treatment failure for patients infected with HIV-1.

Objective: To describe the efficacy and safety of tenofovir disoproxil fumarate (tenofovir DF) compared with placebo in patients with detectable viral replication despite current antiretroviral therapy.

Design: Randomized, double-blind, placebo-controlled study through 24 weeks. After 24 weeks, all patients received open-label tenofovir DF for the remainder of the 48-week study.

Setting: 75 North American, European, and Australian HIV clinics.

Patients: 552 HIV-1-infected adults who were receiving antiretroviral therapy and had stable HIV-1 RNA levels ranging from 400 to 10 000 copies/mL.

Measurements: Change in HIV-1 RNA level (time-weighted average from baseline through week 24); proportion of patients with grade 3 or 4 laboratory abnormalities and adverse events; and genotypic HIV-1 resistance testing in a separate substudy at baseline, week 24, and week 48.

Results: A statistically significant decrease in HIV-1 RNA level through week 24 (the primary end point) was observed in the tenofovir DF group versus the placebo group ($-0.61 \log_{10}$ copies/mL vs. $-0.03 \log_{10}$ copies/mL, respectively [$P < 0.001$]; difference, $-0.58 \log_{10}$ copies/mL [95% CI, -0.68 to $-0.49 \log_{10}$ copies/mL]). In a virologic substudy, 94% of 253 patients had plasma isolates expressing reverse transcriptase mutations associated with nucleoside resistance mutations at baseline. Through week 24, the incidence of clinical adverse events was similar between patients receiving placebo and those receiving tenofovir DF (14% vs. 13%). No evidence of tenofovir DF-related toxicity was seen through week 48.

Conclusion: In treatment-experienced patients with suboptimal viral suppression, tenofovir DF significantly reduced HIV-1 RNA level and had a safety profile similar to that of placebo.

Ann Intern Med. 2003;139:313-320.

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Combination antiretroviral therapy has decreased mortality rates for patients with HIV-1 infection (1, 2). Suppression of HIV-1 viral load has been shown to be highly predictive of slower clinical disease progression (3). However, the clinical utility of a combination antiretroviral regimen typically wanes, often because of one or more factors such as drug resistance or poor adherence (4). These factors are even more important for patients who have previously used antiretroviral drugs. This difficult-to-treat population has limited treatment options and often experiences drug-related adverse effects due to previous antiretroviral therapy regimens (5–7). Given these limitations, novel potent agents that combine ease of dosing with favorable safety and resistance profiles are needed to increase the long-term durability of combination antiretroviral therapy (8, 9). Simplifying HIV treatment regimens using once-daily antiretroviral drugs may improve adherence and therapeutic outcomes (10, 11).

Tenofovir disoproxil fumarate (tenofovir DF) is an oral prodrug of tenofovir, a novel, acyclic nucleotide analogue with in vitro activity against HIV-1 and HIV-2 (12, 13). Unlike nucleoside analogues, tenofovir is active in both active and resting lymphoid cells and macrophages (13). In rhesus macaques acutely infected with simian immunodeficiency virus, tenofovir administered as monotherapy 24 hours after inoculation eradicated viral DNA from lymph nodes, plasma, and leukocytes (14). Tenofovir has also demonstrated in vitro activity against wild-type

and lamivudine-resistant hepatitis B virus (15). In a 186-patient phase II dose-ranging study, Schooley and colleagues (16) demonstrated the potency and favorable safety profile of tenofovir DF, 300 mg, highlighting its potential for further study in larger phase III trials. Tenofovir has a favorable resistance profile with activity against most nucleoside analogue-resistant viruses and infrequent (3%) emergence of the K65R resistance mutation through 96 weeks (17–21). Our study was designed to confirm the antiviral efficacy and safety of tenofovir DF compared with placebo in treatment-experienced patients who had detectable HIV-1 RNA levels despite combination antiretroviral therapy.

METHODS

Study Sample

Institutional review boards at all study sites approved the study protocol and informed consent statements. Recruitment began in October 1999 and continued until June 2000 at 75 HIV clinics in western Europe, North America, and Australia. All patients gave written informed consent. Patients 18 to 65 years of age were eligible if they had received antiretroviral therapy (four agents or fewer) for at least 8 weeks before randomization and had stable plasma HIV-1 RNA levels of 400 to 10 000 copies/mL on the Roche Amplicor HIV-1 Monitor UltraSensitive test, version 1.0 (Roche Diagnostics, Branchburg, New Jersey).

Context

Resistance to antiretroviral drugs is a leading cause of treatment failure in HIV-1-infected patients.

