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Effects of Tenofovir Disoproxil Fumarate in Hepatitis B e Antigen-Positive Patients With Normal Levels of Alanine Aminotransferase and High Levels of Hepatitis B Virus DNA

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BACKGROUND & AIMS: Little is known about the benefit of antiviral therapy for hepatitis B e antigen (HBeAg)−positive patients with high viral load and normal levels of alanine aminotransferase. We evaluated the effects of single and combination therapies in immune-tolerant patients with chronic hepatitis B. **METHODS:** In a double-blind study, nucleos(t)ide-naïve patients with high levels of hepatitis B virus (HBV) DNA who were positive for HBeAg and had normal levels of alanine aminotransferase were randomly assigned to groups given either oral tenofovir disoproxil fumarate (TDF, 300 mg) and placebo (n = 64) or a combination of TDF (300 mg) and emtricitabine (200 mg, n = 62) for 192 weeks. The primary end point was proportion of patients with serum levels of HBV DNA <69 IU/mL at week 192. **RESULTS:** The study population (mean age was 33 years; 89% were Asian) was predominantly infected with HBV genotypes B and C (93%), 99% were HBeAg positive with a mean baseline level of HBV DNA of 8.41 $\log_{10}$ IU/mL. At week 192, 55% of patients (35 of 64) in the TDF+placebo group and 76% of patients (47 of 62) in the TDF+emtricitabine group had levels of HBV DNA <69 IU/mL ($P = .016$). No patients were found to have viral resistance to therapy. HBeAg seroconversion occurred in 3 patients (5%), all in the TDF+placebo group; no patient had loss of hepatitis B surface antigen. In multivariate analysis, female sex (odds ratio = 7.05; $P = .002$) and TDF+emtricitabine treatment (odds ratio = 3.9; $P = .01$) were associated with a favorable response. Both regimens were well tolerated. **CONCLUSIONS:** In HBeAg-positive patients with chronic HBV infection, high viral loads, normal levels of alanine aminotransferase, and therapy with the combination of TDF and emtricitabine provided better viral suppression than TDF alone, although rates of HBeAg seroconversion and hepatitis B surface antigen loss were low.

Keywords: Immune Tolerant; Combination Therapy; FTC; HBsAg.

Infection with hepatitis B virus (HBV) is a global concern, with approximately 350 million people infected chronically worldwide.[1] Although >95% of newly infected adults do not progress beyond self-limited acute infection, the majority of neonates and children who are exposed to the virus develop chronic infection. The earliest stage of chronic infection in these patients is characterized by very high levels of viral replication, the presence of HBV e antigen (HBeAg) in the serum, and little or no elevation in liver transaminases.[1,2] This is often referred to as the immune-tolerant phase, which typically lasts for 20−30 years.[3] Most patients do not have significant disease activity or disease progression during the immune-tolerant phase.[4] After loss of immune tolerance, serum liver enzymes frequently rise and there might be necroinflammatory activity on liver histology.[5,6]

The goal of chronic hepatitis B (CHB) treatment is to reduce viral replication, subsequent liver inflammation and fibrosis, and risk of developing cirrhosis and hepatocellular carcinoma (HCC). Treatment guidelines use the presence of active liver inflammation on histology or elevated serum levels of liver transaminases as the criteria for initiating treatment.[7–9] However, for immune-tolerant patients, alanine aminotransferase (ALT) might not be a reliable marker of disease activity, and some patients might have intermittently elevated ALT that can escape typical clinical surveillance. Therefore, although no formal treatment recommendations exist for immune-tolerant patients, liver biopsy should be considered regardless of ALT levels if there is a family history of liver cancer or cirrhosis, or if the patient is older than 30 years of age, because the risk for liver cancer

Abbreviations used in this paper: **ALT, alanine aminotransferase; CHB, chronic hepatitis B; FTC, emtricitabine; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; pol/RT, polymerase/reverse transcriptase; TDF, tenofovir disoproxyl fumarate; ULN, upper limit of normal.**

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and cirrhosis increases at that time.[7] In a histologic series of 73 HBeAg-positive patients with persistently normal ALT levels, 40% of patients demonstrated significant liver fibrosis on liver biopsy.[10] In another series using Fibroscan among 243 HBeAg-positive patients older than 35 years of age with normal ALT, 35% had liver stiffness suggestive of advanced liver fibrosis.[11] Therefore, disease activity in immune-tolerant patients might be under-represented by assessing HBV DNA and ALT levels alone.[10,12]

