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Long-Term Therapy With Tenofovir Is Effective for Patients Co-Infected With Human Immunodeficiency Virus and Hepatitis B Virus

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BACKGROUND & AIMS: We investigated the long-term efficacy and renal safety of tenofovir disoproxil fumarate (TDF), administered to patients co-infected with human immunodeficiency virus and hepatitis B virus (HBV) as part of an antiretroviral therapy. **METHODS:** We performed a multicenter, prospective cohort study of 102 patients co-infected with human immunodeficiency virus and HBV who were treated with TDF. **RESULTS:** At baseline, 80% of patients had a detectable viral load (HBV DNA > 20 IU/mL). Among patients positive for hepatitis B e antigen (HBeAg) (n = 67), 92% had a virologic response (HBV DNA < 20 IU/mL) after 5 years of treatment. There was no difference between patients with or without lamivudine resistance at baseline (P = .39). Loss rates of HBeAg and hepatitis B s antigen (HBsAg) were 46% and 12%, respectively. Among HBeAg-negative patients (n = 15), 100% had a virologic response after 4 years of treatment and 2 (13%) lost HBsAg. Twenty subjects (20%, all HBeAg-negative) had undetectable HBV DNA at baseline; during a median follow-up period of 52 months (range, 41–63 mo), 19 (95%) maintained a virologic response and 2 (10%) lost HBsAg. Overall, one patient acquired a combination of resistance mutations for anti-HBV drugs and experienced a virologic breakthrough. Three (3%) patients discontinued TDF because of increased serum creatinine levels. The estimated decrease in renal function after 5 years of TDF therapy was 9.8 mL/min/1.73 m^2, which was most pronounced shortly after TDF therapy was initiated. **CONCLUSIONS: TDF, administered as part of antiretroviral therapy, is a potent anti-HBV agent with a good resistance profile throughout 5 years of therapy. Only small nonprogressive decreases in renal function were observed.**

Keywords: Highly Active Antiretroviral Therapy; Nephrotoxicity; Entecavir; Liver Disease.

Tenofovir disoproxil fumarate (TDF) was licensed for the treatment of human immunodeficiency virus (HIV) infection in 2001, and since then has played a pivotal role in HIV management. Currently, the combination of TDF and emtricitabine is the most widely prescribed nucleos(t)ide analogue reverse-transcriptase inhibitor backbone in Europe. Because HIV and hepatitis B virus (HBV) share similar routes of transmission, prevalence of HBsAg carriership is more than 5-fold higher among HIV-infected patients compared with the general population.[1,2] Furthermore, HIV/HBV co-infected patients are at increased risk for development of cirrhosis and hepatocellular carcinoma, and have higher overall mortality rates compared with HIV-monoinfected patients.[3–6]

The efficacy of TDF in HBV therapy was first described in studies including mainly patients with HIV-1 co-infection.[7–11] Recent data showed the efficacy of TDF in the treatment of chronically HBV-monoinfected patients as well.[12] TDF was superior to adefovir dipivoxil in both nucleos(t)ide-naive hepatitis B e antigen (HBeAg)-positive and HBeAg-negative HBV patients, and appeared to be one of the most potent anti-HBV agents so far. Several reports showed that TDF also was effective in the nucleos(t)ide-experienced population, although conflicting results have been presented concerning patients with genotypic resistance to adefovir dipivoxil.[13–16] Moreover, TDF has a good resistance profile, and no convincing proof of HBV-resistant mutants to TDF has been presented so far.[17]

Long-term therapy is indicated for all HIV/HBV co-infected and most HBV mono-infected patients treated with oral nucleos(t)ide analogues because a sustained response after cessation of therapy is rare.[18,19] However, follow-up evaluation in studies investigating the efficacy of TDF in HIV/HBV co-infected and HBV-monoinfected patients is limited to only 2 years. In addition, there are concerns about the risk of renal toxicity with TDF.[20–25]

Abbreviations used in this paper: eGFR, estimated glomerular filtration rate; ETV, entecavir; HIV, human immunodeficiency virus; LAM, lamivudine; TDF, tenofovir disoproxil fumarate.