Contribution

In this multicenter, double-blind trial, 552 treatment-experienced patients with detectable RNA levels despite continuing antiretroviral therapy were randomly assigned to receive tenofovir DF (a nucleotide analogue) or placebo. At 6 months, patients given tenofovir DF had greater reductions in HIV-1 RNA levels than those given placebo. Nearly 15% of patients in both groups had clinical adverse events, such as severe diarrhea, pain, or depression.

Implications

In HIV-infected patients with suboptimal viral suppression, adding tenofovir DF to ongoing antiretroviral therapy reduces viral loads.

—The Editors

The lower limit of quantification for this test was 50 copies/mL. We placed no entry restrictions on CD4 cell count. Additional inclusion criteria were serum creatinine concentration of 133 $\mu\text{mol/L}$ or less (≤ 1.5 mg/dL), calculated creatinine clearance (using the Cockcroft–Gault formula [22]) of at least 1.00 mL/s (≥ 60 mL/min), absolute neutrophil count of at least 1.000×10^9 cells/L, platelet count of at least 50.0×10^9 cells/L, hemoglobin level of at least 80 g/L, total bilirubin level of 26 $\mu\text{mol/L}$ or less (≤ 1.5 mg/dL), serum phosphorus level of at least 0.71 mmol/L (≥ 2.2 mg/dL), alanine aminotransferase level less than 108 U/L, aspartate aminotransferase level less than 90 U/L, negative results on a serum pregnancy test for women of childbearing potential, and life expectancy of more than 1 year. We excluded patients who had previously participated in clinical trials with intravenous tenofovir, tenofovir DF, or adefovir dipivoxil; had been immunized within 30 days of baseline; had an active AIDS-defining condition within 30 days of baseline; or were receiving aminoglycoside antibiotics, amphotericin B, cidofovir, cisplatin, fosfarnet, intravenous pentamidine, vancomycin, ganciclovir, systemic chemotherapeutic agents, oral corticosteroids, probenecid, or other investigational agents on an ongoing basis. Patients who were receiving their first antiretroviral regimen were not excluded.

We assessed 976 patients for eligibility and randomly assigned 552 to study groups (Figure). Four hundred twenty-four patients were excluded. Of the 380 who did not meet inclusion criteria, the most common reason ($n = 269$) was HIV-1 RNA levels outside the range of 400 to 10 000 copies/mL. Among the remaining 44 patients, 22 elected not to participate in the study and the other 22 were excluded because of investigator decision, concomitant participation in other investigational studies, screening after the cutoff date for enrollment, incomplete laboratory

values, lack of follow-up, or family emergency. No statistical analyses were performed for patients who were not randomly assigned to a study group.

Design

Through an interactive voice response system that centralized patient randomization, Interactive Clinical Technologies, Inc. (San Francisco, California), generated the random allocation sequence, assigned patients to their treatment groups, and blinded kit numbers to conceal the allocation sequence. Patients were stratified according to HIV-1 RNA level (< 5000 copies/mL or ≥ 5000 copies/mL), CD4 cell count ($< 0.2 \times 10^9$ cells/L or $\geq 0.2 \times 10^9$ cells/L), and number of antiretroviral drugs taken before study entry (fewer than four or at least four). Patients were then randomly assigned in a 2-to-1 ratio to add tenofovir DF, 300 mg, or identical-appearing placebo to their existing antiretroviral regimen. Randomization was not stratified by study center. Changes to the background regimen were discouraged during the first 24 weeks. After week 24, all patients received open-label tenofovir DF for the remainder of the 48-week study.