Entecavir and tenofovir are effective treatment options for chronic hepatitis B. More than two thirds of treatment-naïve, HBeAg-positive patients treated with tenofovir have HBV DNA suppression at the end of 1 year, and the effectiveness of the suppression tends to improve with time.[13,14] Similar rates of HBV DNA suppression in treatment-naïve HBeAg-positive CHB patients have been seen after 1 year of treatment with entecavir.[15] In a case series of 160 HBeAg-positive patients receiving entecavir therapy, among those with detectable HBV DNA after 1 year of therapy, only 46% had undetectable HBV DNA at the end of 3 years.[16] Recent data have underscored the importance of complete viral suppression, in particular among cirrhotic patients; patients with incomplete viral suppression after entecavir therapy were found to have a higher risk of liver-related complications and HCC.[17,18] Elevated DNA levels also have been shown to independently predict HCC incidence in a large cohort of Taiwanese CHB patients, although most of these patients were not in the immune-tolerant phase of disease.[3,19]

Previous studies on combination therapy have failed to show improved viral suppression as compared with monotherapy.[20,21] In a recent study examining the efficacy of entecavir with or without tenofovir in the treatment of CHB, combination therapy had efficacy similar to monotherapy overall, but was possibly more efficacious in patients with high viral load. It should be noted that this study lacked a tenofovir monotherapy arm.[22]

In this study, we evaluated treatment with either tenofovir disoproxil fumarate (TDF) and placebo or a combination of TDF and emtricitabine (FTC) for the treatment of patients with CHB, high viral load, and normal liver aminotransferase levels to determine the role of antiviral therapy in the treatment of these patients. We addressed the efficacy of these regimens in achieving virologic and serologic end points, the safety of the treatments, and the rates of viral resistance to treatment during a 192-week study period.

Methods

Study Design

This was a phase 2, randomized, double-blind, multicenter study in nucleos(t)ide-naïve patients with high HBV viral load (HBV DNA $\geq 1.7 \times 10^7$ IU/mL) and ALT levels less than the upper limit of normal (ULN). Patients were randomized in a 1:1 ratio to receive TDF 300 mg orally once daily plus FTC 200 mg once daily, or TDF 300 mg once daily plus placebo once daily for 192 weeks. The initial protocol was for a 48-week study. The protocol was subsequently modified to extend treatment to 96 weeks, and then eventually to 192 weeks, in order to better evaluate the long-term response to treatment in this population. All patients who discontinued study drug on or before week 192 were followed for 24 weeks off treatment or until initiation of alternative HBV therapy, whichever occurred first. When the last subject reached week 192, the sponsor provided open-label TDF for study patients in countries where the drug was not commercially available and treatment of these patients was at the discretion of the investigator.

Patients

All patients enrolled were HBV treatment naïve. Major inclusion criteria included age 18 to 69 years, serum HBV DNA $>1.7 \times 10^7$ IU/mL, serum ALT less than or equal to the ULN (ULN: 43 U/L for men and 34 U/L for women), hepatitis B surface antigen (HBsAg) positive, with a calculated creatinine clearance $\geq$70 mL/min. Major exclusion criteria included decompensated liver disease, history of HCC, and co-infection with hepatitis C virus, hepatitis D virus, or human immunodeficiency virus. All patients provided written consent, and the protocol was approved by the Institutional Review Boards of each participating institution.

Virologic Response

The primary efficacy end point was the proportion of patients achieving HBV DNA <69 IU/mL at week 192. This level was chosen because it concurred with the primary end point of the pivotal studies of TDF.[13] HBV DNA was measured using the Roche COBAS TaqMan (Roche Diagnostics, West Sussex, UK) assay (lower limit of quantification 29 IU/mL). HBsAg levels were determined using the Abbott Architect Assay (Abbott Laboratories, Abbott Park, IL).

Virologic Resistance

Population sequencing of the HBV polymerase/reverse transcriptase (pol/RT) at baseline and for viremic patients with HBV DNA >172 IU/mL at weeks 48, 96, 144, and 192 or last visit, was used to assess evidence of virologic resistance. The 1032-bp pol/RT domain was sequenced and compared with baseline sequences by methods reported previously.[23] Phenotyping was done for viremic patients with an amino acid substitution at a conserved site of the HBV pol/RT. Phenotypic analysis was done using an in vitro system of HBV replication using the pol/RT sequence from the patient in the presence of TDF for 50% effective concentration calculations; a 2.5-fold or greater increase in 50% effective concentration was considered as TDF resistance, as described previously.[23]

Safety Analyses

Adverse events, serious adverse events, laboratory abnormalities, study drug discontinuation due to adverse events, and deaths were evaluated for the entire study period. Hepatic flares were defined as either serum ALT >2× the baseline and >10× ULN, a confirmed ALT elevation with

other confirmed laboratory findings suggestive of hepatic decompensation (eg, total bilirubin ≥34.2 umol/L above baseline, prothrombin time ≥2 seconds or international normalized ratio ≥0.5 above baseline, or serum albumin ≥10 g/L below baseline), or any clinical manifestation of hepatic decompensation.