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We investigated the long-term efficacy and renal safety of TDF administered as a part of an antiretroviral therapy in a large cohort of HIV/HBV co-infected patients.

Materials and Methods

Study Populations

Six Dutch centers specializing in HIV management participated in this multicenter cohort study. From 2001 to July 2006 all consecutive adult HIV-infected patients positive for hepatitis B surface antigen (HBsAg) for more than 6 months, and treated with TDF as a part of antiretroviral therapy for at least 6 months, were included. Patients were excluded if they had hepatitis C or hepatitis delta co-infections, or received concomitant treatment with (pegylated) interferon during the on-treatment follow-up period. Patients were categorized as those with or without the presence of detectable HBV DNA at baseline.

Follow-Up Evaluation of Participants

Virologic, hematologic, and biochemical parameters were recorded at 6-month intervals (or shorter) in the first 2 years of follow-up evaluation and at yearly intervals thereafter. At every visit a routine examination with measurement of serum alanine aminotransferase (ALT), creatinine, CD4 cell count, serum HIV RNA, serum HBV DNA, HBeAg, and antibody to HBeAg (anti-HBe) took place. HBsAg status was measured in case of the combined presence of undetectable HBV DNA and negative HBeAg. A mutation analysis was performed (1) at baseline in all lamivudine (LAM)-experienced HBV patients; (2) in case of virologic breakthrough, defined as an increase in serum HBV-DNA level greater than 1 $\log_{10}$ (10-fold) above nadir on at least 2 occasions after initial virologic response; or (3) in case of serum HBV-DNA level greater than 200 IU/mL at the end of follow-up evaluation. HBV genotype was determined at baseline. At baseline, the diagnosis of cirrhosis was based on the treating physician's judgment. An abdominal ultrasound was performed if there was clinical suspicion of progression to cirrhosis, development of decompensated liver disease, or hepatocellular carcinoma.

End Points

The primary outcome was virologic response, defined as serum HBV-DNA levels less than 20 IU/mL during the on-treatment follow-up period. Secondary end points were HBsAg loss, HBeAg loss for HBeAg-positive patients, ALT normalization, and emergence of antiviral-resistant mutations. Progression to cirrhosis was defined on clinical grounds, that is, an albumin level less than 3.5 g/dL, a platelet count less than 100,000 mm^3, clinical decompensation, and ultrasound demonstration of surface nodularity, splenomegaly, and greater than 15-mm portal vein diameter. Clinical decompensation was defined as development of ascites, encephalopathy, jaundice, or gastrointestinal bleeding, defined according to international criteria.[26] Renal function was assessed by monitoring the estimated glomerular filtration rate (eGFR) in mL/min/1.73 m^2, which was calculated using the Modification in Diet in Renal Disease equation, based on the serum creatinine level, age, sex, and rac.

Laboratory Tests

Serum ALT and creatinine levels were measured using automated techniques. Absolute numbers of CD4 T lymphocytes were assessed on whole blood by flow cytometry. HBsAg, HBeAg, and anti-HBe were determined using commercially available enzyme immunoassays. HIV RNA was assessed quantitatively with the Cobas Ampliprep/Cobas Amplicor version 1.5 (lower limit of detection: 50 copies/mL; Roche Molecular Systems, Penzberg, Germany). HBV DNA was quantified in serum as previously described.[27,28] The lower limit of this assay was recently determined at 20 IU/mL by probit analysis (M. Schutten, unpublished data). HBV genotype was determined by Sanger sequencing on a 752–base pair fragment in the S gene as previously described.[29] Antiviral resistance–associated mutations were determined using the Inno-LIPA HBV DR v2 (Innogenetics NV, Zwijnaarde, Belgium) for highly sensitive detection of mutant species and by Sanger sequencing of the HBV reverse transcriptase gene to detect mutations not present on the Inno-LIPA HBV DR v2 (rtT184, rtA194, rtS202, rtI233, rtM250).