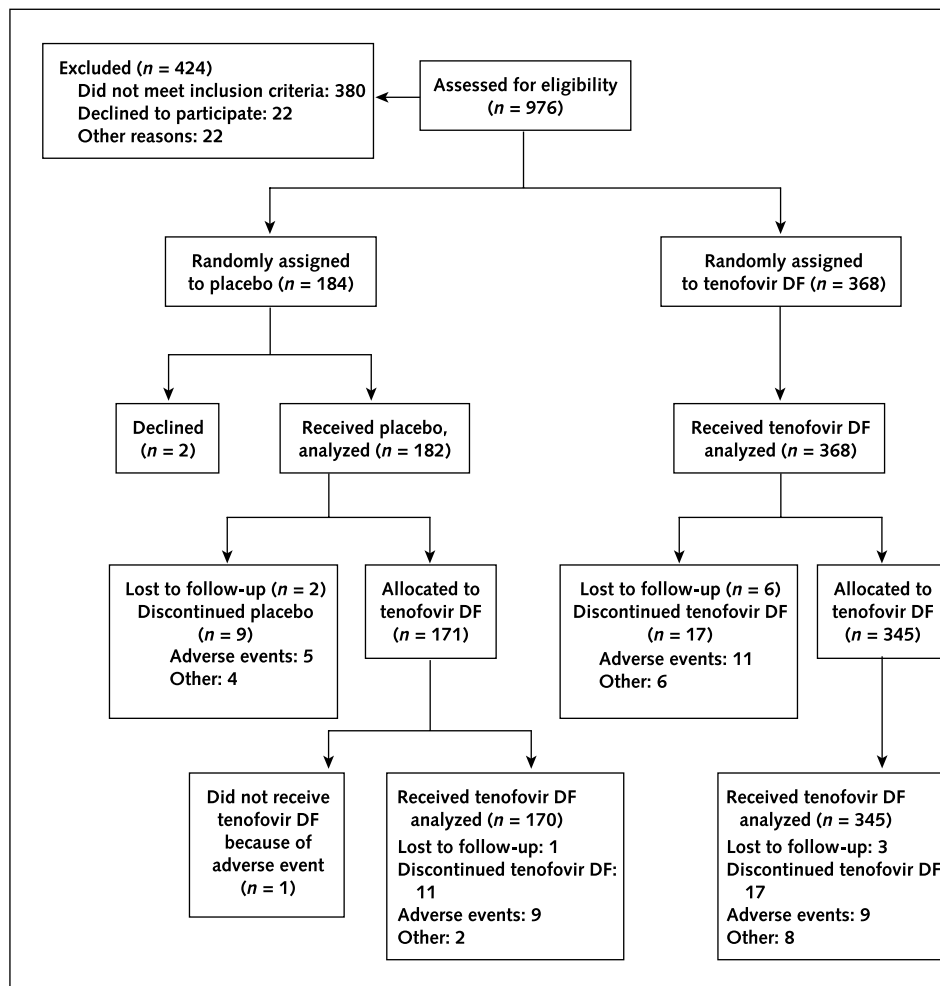
As predefined in both the study protocol and the statistical analysis plan, the single end point for primary efficacy was the time-weighted average change in HIV-1 RNA level from baseline to week 24. Secondary efficacy end points included the proportion of patients with HIV-1 RNA levels of 50 copies/mL or less and 400 copies/mL or less at weeks 24 and 48, the time-weighted average change in HIV-1 RNA level from baseline to week 48, and CD4 cell count at weeks 24 and 48. Adherence to treatment was measured by using pill counts at each study visit, but pill counts were not formally analyzed and drug concentrations were not measured during routine study visits.

Approximately half of the patients ($n = 274$) were randomly assigned to a virologic genotyping substudy at baseline. HIV-1 reverse transcriptase nucleotides 1 to 1200 and all of the HIV-1 protease gene were sequenced by following the reverse transcriptase polymerase chain reaction from plasma HIV-1 RNA (VircoGen, Virco, Mechelen, Belgium). All plasma HIV-1 samples that were obtained at baseline, at week 24 or 48, or at early study termination and had more than 50 copies of HIV-1 RNA per mL were analyzed in a blinded fashion. A polymerase chain reaction product could not be obtained from 21 patients at baseline (14 in the tenofovir DF group, 7 in the placebo group). Plasma HIV-1 RNA was insufficient for genotypic analysis in 58 patients at week 24 (43 patients in the tenofovir DF group, 15 in the placebo group) and 129 patients at week 48.

Assessments

The incidences of grade 3 or 4 clinical adverse events or laboratory abnormalities were evaluated as safety end points. At each postbaseline study visit (weeks 2, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, and 48), the investigator assessed and recorded all adverse events found during history and physical examination as well as laboratory toxic-

Figure. Recruitment and disposition flow chart.



Tenofovir DF = tenofovir disoproxil fumarate.

ties found on chemical and hematologic tests and urinalysis, including date of onset and resolution, severity, relationship to study drug, outcome, and action taken with study medication. Severity was recorded and graded according to the modified severity grading for adult adverse experiences outlined by the National Institutes of Allergy and Infectious Diseases, Division of AIDS. Plasma levels of HIV-1 RNA and CD4 cell counts were measured at all study visits except weeks 28, 36, and 44.

Statistical Analysis

Data were analyzed for the intention-to-treat sample and the as-treated sample. The intention-to-treat sample included all enrolled patients who received at least one dose of study medication and was the primary sample for analyses of efficacy. The as-treated sample included patients who received at least one dose of study medication but excluded all data obtained after permanent discontinuation of assigned study medication or addition of other antiretroviral medication. The safety analysis sample included all patients who received at least one dose of study medication.