Statistical Analysis

The primary end point was the proportion of patients with virologic suppression, defined as HBV DNA <69 IU/mL. Patients who discontinued study medication before week 192 were considered treatment failures. A sample size of 65 patients per group provided 85% power to detect a relative difference in response rates of HBV DNA suppression at week 192 of 30% on the basis of a 2-sided significance level of .05. Treatment group differences were evaluated using a 2-sided Fisher's exact test. Secondary end points were summarized by visit using descriptive statistics. For continuous secondary end points, treatment group differences were evaluated using a 2-sided Wilcoxon rank-sum test. For categorical secondary end points, treatment group differences were evaluated using a 2-sided Fisher's exact test. In a post-hoc analysis examining predictors of treatment response, univariate logistic regression analysis was performed on a predetermined set of predictor variables. A multivariate logistic regression analysis using a forward selection model with a significance level of .05 was used to identify independent significant predictors of treatment response.

Results

Patient Population

Of the 129 patients randomized at 34 sites in 11 countries, 126 received treatment with either TDF+placebo (n = 64) or TDF+FTC (n = 62) (Supplementary Figure 1). The baseline demographics and disease characteristics were similar between treatment groups (Table 1). The patients had a mean age of 33 years and were predominantly Asian (88% and 90% in the TDF and TDF+FTC groups, respectively) with approximately equal numbers of men and women (48% of patients in the TDF arm and 50% of patients in the TDF+FTC arm were male). Almost all were positive for HBeAg (99%) and were infected predominantly with HBV genotype B and C (89% in TDF+placebo and 97% in TDF+FTC). Mean baseline HBV DNA level was 8.41 $\log_{10}$ IU/mL. Mean ALT at entry to the study was below the ULN and comparable in both groups.

Virologic Response

Both regimens showed potent antiviral activity with a similar change in HBV DNA levels by week 4 of treatment (−3.16 $\log_{10}$ IU/mL for TDF+placebo and −2.99 $\log_{10}$ IU/mL for TDF+FTC; $P = .25$), as well as at week 192 of treatment (−6.32 $\log_{10}$ IU/mL for TDF+placebo and −6.70 $\log_{10}$ IU/mL for TDF+FTC; $P = .07$) (Figure 1).

The proportion of patients who achieved the primary end point of HBV DNA <69 IU/mL at week 192 were greater among patients receiving TDF+FTC than among those receiving TDF alone (76% vs 55%; $P = .011$, Table 2 and Figure 2). A per-protocol analysis also demonstrated that more patients receiving TDF+FTC had HBV DNA <69 IU/mL throughout the study than those receiving TDF alone (Table 2 and Supplementary Figure 2). In general, most patients who achieved viral suppression at week 192 had HBV DNA <69 IU/mL within the first year of therapy (Supplementary Tables 1 and 2). Of those who did not meet the primary end point, the majority had low levels of ongoing HBV replication, with 6 of 7 patients in the TDF+FTC arm and 12 of 18 in the TDF+placebo having HBV DNA levels <500 IU/mL (Supplementary Figure 3 and Supplementary Table 3). A multivariate regression analysis was performed to identify factors associated with achieving the primary end point at week 192 of treatment. Univariate analysis using either 2-sided Fisher's exact test or 2-sided Wilcoxon rank-sum test; of those with week 192, HBV DNA values showed that achieving the primary end point was associated with female sex, lower baseline HBV DNA levels, treatment with TDF+FTC, lower body mass index, and lower baseline HBsAg levels (Table 3). In a multivariate regression analysis using a forward selection model, only female sex and TDF+FTC treatment remained significantly associated with a favorable treatment response (Table 4). These results were similar when using HBV DNA <29 IU/mL as a primary outcome (Supplementary Tables 4 and 5). In addition, when using a proportional hazards model to examine the relationship between time to viral suppression and the covariates, in addition to treatment arm and sex, a low baseline HBsAg level also had a statistically significant effect on time to achieving HBV DNA suppression (Supplementary Table 6). Viral suppression was numerically greater for patients who had baseline HBV DNA levels <9 $\log_{10}$ HBV DNA (IU/mL) for both treatment groups (Supplementary Table 7).

