Research Article
Viral Hepatitis

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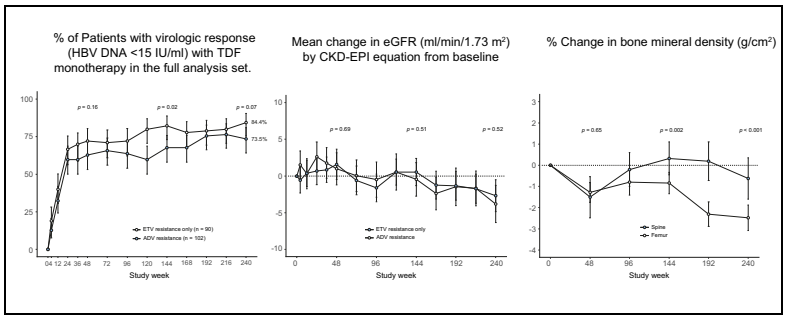
Monotherapy with tenofovir disoproxil fumarate for adefovir-resistant *vs.* entecavir-resistant chronic hepatitis B: A 5-year clinical trial

Graphical abstract



Highlights

- 240 weeks of TDF monotherapy provided an increasing virologic response rate in patients with multidrug-resistant HBV.
- Virologic breakthrough was rare and not associated with emergence of additional resistance mutations.
- The HBeAg seroconversion rate was very low and no patient achieved HBsAg seroclearance up to week 240.
- Prolonged continuous treatment may be needed to maintain viral suppression in these patients.
- Progressive and significant decreases in renal function and bone mineral density after week 144 raise safety concerns.

Authors

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Lay summary

In patients chronically infected with hepatitis B virus resistant to multiple drugs including lamivudine, entecavir, and/or adefovir, tenofovir disoproxil fumarate (TDF) monotherapy showed non-inferior efficacy compared with the combination therapy of TDF plus entecavir. Nonetheless, short-term TDF monotherapy was associated with suboptimal virologic response, and its long-term safety was uncertain. This study displayed that 240 weeks of TDF monotherapy provided a virologic response in most of those patients, but it was associated with poor serological responses and decreasing renal function and bone mineral density.

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Monotherapy with tenofovir disoproxil fumarate for adefovir-resistant *vs.* entecavir-resistant chronic hepatitis B: A 5-year clinical trial

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Background & Aims: Tenofovir disoproxil fumarate (TDF) monotherapy has displayed non-inferior efficacy to TDF plus entecavir (ETV) combination therapy in patients with hepatitis B virus (HBV) resistant to ETV and/or adefovir (ADV). Nonetheless, the virologic response rate was suboptimal in patients receiving up to 144 weeks of TDF monotherapy. We aimed to assess the efficacy and safety of TDF monotherapy given for up to 240 weeks.

Methods: One trial enrolled patients with ETV resistance without ADV resistance (n = 90), and another trial included patients with ADV resistance (n = 102). Most patients (91.2%) also had lamivudine resistance. Patients were randomized 1:1 to receive TDF monotherapy or TDF + ETV combination therapy for 48 weeks, and then TDF monotherapy until week 240. We compared efficacy between the studies and safety in the pooled population at 240 weeks.

Results: At week 240, the proportion of patients with serum HBV DNA <15 IU/ml was not significantly different between the ETV and ADV resistance groups in the full analysis set (84.4% *vs.* 73.5%; $p = 0.07$), which was significantly different by on-treatment analysis (92.7% *vs.* 79.8%; $p = 0.02$). Virologic blips associated with poor medication adherence occurred in 7 patients throughout the 240 weeks. None developed additional HBV resistance mutations. Among the 170 HBV e antigen (HBeAg)-positive patients at baseline, 12 (7.1%) achieved HBeAg seroconversion at week 240. None achieved HBV surface antigen seroclearance. Significant decreases from baseline were observed at week 240 in the estimated glomerular filtration rate (−3.21 ml/min/1.73 m^2 by the CKD-EPI equation, $p < 0.001$) and bone mineral density (g/cm^2) at the femur (−2.48%, $p < 0.001$).

Conclusions: Up to 240 weeks of TDF monotherapy provided an increasing virologic response rate in heavily pretreated patients with HBV resistant to ETV and/or ADV. However, it was associated with poor serological responses and decreasing renal function and bone mineral density.

(ClinicalTrials.gov No, NCT01639066 and NCT01639092).