As the primary efficacy end point, the time-weighted average change in HIV-1 RNA level from baseline to week 24 represents the difference between the time-weighted average postbaseline values and values at baseline. This difference is defined as follows (23, 24). If the area under the curve (AUC) at week 24 (AUC_{24}) is the AUC of \log_{10} [HIV-1 RNA copy number] between the first postbaseline measurement and week 24 (using the trapezoidal rule with available marker data between weeks 0 and 48 inclusive), then time-weighted average change in HIV-1 RNA level at week 24 is defined as $[AUC_{24} - 24 \times Y_0]/24$, where time is measured in weeks and Y_0 denotes the value of \log_{10} [HIV-1 RNA copy number] at time 0, taken to be the average of the prebaseline and baseline values. For patients with marker data only through week w , where w is less than 24, time-weighted average change in HIV-1 RNA level at week 24 is taken to be $[AUC_w - w \times Y_0]/w$. A total of 550 patients randomly assigned to two treatment groups in a 2-to-1 ratio provided at least 90% power to detect a treatment difference of 0.25 \log_{10} copies/mL in time-weighted average change in HIV-1 RNA level at week

Table 1. Baseline Characteristics of the Study Sample*

Characteristic	Placebo Group (n = 182)	Tenofovir DF Group (n = 368)
Mean age, y	42.0	41.3
Men, n (%)	160 (88)	309 (84)
Race, n (%)		
White	118 (65)	261 (71)
Black	34 (19)	58 (16)
Hispanic	26 (14)	42 (11)
HIV risk factors present, n (%)		
Homosexual contact	130 (71)	252 (69)
Heterosexual contact	51 (28)	97 (26)
Injection drug use	10 (6)	21 (6)
Mean CD4 cell count \pm SD, $\times 10^9$ cells/L	0.447 \pm 0.217	0.418 \pm 0.212
Mean HIV-1 RNA level \pm SD, log ₁₀ copies/mL	3.38 \pm 0.49	3.35 \pm 0.52
Mean length of previous antiretroviral therapy use, y	5.3	5.5
Background antiretroviral therapy, n (%)		
PI + NRTI	87 (48)	159 (43)
NNRTI + NRTI	48 (26)	120 (33)
NRTI only	28 (15)	53 (14)
PI + NNRTI + NRTI	14 (8)	34 (9)

* Baseline resistance mutations for all patients in the virologic substudy (n = 253) were 48% for NNRTI, 58% for PI, and 94% for NRTI. NNRTI = non-nucleoside reverse transcriptase inhibitor; NRTI = nucleoside reverse transcriptase inhibitor; PI = protease inhibitor; tenofovir DF = tenofovir disoproxil fumarate.

24 (Wilcoxon rank-sum test). Comparisons of treatment groups were based on the Wilcoxon rank-sum test, and *P* values less than 0.05 were considered statistically significant. Safety results included summaries of grade 3 or 4 toxicity.

Role of the Funding Source

The funding source supported the collection and analysis of the data. The data were interpreted and the decision to submit the manuscript for publication was made in joint consultation between the funding source and the authors. The funding source placed no restrictions on the interpretation of the data or the content of the manuscript.

RESULTS

Study Sample

Of the 75 sites that were eligible to enroll patients in the study, 72 did and 3 did not. Five hundred fifty-two HIV-1–infected adults were enrolled, and of these, 368 were assigned to the tenofovir DF group and 184 were assigned to the placebo group. Enrollment was relatively balanced among all sites: Each enrolled 5% or fewer of the patients in the study with one exception, which enrolled 7%. All baseline characteristics of the two treatment groups were well matched (Table 1). Four hundred sixty-nine patients were men; mean age was 41.6 years. The mean plasma HIV-1 RNA value at baseline was 3.36 log₁₀ copies/mL (2340 copies/mL), and the mean CD4 cell count at baseline was 0.427 $\times 10^9$ cells/L. The mean duration of previous antiretroviral therapy was 5.4 years. At baseline, 86% of patients had previously taken four or more antiretroviral agents. Baseline HIV-1 genotypic data were ob-

tained from 253 patients. Consistent with the extensive treatment experience of the patients in this trial, baseline genotypic analysis revealed that 94% expressed one or more primary nucleoside reverse transcriptase inhibitor–associated resistance mutations, 58% expressed primary protease inhibitor–associated resistance mutations, and 48% expressed primary non-nucleoside reverse transcriptase inhibitor–associated resistance mutations. Similar proportions of patients were receiving existing antiretroviral therapy containing a protease inhibitor or non-nucleoside reverse transcriptase inhibitor; no single combination regimen occurred in more than 15% of patients.