Data Analysis

Continuous variables are expressed as means ± standard deviation or median (interquartile range) where appropriate. Continuous variables were compared using the *t* test or the Mann–Whitney test. Categoric variables were compared using the chi-square or Fisher exact test. Follow-up times were calculated from the date of TDF treatment initiation to the date of event or censorship. The cumulative probabilities of virologic response, HBeAg loss, and HBsAg loss during treatment were calculated by the Kaplan–Meier method. Survival analysis with a Cox regression model was used to analyze which baseline factors were associated with virologic response in patients with a detectable HBV-DNA level at baseline (n = 82). Changes in creatinine level during treatment were analyzed with a repeated-measurement model estimating an overall smooth quadratic decline while allowing for a random intercept and a decline per patient. Differences in decline between baseline characteristics such as the use of ritonavir-boosted protease inhibitors were tested, adding an interaction term with time in the model. All statistical tests were 2-sided, and a *P* value less than .05 was considered statistically significant. SPSS version 15.0 (SPSS, Inc, Chicago, IL) and SAS version 9.2

Table 1. Baseline Characteristics

	Detectable HBV DNA (N = 82)	Undetectable HBV DNA (N = 20)	*P* value
Age, y	42 ± 8.7	43 ± 10	.68
Sex, (male %)	77 (94)	15 (75)	.02
Race			.04
Caucasian	54 (66%)	8 (40%)	
Black	18 (22%)	10 (50%)	
Other	10 (12%)	2 (10%)	
Body mass index	23 ± 5.2	25 ± 3.4	.31
ALT level, ×upper limit of normal	1.6 (1.0–2.7)	0.7 (0.4–1.0)	<.001
HBV-DNA level, $\log_{10}$ copies/mL	7.8 ± 2.1	Undetectable	<.001
HBeAg-positive	67 (82%)	0 (0%)	<.001
Genotype (N = 81)			.15
A	47 (62%)	5 (100%)	
Other	29 (38%)	0 (0%)	
Presence of cirrhosis	12 (15%)	2 (10%)	.66
CD4 count	285 (120–473)	320 (155–460)	.68
HIV RNA, $\log_{10}$ copies/mL	3.1 ± 1.6	2.0 ± 1.3	.002
Creatinine level, mg/dL	0.86 ± 0.17	0.88 ± 0.19	.66
eGFR, mL/min	106 ± 31	102 ± 30	.62
Treatment regimen			.41
2 NRTI + 1 NNRTI	50 (61%)	15 (75%)	
2 NRTI + PI/r	20 (24%)	4 (20%)	
Other	12 (16%)	1 (5%)	
Concomitant anti-HBV therapy			.26
Lamivudine	77 (94%)	20 (100%)	
Emtricitabine	5 (6%)	0 (0%)	
Previous anti-HBV therapy			
LAM experienced	50 (61%)	18 (90%)	.02
LAM resistance at baseline	33 (40%)	0 (0%)	<.001
Duration of LAM therapy, mo	42 (22–74)	45 (24–64)	.73

NNRTI, non–nucleos(t)ide reverse transcriptase inhibitor; NRTI, nucleos(t)ide reverse transcriptase inhibitor.

(SAS Institute, Inc, Cary, NC) were used for all statistical analysis.

Results

Baseline characteristics of the study population are presented in Table 1. A total of 102 patients were included in this analysis. Ninety-two (90%) subjects were men and the mean age of subjects was 42 ± 8.9 years. The treatment regimens that were used in addition to TDF were for most patients either a nucleos(t)ide analogue reverse-transcriptase inhibitor and a non–nucleos(t)ide analogue reverse-transcriptase inhibitor regimen (64%) or a nucleos(t)ide analogue reverse-transcriptase inhibitor and ritonavir-boosted protease inhibitor regimen (24%). During the on-treatment follow-up evaluation all patients received concomitant treatment with either LAM or emtricitabine. The median follow-up period of the whole study population was 55 months (42–64 mo).