Resistance and Virologic Breakthrough

During the 192-week treatment period, a total of 69 patients, with serum samples from 145 time points genotyped for resistance testing (Supplementary Table 8). Of the 122 available paired baseline and post-baseline sequences analyzed, 86 (70%) had no changes in the HBV pol/RT gene from baseline. Conserved-site changes with or without polymorphic-site changes were observed in 11 of 122 (9%) sequences compared with baseline, 6 from the TDF+placebo group and 5 from the TDF+FTC group. Two common conserved-site changes were observed: rtL29F/L in 2 patients in the TDF+placebo group and rtP170P/S in 2 patients in the FTC+TDF group. None of the patients with these changes experienced virologic breakthrough. Unique polymorphic site changes were observed in 25 of 122 sequences (20%), 16 from the TDF+placebo and 9 from the TDF+FTC group. Patients with unique or common (observed in more than 1 patient) conserved-site changes qualified for phenotypic analysis. Of the 6 patients that qualified for phenotypic analysis, none of the conserved-site mutations analyzed conferred resistance to TDF.

Seventeen patients in the TDF arm, including 1 who discontinued medication prematurely, experienced virologic

Table 1. Baseline Demographics and Disease Characteristics

Demographics and disease characterstics	TDF 300 mg + placebo (n = 64)	TDF 300 mg + FTC 200 mg (n = 62)	*P* value
Age, *y*			
Mean (SD)	33 (9.5)	33 (11.2)	.86
Range	18–62	18–58	
Male, n (%)	31 (48.4)	31 (50)	1.00
Race, n (%)			.46
Asian	56 (87.5)	56 (90.3)	
White	4 (6.3)	1 (1.6)	
Black	2 (3.1)	2 (3.2)	
Pacific Islander	1 (1.6)	3 (4.8)	
Other	1 (1.6)	0 (0)	
Region, n (%)			.36
Asia Pacific	37 (57.8)	43 (69.4)	
North America	18 (28.1)	11 (17.7)	
Europe	9 (14.1)	8 (12.9)	
Baseline body mass index			
Mean (SD)	23.5 (3.93)	23.1 (3.71)	.56
Range	17.8–35.4	17.6–33.7	
HBV DNA, log_{10} *IU/mL*			
Mean (SD)	8.42 (0.402)	8.40 (0.395)	.88
Range	8.02–10.22	7.79–9.87	
HBV genotype, n (%)			.63
A	1 (1.6)	0 (0)	
B	33 (51.6)	32 (51.6)	
C	24 (37.5)	28 (45.2)	
C/D	1 (1.6)	0 (0)	
D	2 (3.1)	0 (0)	
E	2 (3.1)	2 (3.2)	
Missing	1 (1.6)	0 (0)	
HBeAg status, n (%)			1.00
HBeAg positive	63 (98.4)	62 (100)	
HBeAg negative	1 (1.6)	0 (00)	
Baseline HBsAg, log_{10} *IU/mL*, mean (SD)	4.72 (0.43)	4.77 (0.37)	.59
Years positive for HBV			
Mean (SD)	9.8 (7.64)	8.3 (7.56)	.22
Range	0.7–29.3	0.3–28.3	
Baseline ALT, *U/L*, mean (SD)	26.9 (14.05)	26.2 (9.88)	.88
Normal ALT, n (%)	60 (94)	56 (90)	.53
Serum creatinine, *mg/dL*			
Mean (SD)	0.80 (.17)	0.81 (0.21)	.72
Range	0.5–1.1	0.5–1.3	
Glomerular filtration rate, *mL/min*			
Mean (SD)	113 (25)	109 (22)	.47
Range	79–213	72–160	
Serum phosphate, *mg/dL*			
Mean (SD)	3.52 (0.50)	3.62 (0.46)	.40
Range	2.20–4.60	2.80–4.40	

breakthrough. Six patients, including the patient who discontinued treatment prematurely, had genotypic changes from baseline outside of conserved sites. Of those 6 patients, the breakthroughs were transient and small in magnitude during 4 years of treatment with subsequent decline in HBV DNA with continued treatment. Another patient with viral breakthrough at the week 192 visit had nonadherence to study drug.

Seven patients in the FTC/TDF arm experienced virologic breakthrough, including 3 who discontinued treatment early. Of the 4 subjects who completed the study, 1 had a conserved-site change and suppressed at week 144 through 192 without changes in treatment regimen. The remaining episodes were associated with documented nonadherence or early discontinuation.