Lay summary: In patients chronically infected with hepatitis B virus resistant to multiple drugs including lamivudine, entecavir, and/or adefovir, tenofovir disoproxil fumarate (TDF) monotherapy showed non-inferior efficacy compared with the combination therapy of TDF plus entecavir. Nonetheless, short-term TDF monotherapy was associated with suboptimal virologic response, and its long-term safety was uncertain. This study displayed that 240 weeks of TDF monotherapy provided a virologic response in most of those patients, but it was associated with poor serological responses and decreasing renal function and bone mineral density.

Introduction

Persistently high serum hepatitis B virus (HBV) DNA levels are an independent risk factor for disease progression to cirrhosis and hepatocellular carcinoma (HCC) in patients with chronic hepatitis B (CHB).[1–3] Multiple studies have shown that long-term treatment with nucleos(t)ide analogue (NUC) therapy reduces the risk of mortality and HCC through inhibition of HBV replication.[3–6] Nevertheless, functional cure of chronic HBV infection (HBV surface antigen [HBsAg] seroclearance) is very rarely achievable, necessitating almost life-long NUC therapy in most patients.[7,8]

Worldwide, many patients with CHB have been exposed to low-potency, low genetic barrier NUCs, *i.e.*, lamivudine (LAM) and adefovir dipivoxil (ADV), and developed multiple resistance mutations of HBV.[9] Treatment options are severely limited for these patients who have HBV resistant to multiple NUCs, such as LAM, ADV, and entecavir (ETV).

Recently, in 2 separate randomized trials for 48 weeks, we have demonstrated that tenofovir disoproxil fumarate (TDF) monotherapy provides a virologic response rate non-inferior

Keywords: Adefovir dipivoxil; Entecavir; Hepatitis B virus; Lamivudine; Resistance; HBV.

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to that of combination therapy with TDF and ETV in patients who acquired multiple HBV mutations resistant to ETV and/or ADV through previous exposure to multiple NUCs.[10,11] However, the virologic response rate was not optimal with up to 144 weeks of TDF therapy in patients resistant to ETV (82.2%) or ADV (67.7%),[12] which raised concerns about the efficacy and safety of long-term TDF monotherapy, especially in those with ADV resistance. Moreover, long-term TDF use may be linked to renal toxicity and reductions in bone mineral density (BMD) in patients with CHB.[13–15]

Therefore, in this study we aimed to compare the antiviral efficacy of long-term (up to 240 weeks) TDF monotherapy between the patients with ETV resistance and those with ADV resistance as extensions of our 2 previous trials. We also aimed to assess the renal and bone safety of TDF monotherapy up to 240 weeks in the pooled population.

Patients and methods

Study design

This was an extension study combining 2 multicenter open-label randomized trials in patients with CHB, detectable serum HBV DNA levels, and genotypically confirmed resistance mutations to ADV (IN-US-174-0202) or ETV (IN-US-174-0205). The trials were originally designed to compare the efficacy and safety of TDF monotherapy with that of TDF + ETV combination therapy in patients with multiple drug-resistant HBV infection. The 2 trials were identical in design, except that IN-US-174-0202 enrolled only patients with documented ADV-resistant HBV mutations (ClinicalTrials.gov ID NCT01639092) and IN-US-174-0205 enrolled only patients with documented HBV mutations resistant to ETV without resistance mutations to ADV (ClinicalTrials.gov ID NCT01639066). After 48 weeks, TDF monotherapy was shown in both studies to be non-inferior to TDF + ETV combination therapy in antiviral efficacy, as measured by rates of suppression of HBV DNA expression to <15 IU/ml.[10,11] All who completed 48 weeks in either study were rolled over to TDF monotherapy for 192 additional weeks. Study conduct and methods were previously described.[10–12]

This study was approved by the institutional review board of each investigational site.

Study participants

Study patients were recruited between September 2012 and August 2013 from 5 tertiary referral hospitals in Korea. Patients had to have a positive result in serum HBsAg test for ≥6 months and serum HBV DNA levels >60 IU/ml, despite continued treatment with various NUCs other than TDF. All the patients had documented ETV-resistance mutations (rtT184A/C/F/G/I/L/S, rtS202G, or rtM250L/V, on the presence of rtM204V/I) or ADV-resistance mutations (rtA181V/T and/or rtN236T). They were 20–75 years old and had serum creatinine <1.5 mg/dl. Patients were excluded if they had prior exposure to tenofovir, evidence or history of decompensated hepatic function, malignancy, or coinfection with other hepatotropic virus or human immunodeficiency virus.