Virologic Response in Patients With Detectable HBV DNA at Baseline

Of 82 patients with detectable HBV DNA at baseline, 67 (82%) subjects were HBeAg-positive at the initiation of TDF, and the mean HBV-DNA level was 7.0 ± 2.1 $\log_{10}$ IU/mL. Fifty (61%) patients were treated previously with LAM for a median duration of 42 months (22–74 mo). TDF was added to LAM therapy as a second anti-HBV drug in 45 (90%) of 50 patients and in 5 patients LAM was reintroduced in combination with TDF. In 33 (66%) subjects LAM-resistant mutations could be detected at the initiation of TDF. During a median follow-up period of 56 months (43–64 mo), 72 (88%) patients achieved virologic response. For HBeAg-positive patients (n = 67), the cumulative probability of achieving virologic response at 1, 2, 3, 4, and 5 years of treatment was 31%, 70%, 83%, 88%, and 92%, respectively (Figure 1). There was no significant difference between patients with or without LAM resistance at baseline (P = .39) (Figure 2). In univariate analysis only HBeAg negativity at baseline showed a trend toward a higher chance of achieving undetectable HBV DNA (P = .09). HBeAg loss and HBsAg loss rates increased to 46% and 12% after 5 years of TDF therapy. For HBeAg-negative patients (n = 15), the cumulative probability of achieving virologic response at 1, 2, 3, and 4 years of treatment was 47%, 85%, 85%, and 100%, respectively (Figure 1). During the follow-up period, 2 (13%) of 15 HBeAg-negative patients achieved HBsAg loss. Of 59 patients with increased ALT levels at baseline, 46 (78%) showed ALT normalization at the end of the follow-up period. Three (4%)

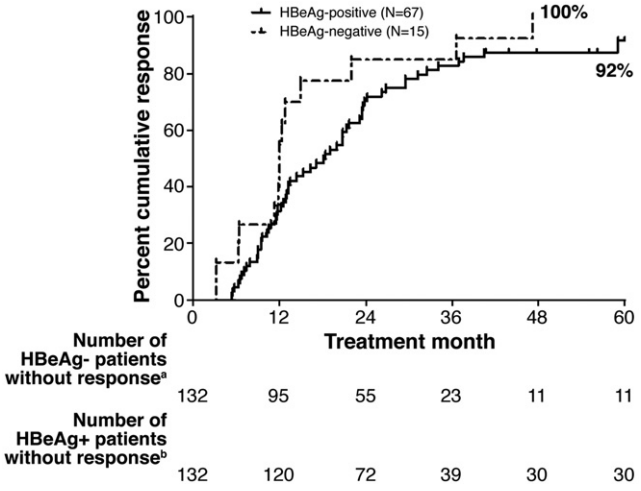

Figure 1. Kaplan–Meier curve for the cumulative probabilities of achieving virologic response, defined as HBV-DNA levels less than 20 IU/mL, for HBeAg-positive (n = 67) and HBeAg-negative (n = 15) HIV/HBV with patients with detectable HBV DNA at baseline (n = 82).

patients experienced a virologic breakthrough during the observation period. In 2 subjects no genotypic resistance could be detected; 1 patient showed the combined presence of rtM204I, rtL80I, rtL180M, and rtA181V in the HBV polymerase gene (Figure 3*B*).

Virologic Response in Patients With Undetectable HBV DNA at Baseline

Twenty patients (100% HBeAg-) had undetectable HBV DNA at baseline. Two patients were treatment-naive; 18 patients were pretreated with LAM for a median duration of 38 months (24–64 mo). In all patients TDF was added as a second anti-HBV drug per internal protocol. During a median follow-up period of 52 months (41–63 mo) 19 (95%) subjects maintained virologic response, and 2 (10%) patients showed HBsAg loss. One (5%) subject experienced a virologic breakthrough after which a hepatocellular carcinoma was diagnosed. No genotypic resistance could be detected.