Serologic Response

There were 4 patients in the TDF+placebo arm (6%) and 1 subject in the TDF+FTC arm (2%) that had HBeAg loss during the 4-year study period, and of these 5 patients, 3 had HBeAg seroconversion, all in the TDF+placebo arm (Table 2). HBeAg loss (with seroconversion) occurred at week 72 (2 patients) and at week 144. There were no

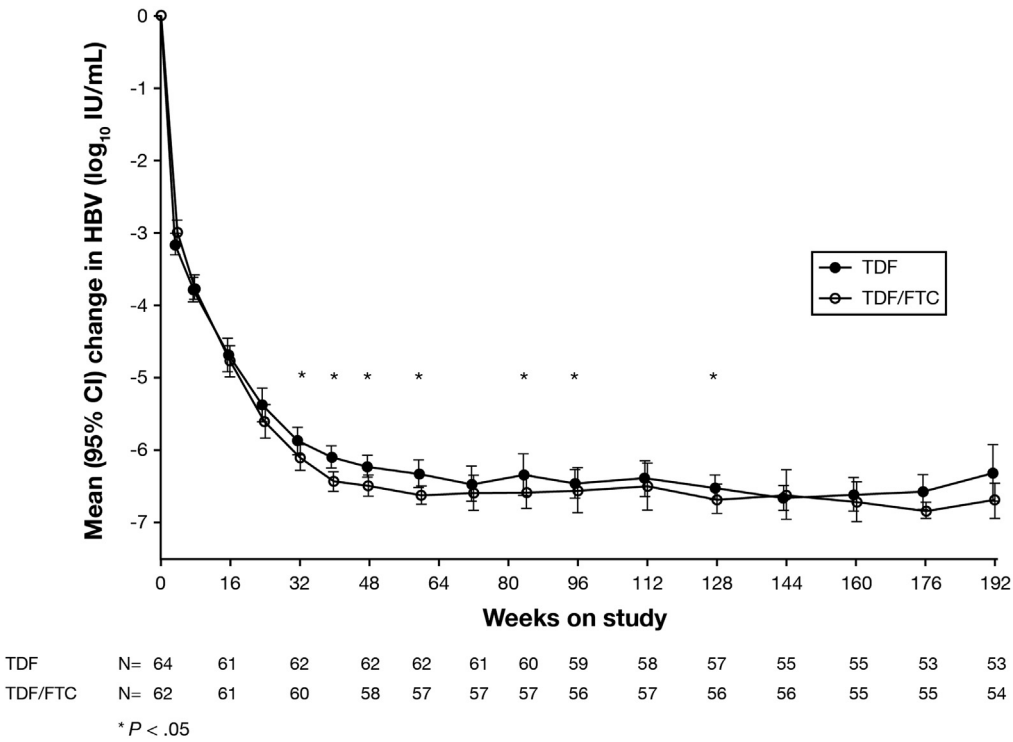

Figure 1. Mean reduction in HBV DNA over time.

occurrences of HBsAg loss or seroconversion in either group during the 192-week study period.

Quantitative HBsAg levels were determined for patients throughout the study period. Mean baseline HBsAg levels were 4.72 $\log_{10}$ IU/mL in the TDF arm and 4.77 $\log_{10}$ IU/mL in the TDF+FTC arm. Only 4 patients in the TDF arm (3%) and no patients in the TDF+FTC arm achieved a >0.5 $\log_{10}$ IU/mL reduction in HBsAg titer by week 24. However, by week 192, 61% of patients remaining in the study, for whom HBsAg data were available at baseline and at week 192 (30 of 49 in the TDF arm and 30 of 50 in the TDF+FTC arm), and had achieved a >0.5 $\log_{10}$ IU/mL reduction in HBsAg titer. The proportion of patients with >0.5 $\log_{10}$ IU/mL decline from baseline increased notably after week 48 (Supplementary Figure 4). A model using the slope of HBsAg decline between week 144 and week 192 suggested that HBsAg levels would reach undetectable levels 26 years from drug initiation in the TDF+placebo arm and 33 years from drug initiation in the TDF+FTC arm (Supplementary Figure 5).

Table 2. Virologic, Biochemical, and Serologic Responses at Week 192

Virologic, biochemical, and serologic responses	TDF 300 mg+placebo (n = 64)	TDF 300 mg+FTC 200 mg (n = 62)	*P* value
Intention to treat analysis			
HBV DNA change from baseline, *log 10 IU/mL*, mean (SD)	−6.32 (1.463)	−6.70 (0.913)	.070
HBV DNA <69 IU/mL , n/N (%)	35/64 (54.7)	47/62 (75.8)	.016
HBV DNA <29 IU/mL , n/N (%)	29/64 (45.3)	43/62 (69.4)	.007
Normal ALT, n/N (%)	41/64 (64.1)	44/62 (71.0)	.451
HBeAg loss, n/N (%)	4/63 (6.3)	1/62 (1.6)	.365
HBeAg seroconversion , n/N (%)	3/63 (4.8)	0/62 (0)	.244
Per-protocol analysis			
HBV DNA change from baseline, *log 10 IU/mL*, mean(SD)	−6.32 (1.463)	−6.70 (0.913)	.070
HBV DNA <69 IU/mL , n/N (%)	35/53 (66.0)	47/54 (87.0)	.012
HBV DNA <29 IU/mL , n/N (%)	29/53 (54.7)	43/54 (79.6)	.008
Normal ALT, n/N (%)	41/53 (77.4)	44/54 (81.5)	.639
ALT U/L, median (range)	29 (7-104)	24.5 (10-100)	.515
ALT U/L, week 192 change from baseline, median (range)	4 (−98 to 58)	2.5 (−30 to 66)	.354
HBeAg loss, n/N (%)	4/52 (7.7)	1/54 (1.9)	.201
HBeAg seroconversion, n/N (%)	3/52 (5.8)	0/54 (0)	.115
HBsAg <2000 IUl/mL, n/N (%)	5/49 (10.2)	5/52 (9.6)	.921