Accountability

Two of 552 enrolled patients, both in the placebo group, never received the study drug. Therefore, the intention-to-treat sample included 550 patients. Rates of study drug discontinuation were 6% in both groups through week 24. The most common reason for premature discontinuation was an adverse event or intercurrent illness, which occurred at the same rate (3%) in both groups. For patients originally assigned to receive tenofovir DF at baseline, the study drug discontinuation rate did not increase with longer dosing duration during weeks 24 to 48 compared with the rate observed during the first 24 weeks (5% vs. 6%).

Plasma HIV-1 RNA Response

For the primary efficacy end point (time-weighted average change in HIV-1 RNA level from baseline to week 24), levels of HIV-1 RNA changed significantly in the tenofovir DF group compared with the placebo group (−0.61 log₁₀ copies/mL vs. −0.03 log₁₀ copies/mL, respectively [*P* < 0.001]; difference, −0.58 log₁₀ copies/mL [95% CI, −0.68 to −0.49 log₁₀ copies/mL]). Patients who crossed over to tenofovir DF for 24 weeks after receiving placebo demonstrated a time-weighted average change from baseline of −0.60 log₁₀ copies/mL at week 48.

At week 24, the mean change in HIV-1 RNA level was similar to the time-weighted average change from baseline. The tenofovir DF group demonstrated a significant change in HIV-1 RNA level compared with the placebo group (−0.59 log₁₀ copies/mL vs. −0.01 log₁₀ copies/mL, respectively [*P* < 0.001]; difference, −0.58 log₁₀ copies/mL [CI, −0.70 to −0.46 log₁₀ copies/mL]). This antiviral response was sustained through week 48 with a mean change of −0.53 log₁₀ copies/mL. Of note, patients who began taking tenofovir DF at week 24 after receiving placebo demonstrated a mean change in HIV-1 RNA level of −0.64 log₁₀ copies/mL at week 48.

When we used all available data and excluded missing observations, the tenofovir DF group demonstrated a significant difference compared with the placebo group at week 24 in the percentage of patients who achieved HIV-1 RNA levels of 400 copies/mL or less (45% vs. 13% [*P* < 0.001]; difference, 32 percentage points [CI, 24 to 39 percentage points]). Through 48 weeks, 41% of tenofovir

Table 2. Grade 3 or 4 Adverse Events and Laboratory Abnormalities at Week 24 and Week 48*

Variable	Placebo Group through Week 24 (n = 182)	Tenofovir DF Group through Week 24 (n = 368)	Tenofovir DF Group through Week 48 (n = 368)	Placebo to Tenofovir DF Crossover Group, Week 24 to Week 48 (n = 170)
	← n (%) →			
Any grade 3 or 4 adverse event	25 (14)	49 (13)	73 (20)	23 (14)
Type of grade 3 or 4 adverse event				
Diarrhea†	3 (2)	3 (<1)	5 (1)	1 (<1)
Pain‡	1 (<1)	3 (<1)	6 (2)	1 (<1)
Depression‡	1 (<1)	1 (<1)	2 (<1)	3 (2)
Any grade 3 or 4 laboratory abnormality	69 (38)	92 (25)	128 (35)	56 (33)
Type of grade 3 or 4 laboratory abnormality				
Creatine kinase level	26 (14)	24 (7)	44 (12)	21 (12)
Triglyceride level	24 (13)	30 (8)	39 (11)	16 (9)
Serum amylase level	13 (7)	21 (6)	27 (7)	11 (7)
Urine glucose level	6 (3)	11 (3)	12 (3)	4 (2)
Aspartate aminotransferase level	5 (3)	10 (3)	15 (4)	9 (5)
Hyperglycemia	8 (4)	7 (2)	10 (3)	5 (3)
Alanine aminotransferase level	3 (2)	8 (2)	12 (3)	12 (7)

* Occurring in $\geq 2\%$ of patients in any group. Tenofovir DF = tenofovir disoproxil fumarate.

† Grade 3 or 4 diarrhea = bloody diarrhea, diarrhea requiring intravenous therapy, ≥ 7 loose stools/d, diarrhea with orthostatic hypotension, hospitalization required, or diarrhea with hypotensive shock.

‡ Grade 3 pain or depression = marked limitation in activity, some assistance usually required; medical intervention or therapy required, hospitalization possible. Grade 4 pain or depression = extreme limitation in activity, significant assistance required; significant medical intervention/therapy required, hospitalization or hospice care probable.