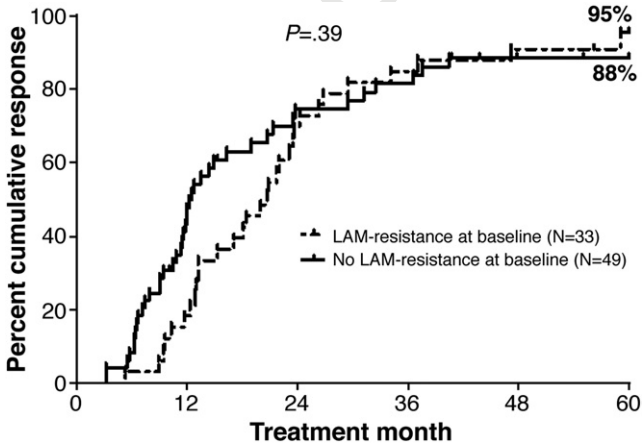

Figure 2. Kaplan–Meier curve for the cumulative probabilities of achieving virologic response, defined as HBV-DNA levels less than 20 IU/mL, for HIV/HBV patients with detectable HBV DNA at baseline (n = 82) with lamivudine-resistant (n = 33) or no lamivudine-resistant mutations (n = 49) at the initiation of TDF.

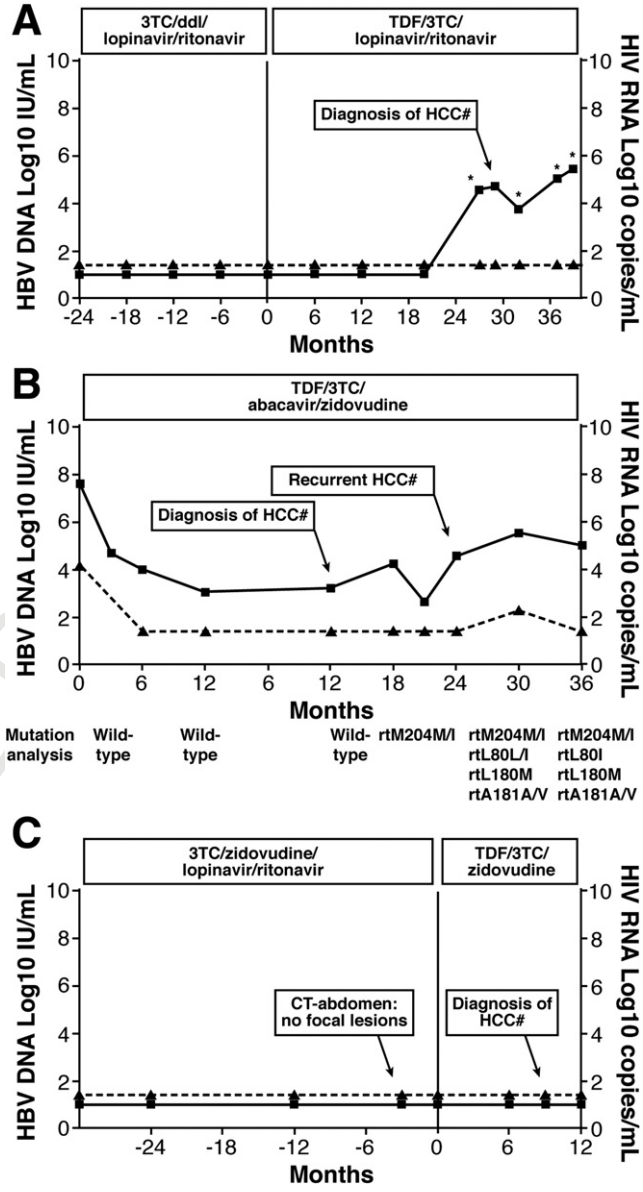

Figure 3. Clinical course of 3 patients who developed hepatocellular carcinoma throughout the follow-up evaluation. Two patients showed a virologic breakthrough as well. (*A*) A magnetic resonance image of the abdomen showed diffuse hepatocellular carcinoma in segments 4–8, with infiltration of the portal vein. In addition, there was a focal lesion in segment 2 with a diameter of 8 mm, suspicious for hepatocellular carcinoma. *Mutation analysis showed wild-type HBV. (*B*) A magnetic resonance image of the abdomen showed a focal lesion in segment 4a, suspicious for hepatocellular carcinoma with a diameter of 3.3 cm, for which the patient received treatment with radiofrequent ablation. After 36 months recurrent hepatocellular carcinoma was diagnosed. (*C*) A magnetic resonance image of the abdomen showed a focal lesion in segment 8, suspicious for hepatocellular carcinoma, with a diameter of 13 cm. Two other focal lesions suspicious for hepatocellular carcinoma were observed in segments 2 and 3, with a diameter of 1 cm.