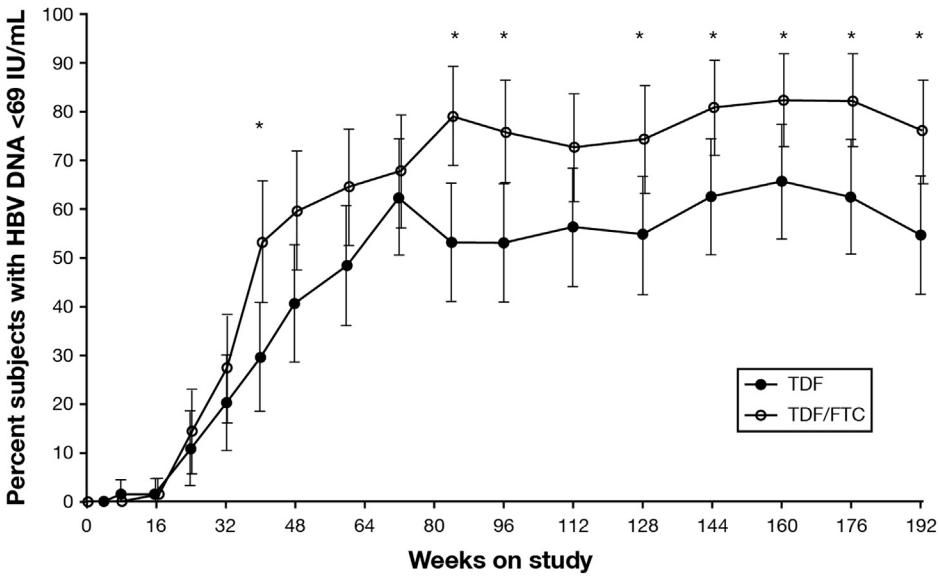

Figure 2. Proportion of patients with HBV DNA <69 IU/mL over time.

Safety

The majority of patients completed 192 weeks of therapy (TDF+placebo, 83%; TDF+FTC, 87%). Overall, 2 patients in the TDF+FTC group (3%) and 1 patient in the TDF+placebo group discontinued the study due to adverse events (Table 5). The adverse events leading to treatment discontinuation were depression (TDF+FTC), spontaneous abortion (TDF+FTC), and homicide (TDF alone). The overall safety profiles were similar between the treatment groups. There was 1 death in the TDF group that was listed as a homicide and unrelated to the study. No patients in either treatment group had evidence of renal function decline throughout the study period, as measured by either an increase in serum creatinine of >0.5 mg/dL or a decrease in creatinine clearance to <50 mL/min, although 19% of patients overall had at least a 20% decline in glomerular filtration rate at some point within the study period. Only 1 patient in the TDF+placebo group had a confirmed serum phosphorus of <2 mg/mL, which occurred at weeks 16 and 24 and resolved by the end of the study with no change in dosing of study drug. There were minimal overall effects on serum phosphate levels otherwise (Supplementary Table 9).

Hepatic flares were seen in only 1 patient in the TDF+placebo arm. This patient had a grade 4 ALT of 368 IU/mL at week 128 of the study, with no associated abnormalities in albumin, international normalized ratio, or total bilirubin. This patient had a documented nonadherence to study drug before the flare and the ALT elevation was accompanied by an increase in HBV DNA from undetectable to 3.67 $\log_{10}$ IU/mL. Upon reinitiating study drug treatment,

Table 3. Bivariate Analysis of Associations of Baseline Characteristics With Achieving the Primary End Point Among Those Subjects With Week 192 Values