DF-treated patients had HIV-1 RNA levels of 400 copies/mL or less. Similarly, at week 24, the tenofovir DF group differed significantly from the placebo group in the percentage of patients who achieved HIV-1 RNA levels of 50 copies/mL or less (22% vs. 1% [$P < 0.001$]; difference, 21 percentage points [CI, 16 to 25 percentage points]). Through 48 weeks, 18% of tenofovir DF-treated patients had HIV-1 RNA levels of 50 copies/mL or less. Among patients who crossed over from placebo to tenofovir DF at week 24, 44% and 23% had HIV-1 RNA levels of 400 copies/mL or less and 50 copies/mL or less, respectively. Of importance, the proportion of tenofovir DF-treated patients with HIV-1 RNA levels of 50 copies/mL or less at week 24 differed when stratified by baseline HIV-1 RNA level (43% for those with initial HIV-1 RNA levels < 1000 copies/mL, 27% for those with levels from 1001 to 2500 copies/mL, 11% for those with levels from 2501 to 5000 copies/mL, and 6% for those with levels > 5000 copies/mL).

Background antiretroviral therapy was changed in 11% of patients in both the tenofovir DF and placebo groups through week 24. Thus, the efficacy analyses for the as-treated sample were consistent with those of the intention-to-treat sample.

CD4 Cell Count Response

Time-weighted average changes in CD4 cell count were limited but were significantly higher for the tenofovir DF group than for the placebo group. Mean changes were 0.013×10^9 cells/L and -0.011×10^9 cells/L, respectively ($P < 0.001$; difference, 0.024×10^9 cells/L [CI, 0.009 to 0.038×10^9 cells/L]). The time-weighted average change in

CD4 cell count remained stable through 48 weeks of tenofovir DF treatment (mean change, 0.013×10^9 cells/L). For patients who crossed over from placebo to tenofovir DF at week 24, the time-weighted average change in CD4 cell count was 0.007×10^9 cells/L after 24 weeks of taking the active drug. Through week 24, the tenofovir DF-treated patients who achieved a plasma HIV-1 RNA level less than 50 copies/mL had a significantly greater mean increase in CD4 cell count from baseline compared with those in the placebo group (0.057×10^9 cells/L vs. -0.001×10^9 cells/L [$P < 0.001$]; difference, 0.058×10^9 cells/L [CI, 0.027 to 0.089×10^9 cells/L]).

Adverse Event Profile

Through 24 weeks, the incidence of grade 3 or 4 clinical adverse events was similar in both the tenofovir DF and placebo groups (13% vs. 14%). For events reported in at least 2% of patients, none occurred at a higher frequency in the tenofovir DF group than in the placebo group (Table 2). Through 48 weeks of tenofovir DF dosing, the clinical adverse event profile did not change.

Through 24 weeks, the incidence of grade 3 or 4 laboratory abnormalities was greater for the placebo group (38%) than for the tenofovir DF group (25%). For laboratory abnormalities reported in at least 2% of patients in either group, none occurred at a higher frequency in the tenofovir DF group than in the placebo group (Table 2). Through 48 weeks of tenofovir DF dosing, the overall incidence (35%) and profile of grade 3 or 4 laboratory abnormalities were similar to those in the placebo group through 24 weeks. The incidences of elevated serum creatinine concentrations ($> 177 \mu\text{mol/L}$ [$> 2.0 \text{ mg/dL}$]) and

Table 3. HIV RNA Responses according to Baseline Resistance Mutations (Genotyping Substudy, Intention-To-Treat)*

Baseline Mutations	Time-Weighted Average Change in HIV-1 RNA Level from Baseline to Week 24		Time-Weighted Average Change in HIV-1 RNA Level from Baseline to Week 48†	
	Placebo Group	Tenofovir DF Group	Placebo to Tenofovir DF Crossover Group	Tenofovir DF Group
	← \log_{10} copies/mL (n) →			
All patients	-0.03 (84)	-0.59 (168)	-0.70 (80)	-0.56 (166)
M184V	-0.05 (54)	-0.68 (117)	-0.81 (51)	-0.64 (117)
M184V/no TAMs‡	-0.16 (16)	-0.97 (42)	-1.23 (16)	-0.90 (42)
No TAMs‡	-0.18 (23)	-0.85 (54)	-1.06 (22)	-0.79 (54)
TAMs‡	0.03 (61)	-0.47 (114)	-0.57 (58)	-0.45 (114)
TAMs + no M184V‡	0.09 (23)	-0.39 (39)	-0.49 (23)	-0.35 (39)
TAMs + M184V‡	-0.01 (38)	-0.51 (75)	-0.62 (35)	-0.50 (75)
Non-nucleoside reverse transcriptase inhibitor resistance§	0.02 (44)	-0.49 (77)	-0.64 (42)	-0.47 (77)
Protease inhibitor resistance	-0.00 (52)	-0.55 (96)	-0.75 (50)	-0.54 (96)

* Numbers in parentheses are numbers of patients. $P < 0.001$ (Wilcoxon rank-sum test) for tenofovir DF compared with placebo in the same mutation group. TAM = thymidine analogue mutation; tenofovir DF = tenofovir disoproxil fumarate.