Table 2. Summary of Patients With Persistent HBV Replication in Whom Entecavir Was Added as Rescue Therapy

	Patient 1	Patient 2	Patient 3	Patient 4
Age, y	26	43	41	37
Sex	Male	Male	Male	Male
Previous therapy with LAM	No	Yes	Yes	No
At start of tenofovir				
HBeAg status	Positive	Positive	Positive	Positive
HBV DNA, $\log_{10}$ IU/mL	8.9	7.3	7.8	8.2
HIV RNA, $\log_{10}$ copies/mL	4.6	3.5	UD	3.5
HBV genotype	A	A	A	A
Concomitant anti-HBV therapy	LAM	LAM	LAM	FTC
Virologic breakthrough	No	No	No	No
At time of initiation of entecavir				
Follow-up period, mo	42	48	48	15
HBV DNA, $\log_{10}$ copies/mL	5.3	3.3	4.2	4.2
HBeAg status	Positive	Positive	Positive	Positive
HIV RNA, $\log_{10}$ copies/mL	UD	UD	UD	UD
Mutation analysis	Wild-type	Wild-type	Wild-type	Wild-type
Noncompliance	No	No	No	No
Response to salvage therapy				
Salvage therapy	Addition of ETV	Addition of ETV	Addition of ETV	Addition of ETV
Follow-up period, mo	15	27	15	12
HBV DNA, $\log_{10}$ IU/mL at last F/U	UD	UD	UD	UD
HBeAg status at last F/U	Positive	Negative	Positive	Positive

UD, undetectable; F/U, follow-up.

HBV Resistance Surveillance

During a median follow-up period of 55 months (42–64 mo), 9 of 67 (13%) HBeAg-positive and 1 of 15 (7%) HBeAg-negative patients with a detectable HBV DNA at baseline did not achieve virologic response. Of these 10 subjects, 3 experienced a virologic breakthrough as well. One of 20 patients with undetectable HBV DNA at baseline showed a virologic breakthrough. None of the subjects with a virologic breakthrough showed LAM-resistant mutations at baseline. In 2 patients nonadherence was suspected because a simultaneous rebound HIV RNA was observed. A hepatocellular carcinoma was diagnosed in the other 2 patients, of whom 1 subject also showed multiple anti-HBV drug-resistant mutations (rtM204I, rtL80I, rtL180M, and rtA181V). Of the patients with a detectable viral load at the end of the follow-up period without fulfilling the criteria of virologic breakthrough (n = 7), 4 subjects showed LAM resistance at baseline, and in 1 patient these substitutions persisted throughout the observation period. No therapy-resistant mutations were observed in the other patients at the end of the follow-up period.

Progression of Hepatitis B and Survival

Of the 14 cirrhotic patients at baseline, 3 developed de novo hepatocellular carcinoma after 10–32 months (Figure 3), and 2 subjects developed decompensated liver disease after 42 and 48 months of follow-up evaluation. In total, 4 patients died of hepatocellular carcinoma progression (n = 3) or complications related to end-stage liver disease (n = 1). Of the 88 noncirrhotic patients, none progressed clinically to cirrhosis or developed de novo hepatocellular carcinoma. Three patients died of HBV-unrelated causes.