Baseline characteristcs	HBV DNA ≥69 IU/mL (n = 25)	HBV DNA <69 IU/mL (n = 82)	*P* value[a]
Female sex, n/N (%)	5/25 (20)	46/82 (56.1)	*.002*
Baseline HBsAg, *log*$_{10}$, mean	4.88	4.69	*.01*
TDF/FTC treatment, n/N (%)	7/25 (28)	47/82 (57.3)	*.012*
Baseline HBV DNA, *log*$_{10}$, mean	9.26	9.13	*.036*
Age >30 y, n/N (%)	13/25 (52)	46/82 (56.1)	.819
Genotype B, n/N (%)	13/25 (52)	38/82 (46.3)	.18
Asian race, n/N (%)	20/25 (80)	75/82 (91.5)	.146
Baseline BMI, mean	24.99	22.81	*.017*
Baseline ALT, *IU/mL*, mean	28.24	26.65	.178
Years HBV positive, mean	9.81	9.25	.59
Age, *y*, mean	31.92	34.26	.437

BMI, body mass index.
[a]*P* values in italic are statistically significant.

Table 4. Odds Ratio of Baseline Characteristics From Logistic Regression Analysis of Those Subjects With Week 192 Value

Baseline characteristics	Odds ratio	Confidence interval
Female sex	7.05	2.10–23.66
TDF/FTC treatment	3.95	1.36–11.47

this patient had resolution of the elevated ALT by week 144 with no additional increases for the remainder of the study and achieved viral suppression of HBV. No other liver-related events, including HCC or hepatic decompensation, occurred during this study.

Treatment-Free Follow-Up

A limited number of patients entered a 24-week treatment-free follow-up period to determine virologic and biochemical effects of cessation of therapy. A total of 26 patients in the TDF+placebo arm and 29 patients in the TDF+FTC arm entered this period, with 51 of 52 patients having a rapid increase in HBV DNA by week 4 of treatment-free follow-up. Only 1 patient had a biochemical ALT flare as predefined in the protocol; no patients achieved HBeAg loss or seroconversion during follow-up.

Discussion

In this study, we examined the safety and efficacy of treating patients with CHB, high HBV DNA levels, and normal ALT levels with either TDF or a combination of TDF and FTC. Both regimens had a potent antiviral effect, but significantly more patients on the TDF+FTC combination therapy than patients on TDF monotherapy achieved the primary end point of viral suppression. Rates of HBsAg and HBeAg loss/seroconversion were low in both groups. No evidence of virologic resistance could be detected in patients with ongoing low levels of viral replication.

The superior efficacy of FTC+TDF over that seen with TDF monotherapy is likely due to the combined efficacy of the 2 agents; FTC monotherapy is unlikely to provide comparable viral suppression, given its lower antiviral efficacy compared with TDF.[24] This trial provides evidence that for viral suppression, combination therapy can be superior to monotherapy, a finding more easily demonstrated in patients with very high viral load due to the high antiviral potency of TDF. A previous study suggested that the combination of entecavir and TDF therapy was superior to entecavir monotherapy in HBeAg-positive patients with very high viral loads ($>10^9$ IU/mL), but this was only a subgroup analysis and there was no direct comparison of the combination therapy with tenofovir monotherapy.[22]

Interestingly, the majority of patients who did not achieve the primary end point had significant reductions in viral load and very few had a viral load $>10^5$ IU/mL at the end of the study. Despite persisting viremia in the presence of study drug, no confirmed TDF resistance could be found by viral genotypic or phenotypic analyses. The mechanism of continued viral replication in the presence of drug remains unclear; potential explanations include differences in host genetics, status of the immune response to the virus, or more subtle changes in the viral sequence or life cycle that allows for low-level viral replication.

No patients experienced HBsAg loss or seroconversion during this study and only a small number of patients had HBeAg loss and seroconversion. This might be due to the fact that, at the time of treatment initiation, a high proportion of patients were in the immune-tolerant phase, during which rates of HBeAg loss are low.[4] The percentage of patients with declines in serum HBsAg levels with treatment increased in both groups with 61% having >0.5 $\log_{10}$ decline by week 192. Most patients achieved this HBsAg decline after 48 weeks of therapy, which is in contrast to immune-active HBeAg-positive patients treated with nucleos(t)ide polymerase inhibitors, for whom >0.5 $\log_{10}$ IU/mL declines in serum HBsAg occur within the first 24 weeks of therapy.[25] This delay in the kinetics of HBsAg decline in the