† From baseline to week 48 for the tenofovir DF group and from week 24 to week 48 for the placebo to tenofovir DF crossover group.

‡ Thymidine analogue mutations are M41L, D67N, K70R, L210W, T215Y/F, or K219Q/E/N in reverse transcriptase.

§ Non-nucleoside reverse transcriptase inhibitor resistance mutations are K103N, V106A, V108I, Y181C/I, Y188C/L/H, G190A/S/E, or P236L in reverse transcriptase.

|| Protease inhibitor resistance mutations are D30N, V32I, G48V, I50V, V82A/F/T/S, I84V, or L90M in protease.

hypophosphatemia (phosphorus level < 0.48 mmol/L [< 1.5 mg/dL]) were similar in the tenofovir DF and placebo groups through 24 weeks and did not change through 48 weeks (0% and $< 1\%$). No patient withdrew from the study because of abnormalities in serum creatinine concentration or phosphorus level.

Virologic Substudy

Baseline genotypic results were obtained from 253 patients randomly assigned to a virologic substudy. Nearly all patients (94%) expressed nucleoside-associated mutations, while 69% and 68% of plasma viral isolates expressed thymidine analogue-associated mutations (M41L, D67N, K70R, L210W, T215Y/F, or K219Q/E/N; mean, 2.8 mutations per patient) or lamivudine-associated resistance mutations (M184V/I), respectively. As determined by the primary efficacy end point, patients in the tenofovir DF group who expressed these common nucleoside reverse transcriptase inhibitor mutation patterns, combined patterns, non-nucleoside reverse transcriptase inhibitor-associated mutations, or protease inhibitor-associated mutations achieved significant reductions in the time-weighted average change in HIV-1 RNA level (Table 3).

The K65R mutation, selected by tenofovir in vitro, developed in five tenofovir DF-treated patients by week 24 and in an additional three patients by week 48 (3% of substudy patients). Didanosine and abacavir can also select the K65R mutation. Among the eight patients who developed the K65R mutation, three were concomitantly taking abacavir along with lamivudine ($n = 2$) or didanosine ($n = 1$) and the other five were taking either zidovudine ($n = 3$) or stavudine ($n = 2$) along with lamivudine. The development of the K65R mutation was associated with a variable treatment response to tenofovir DF (mean time-weighted average change in HIV-1 RNA level at week 24, -0.29 \log_{10} copies/mL [range, -1.10 to 0.72 \log_{10} copies/mL]).

By week 24, patients in the tenofovir DF group and in the placebo group developed primary nucleoside-associated resistance mutations (16% vs. 24%; $P = 0.17$), non-nucleoside-associated resistance mutations (5% vs. 10%; $P > 0.2$), and protease inhibitor-associated resistance mutations (2% vs. 8%; $P = 0.02$). These findings were consistent with effective reduction of viral replication in the tenofovir DF group.

DISCUSSION

In this study, we compared tenofovir DF with placebo when either was added to existing antiretroviral therapy in patients with stable HIV-1 RNA levels between 400 and 10 000 copies/mL. This design allowed us to rigorously assess the efficacy and safety profiles of tenofovir DF in treatment-experienced patients who had low but stable levels of viral replication despite ongoing treatment. Patients with HIV-1 RNA levels greater than 10 000 copies/mL were excluded from the study. The 6-month use of a placebo control is not consistent with current clinical practice in patients with an unstable, failing antiretroviral regimen and would put them at undue risk for rapid development of additional HIV-1 resistance mutations and probable clinical progression of HIV disease.

We found statistically significant reductions at week 24 in the time-weighted average change in HIV-1 RNA level, although nearly all patients had nucleoside resistance mutations at baseline. For several reasons, we defined this metric—time-weighted average change in HIV-1 RNA level at week 24—a priori as the single primary efficacy end point in both the protocol and statistical analysis plan. A previous study demonstrated that the percentage decrease in the rate of disease progression has a log-linear relationship to change in HIV-1 RNA levels (25). In our

study sample, in which the mean length of previous antiretroviral treatment was 5.4 years and the prevalence of nucleoside resistance mutations was high, the antiviral effect of a single additional agent was expected to be limited. Thus, using a metric of change from baseline was expected to be more sensitive than selecting a percentage below the limit of detection, since the former measure allowed all patients to contribute to the primary end point and power of the study.