Entecavir as Rescue Therapy in Patients With Persisting HBV Replication

In 4 patients who showed persistent HBV replication during antiviral therapy, entecavir (ETV) (dosed at 1 mg once daily) was added to the treatment regimen as rescue therapy (Table 2). Patients were compliant with the treatment regimen, which is supported by the undetectable HIV-RNA levels in these 4 patients at the moment ETV was added. The addition of ETV resulted in undetectable HBV DNA in all subjects after 3–15 months of follow-up evaluation; 1 patient also achieved HBeAg loss.

HIV RNA and CD4 Cell Count Changes

At the initiation of TDF, the mean HIV RNA was 2.9 ± 1.6 $\log_{10}$ copies/mL, and 51 patients (50%) showed serum HIV-RNA levels less than 50 copies/mL. At the end of the follow-up period a significantly increased proportion of patients (84%; $P < .001$) showed undetectable HIV RNA. The median CD4 cell count increased from 293 cells/mm^3 (138–470 cells/mm^3) at baseline to 455 cells/mm^3 (340–643 cells/mm^3) at the end of the follow-up period ($P < .001$).

Renal Safety

Two patients (2%) experienced an increase in serum creatinine level greater than 0.5 mg/dL after 5 (peak creatinine level, 1.5 mg/dL; eGFR, 54 mL/min) and 16 (peak creatinine level, 2.2 mg/dL; eGFR, 32 mL/min)

months of follow-up evaluation, respectively. In both patients TDF was stopped, after which serum creatinine levels stabilized, but did not return to normal in both patients. In one of these patients this also can be explained by polyarteritis nodosa related to HBV with associated renal insufficiency. In 1 additional subject TDF was discontinued after 45 months because of an increase in serum creatinine of 0.38 mg/dL from baseline. The mean eGFR at baseline was 105 ± 30 mL/min/1.73 m^2. The estimated decrease after 5 years of TDF therapy was 9.8 mL/min/1.73 m^2 (95% confidence interval, 5.4–14.2 mL/min/1.73 m^2). The major part of decline in renal function occurred shortly after initiation of TDF therapy ($P = .02$), and was observed especially in those subjects with a baseline eGFR greater than 100 mL/min/1.73 m^2 ($P < .001$). The use of ritonavir-boosted protease inhibitors was not related to decline in eGFR ($P = .60$).

Discussion

This study assessed the long-term efficacy of TDF administered as part of antiretroviral therapy in a large cohort of HIV/HBV co-infected patients. Previous studies on the efficacy of TDF in both HIV/HBV co-infected and HBV-monoinfected patients were limited by a relatively short follow-up period for up to 2 years.[7,8,12,14] In our study, there was a median follow-up period of almost 5 years, and we show a large cohort of HIV/HBV co-infected patients treated with TDF. We showed that after 5 years of follow-up evaluation, approximately 90% of patients achieved undetectable HBV DNA (<20 IU/mL), almost 50% of HBeAg-positive patients showed HBeAg loss, and HBsAg loss was even observed in approximately 10% of subjects. There was no significant difference between patients with or without LAM resistance at baseline. More importantly, only 1 patient showed a combination of known anti-HBV drug-resistant mutations, and experienced a virologic breakthrough thereafter. In 3 patients TDF was discontinued because of increases of serum creatinine levels. The estimated decrease in renal function after 5 years of TDF therapy was approximately 10 mL/min/1.73 m^2, and was most pronounced directly after initiation of TDF therapy.

The widespread use of highly active antiretroviral therapy has significantly increased the life expectancy of HIV-infected patients, and liver disease has now emerged as a significant cause of non–acquired immune deficiency syndrome–related death.[3,5] A large prospective cohort study showed active HBV infection to be associated strongly with liver-related mortality.[3] Current guidelines therefore recommend inclusion of HBV-active agents within the highly active antiretroviral therapy regimen, and to initiate highly active antiretroviral therapy early if an indication to treat HBV infection exists.[30] However, the benefits of long-term treatment may be negated by the development of anti-HBV drug resistance, which can lead to reversion of virologic and histologic improvement. In 2 recently performed randomized clinical trials in HBV–mono-infected patients, TDF resulted in HBV-DNA levels less than 400 copies/mL in 76% and 93% of HBeAg-positive and HBeAg-negative patients, respectively.[12] Continued therapy produced additional viral suppression, HBeAg- and HBsAg-loss at weeks 72 and 96, respectively.[31,32] Our study now shows TDF, combined with either LAM or emtricitabine, to be an effective anti-HBV agent through 5 years of therapy with 90% of HIV/HBV co-infected subjects achieving undetectable HBV DNA.