Table 5. Cumulative Safety Through Week 192

	TDF 300 mg + placebo (n = 64), n (%)	TDF 300 mg + FTC 200 mg (n = 62), n (%)	*P* value
All adverse events	58 (90.6)	45 (72.6)	.011
Serious adverse events	6 (9.4)	3 (4.8)	.492
Infections and infestations	4 (6.3)	1 (1.6)	
Neoplasm[a]	1 (1.6)	0 (0)	
Miscellaneous	1 (1.6)	2 (3.2)	
Study drug-related adverse event			
Grade 2	4 (6.3)	5 (8.1)	.742
Grade 3 or 4	0 (0)	0 (0)	
Discontinuation due to adverse event	1 (1.6)	2 (3.2)	.24
ALT flares	0 (0)	0 (0)	1
Increase in serum creatinine by ≥0.5 mg/dL	0 (0)	0 (0)	
Decrease in creatinine clearance to <50 mL/min	0 (0)	0 (0)	
Deaths	1 (1.6)	1(1.6)	1

[a]Uterine leiomyoma.

study population might be due to the underlying immune state of immune-tolerant patients compared with immune-active patients. The decrease in HBsAg might represent a decrease in the presence of covalently closed circular DNA within the hepatocytes or a reduction of transcription from existing covalently closed circular DNA. These data suggest that a longer duration of treatment (26 to 33 years) might be needed to achieve serologic end points of HBeAg loss and HBsAg loss in this patient population. The majority of patients maintained a normal ALT during this study. However, 19%−23% of patients had abnormal ALT values at the end of the study, of which only 36% had elevated HBV DNA as well. It is not clear if this represents a treatment effect or the natural history of these patients who were undergoing transition to immune-active disease.

Female sex was found on secondary analysis to be associated with a favorable treatment response to antivirals, independent of the treatment received. The reasons for this association remain unclear and additional analyses of this in subsequent trials are needed.

In conclusion, this study demonstrates that treatment of HBeAg-positive patients who have high viral load and normal ALT with highly potent antiviral drugs is safe, efficacious, and does not promote viral resistance. Combination therapy with FTC can provide improved viral suppression among patients who cannot achieve undetectable HBV DNA by TDF alone. As the rates of HBeAg seroconversion and HBsAg loss remain low, long-term therapy beyond 4 years might be required. Additional research into the long-term benefits of treatment, including changes in rates of cirrhosis and HCC is needed. Routine treatment of HBeAg-positive patients with high HBV DNA and normal ALT levels with antiviral medications is not supported by the current study results. The indication of antiviral therapy should still be based on liver histology in this population.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2014.01.044.

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Conflicts of interest
These authors disclose the following: Henry L. Y. Chan has received grants and done research for Roche; consulted for Bristol-Myers Squibb, Gilead Sciences, Merck Sharp & Dohme, Novartis, and Roche; been a speaker for Bristol-Myers Squibb, Echosens, Gilead Sciences, GlaxoSmithKline, Merck Sharp & Dohme, Novartis, and Roche. Fred Poordad has received grants and done research for Achillion, Anadys, Boehringer Ingelheim, Bristol-Myers Squibb, Genentech, Gilead Sciences, Human Genome Sciences, Idenix, Inhibitex, Janssen, Merck, Novartis, Pharmasset, Salix, and Vertex; consulted for Achillion, Anadys, Boehringer Ingelheim, Bristol-Myers Squibb, Genentech, Gilead Sciences, Idenix, Inhibitex, Janssen, Kadmon, Merck, Novartis, Salix, and Vertex; been a speaker for Genentech, Gilead Sciences, Kadmon, Merck, Salix, and Vertix. Philippe Mathurin serves on advisory boards at Abbott, Bayer Healthcare, Boehringer Ingelheim, Bristol-Myers Squibb, Gilead Sciences, Janssen-Cilag, Merck Sharp & Dohme, Novartis, and Roche; consulted for Abbott, Bayer Healthcare, Boehringer-Ingelheim, Bristol-Myers Squibb, Gilead Sciences, Janssen-Cilag, Merck Sharp & Dohm, Novartis, and Roche; been a speaker for Abbott, Bayer Healthcare, Boehringer Ingelheim, Bristol-Myers Squibb, Gilead Sciences, Janssen-Cilag, Merck Sharp & Dohme, Novartis, and Roche. Sam Lee has received grants and done research for AbbVie, Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, Gilead Sciences, GlaxoSmithKline, Janssen, Merck, Roche, and Vertex; consulted for AbbVie, Boehringer Ingelheim, Bristol-Myers Squibb, Gilead Sciences, GlaxoSmithKline, Janssen, Merck, Roche, and Vertex; been a speaker for Bristol-Myers Squibb, Gilead Sciences, Merck, Roche, and Vertex. Edward J. Gane serves on advisory boards for AbbVie, Gilead Sciences, Janssen, Novartis, and Roche; been a speaker for Gilead Sciences and Roche. John F. Flaherty, Lanjia Lin, Amy Corsa, Anuj Gaggar, G. Mani Subramanian, and John G. McHutchison are current or former employees of Gilead Sciences. The remaining authors disclose no conflicts.

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