A major goal of antiretroviral therapy is the effective suppression of HIV-1 RNA levels to below the limits of detection; the consequent increase in CD4 cell counts then supports effective cellular immunity (6). As a secondary end point, the proportion of patients with viral loads of 50 copies/mL or less (below the limit of detection) was significantly greater in the tenofovir DF group than in the placebo group (22% vs. 1%). Patients who had lower baseline HIV-1 RNA levels at study entry more often had viral loads less than or equal to 50 copies/mL at follow-up. The increase in CD4 cell count was significantly greater in these patients than in the overall study sample. The modest overall increase in CD4 cell count seen in our study is consistent with the results of similarly designed trials of abacavir and tenofovir DF (16, 26) and with the low proportion of patients who achieved HIV-1 RNA levels less than or equal to 50 copies/mL.

The resistance analyses demonstrated that tenofovir DF has significant activity against common lamivudine and thymidine analogue resistance mutations. Our trial confirms tenofovir DF's antiviral activity against HIV-containing nucleoside-associated resistance mutations, which was previously observed in a phase II study (16, 21). Given the low percentage of patients who achieved undetectable HIV-1 RNA levels in both the tenofovir DF and placebo groups, resistance mutations continued to develop. After 24 weeks, there was a trend toward development of fewer additional nucleoside resistance mutations in the tenofovir DF group compared with the placebo-treated group, but it did not achieve statistical significance. Despite ongoing viral replication in nearly 80% of patients receiving tenofovir DF, the K65R mutation, selected by tenofovir *in vitro*, was seen in only 3% of patients through week 48.

The discontinuation rates and incidences of grade 3 and 4 clinical adverse events and laboratory abnormalities were similar in the tenofovir DF and placebo groups through week 24. With extended observation through week 48, the safety profile of tenofovir DF appeared unchanged and, in fact, no clinical adverse events or laboratory abnormalities could be associated with the drug. Animal studies using high-dose intravenous tenofovir led to nephrotoxicity. In our study, we found no evidence of drug-related nephrotoxicity with close monitoring of serum and urine biomarkers. Follow-up beyond 48 weeks continues in an open-label safety study.

We designed and initiated our trial before genotypic and phenotypic resistance testing were widely available and

before their widespread use. Thus, the present-day clinical applicability of adding a single agent to a stable but failing existing antiretroviral regimen may be limited to levels of viral replication at which resistance testing is not feasible. In this study, patients with HIV-1 RNA levels less than 1000 copies/mL benefited most in CD4 cell count change and in decrease of viral loads to less than or equal to 50 copies/mL. Tenofovir DF is one of the first antiretroviral agents to be administered as a single tablet once daily. This simple dosing regimen is likely to increase adherence and can easily be incorporated in directly observed therapy. Increased adherence to antiretroviral therapy has been shown to lead to superior viral and immunologic treatment outcomes (27, 28) and is likely to decrease the frequency of resistance to antiretroviral agents.

The primary goals of antiretroviral therapy are to maintain an HIV-1 RNA level below the limit of detection, to increase or stabilize CD4 cell counts, and to delay clinical progression of HIV-1 disease (6). However, it becomes increasingly difficult to achieve these goals in antiretroviral-experienced HIV-1-infected patients because of the emergence of drug resistance and the administration of complex regimens requiring large pill burdens that are difficult to tolerate. The durable antiviral activity of tenofovir DF, characterized by potent efficacy against nucleoside reverse transcriptase mutations, infrequent emergence of resistance, a convenient dosing schedule, and placebo-like tolerability, will enhance combination antiretroviral regimens for HIV-1-infected patients.

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Presented in part at the 6th International Congress on AIDS in Asia and the Pacific, Melbourne, Australia, 5–10 October 2001; the 8th European Conference on Clinical Aspects and Treatment of HIV Infection, European AIDS Clinical Society, Athens, Greece, 28–31 October 2001; the 41st Interscience Conference on Antimicrobial Agents and Chemotherapy, American Society for Microbiology, Chicago, Illinois, 16–19 December 2001; the 9th Conference on Retroviruses and Opportunistic Infections, Foundation for Retrovirology and Human Health, Seattle, Washington, 24–28 February 2002; and the 14th International AIDS Conference, International AIDS Society, Barcelona, Spain, 7–12 July 2002.

Acknowledgments: The authors gratefully acknowledge the contributions of all the study site personnel and patients who participated in this study.

Grant Support: By Gilead Sciences, Foster City, California.

Potential Financial Conflicts of Interest: *Employment:* M. Wulfsohn (Gilead Sciences), M.D. Miller (Gilead Sciences), J.J. Toole (Gilead Sciences), D.F. Coakley (Gilead Sciences), A. Cheng (Gilead Sciences);

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