In the phase III trials in HBV–mono-infected patients no evidence of TDF resistance was shown during up to 72 weeks of treatment despite extensive resistance surveillance.[17] Until now TDF resistance has been described in only 2 HIV-HBV co-infected patients showing the A194T mutation in addition to LAM resistance,[33] yet the association between this mutation and TDF resistance was not confirmed in another study.[34] In our study, 4 subjects experienced a virologic breakthrough. In 2 patients this was explained by noncompliance and only 1 patient showed a combination of LAM- and adefovir-resistant mutations in the HBV polymerase gene. The rtA194T mutation was not observed. An interesting phenomenon was that 2 virologic breakthroughs occurred in association with the development of hepatocellular carcinoma. A satisfactory explanation for this relation could not be found. There are many reports that show an association between development of resistance and the risk of hepatocellular carcinoma, which is largely explained by the recurrence of viral replication; only one report noted that significantly more hepatocellular carcinomas were observed shortly after development of LAM resistance.[35]

The recently published EASL guidelines on the management of hepatitis B state that "in patients receiving entecavir or tenofovir with a partial virologic response at week 48, some experts would suggest adding the other drug in order to prevent resistance in the long term."[36] In agreement with the follow-up data of the 2 large phase III trials in HBV-monoinfected patients,[12] our study shows that most patients are still able to achieve undetectable HBV DNA in the second year without changing the treatment regimen. Moreover, this report shows that adding ETV to existing TDF therapy still is effective after at least 15 months of treatment, and resulted in undetectable HBV DNA in all patients. Our study therefore suggests that one can probably wait at least 24 months before adding ETV in patients who are viremic on a TDF-containing treatment regimen.

There have been concerns about the risk of renal toxicity with TDF because of an association between related compounds such as adefovir and nephrotoxicity.[37,38] In our study, a small but significant, increase in serum creatinine levels was observed after 5 years of treatment. Yet, only 3% of patients developed serum creatinine increases that necessitated the discontinuation of TDF.

Furthermore, serum creatinine increases usually occurred early, which suggests that frequent monitoring of renal function is necessary shortly after initiation of TDF treatment, but that thereafter monitoring probably can be decreased.[22] Overall, this study supports the renal safety of TDF as a part of antiretroviral therapy through 5 years of treatment.

To date, no confirmed genotypic substitutions in the HBV polymerase gene associated with decreased sensitivity to TDF have been identified. Although direct sequencing does allow for all mutations to be identified, in vitro phenotypic confirmatory assays are mandatory to detect new substitutions. A limitation of our study, therefore, was that we were only able to search for known anti-HBV drug-resistant mutations. In addition, no liver biopsies were available during follow-up evaluation in all our patients, and abdominal ultrasound was performed only if there was clinical suspicion of progression to cirrhosis, decompensated liver disease, or hepatocellular carcinoma. The frequency of progression of hepatitis B, and, more specifically, the development of cirrhosis and hepatocellular carcinoma, may have been underestimated in our study.

AQ: 27 In conclusion, TDF administered as part of antiretroviral therapy was shown to be a potent anti-HBV agent with a good resistance profile throughout 5 years of therapy. The antiviral efficacy of TDF was not influenced by the presence of LAM resistance. Furthermore, this study supports the renal safety of TDF through 5 years of treatment because only a small, nonprogressive decline in renal function was observed. Nevertheless, close monitoring of renal function still is indicated. Adding ETV to the treatment regimen resulted in achievement of undetectable HBV DNA in patients who show persistent HBV replication during a TDF-containing treatment regimen.

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AQ:4 *Reprint requests*

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Conflicts of interest AQ:6-7

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