Study visits occurred every 12 weeks until treatment week 48, after which study visits occurred every 24 weeks.

Outcomes

The efficacy endpoints for the analysis at 240-weeks were the proportion of patients achieving a virologic response (defined as serum HBV DNA <15 IU/ml), the proportion of patients with serum HBV DNA <60 IU/ml, changes in serum HBV DNA levels from baseline, the proportion of patients with normal alanine aminotransferase (ALT), and the proportion of patients achieving HBV e antigen (HBeAg) seroclearance/seroconversion (only for HBeAg-positive patients) and HBsAg seroclearance/seroconversion. The occurrence of virologic breakthrough (rise of serum HBV DNA levels ≥1 $\log_{10}$ IU/ml by 2 consecutive tests) was assessed during the study. Genotypic analysis for the development of new drug-resistant mutations were conducted for the cases with virologic breakthrough during the study or considerable HBV DNA levels (>60 IU/ml) at study discontinuation and at 240-weeks.

Clinical and laboratory adverse events were assessed during the study period. Estimated glomerular filtration rate (eGFR) was calculated by using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation as follows:[16] for women with serum creatinine ≤0.7 mg/dl, eGFR was calculated as $144 \times (\text{serum creatinine}/0.7)^{-0.329} \times (0.993)^{\text{age}}$; for women with serum creatinine >0.7 mg/dl, eGFR was calculated as $144 \times (\text{serum creatinine}/0.7)^{-1.209} \times (0.993)^{\text{age}}$; for men with serum creatinine ≤0.9 mg/dl, eGFR was calculated as $141 \times (\text{serum creatinine}/0.9)^{-0.411} \times (0.993)^{\text{age}}$; and for men with serum creatinine >0.9 mg/dl, eGFR was calculated as $141 \times (\text{serum creatinine}/0.9)^{-1.209} \times (0.993)^{\text{age}}$. The BMDs of the lumbar spine and femur were measured using dual-energy x-ray absorptiometry (DXA) at baseline and every 48 weeks. We assessed the adherence to study drugs by counting the number of unused pills and empty blister packets returned at each visit. Low adherence was defined as <80%.

Serum assays

Serum levels of HBV DNA were measured by a real-time PCR assay (15 IU/ml to 1×10^9 IU/ml of linear dynamic detection range; Abbott Laboratories, Chicago, IL, USA). Restriction fragment mass polymorphism analyses and direct sequencing of the reverse transcriptase region of the HBV polymerase gene (pol/RT) were used to determine HBV resistance mutations. Serum HBsAg levels were tested quantitatively (lower detection limit, 0.05 IU/ml; Architect assay, Abbott Laboratories). HBeAg, anti-HBe, and anti-HBs were measured using enzyme immunoassays (Abbott Laboratories). Normal ALT was defined as <40 IU/L by local laboratory criteria, and as ≤25 IU/L for females and ≤35 IU/L for males by the American Association for the Study of Liver Diseases (AASLD) criteria.[17]

Statistical analyses

The primary analyses for efficacy and safety were conducted in the full analysis set including all patients who were randomized in accordance with the principle of the intention-to-treat analysis. Individuals who stopped the study early before week 240 were regarded as failures for all endpoints after discontinuation. On-treatment analysis was also performed including only individuals on study with non-missing data at each time point.

Between-group comparisons were conducted using the chi-square test, *t* test, Fisher's exact test, or analysis of variance for categorical or continuous variables as appropriate. The proportion of individuals achieving a virologic response were compared between weeks 48 and 240 by the McNemar test. We conducted subgroup analyses for the baseline ADV-resistance mutations as predefined.

R (http://cran.r-project.org/; v3.0) was used for the statistical analyses. A p value of <0.05 was considered statistically significant.

Results

Patient disposition

Of the 102 ADV-resistant patients originally randomized and treated in the IN-US-174-0202 trial, 94 (92.2%) completed 240 weeks of TDF therapy. Of the 90 ETV-resistant patients originally randomized and treated in the IN-US-174-0205 study, 82 (91.1%) completed 240 weeks of TDF therapy (Fig. 1).

The baseline patient characteristics of the 2 studies were similar except HBV resistance profiles and treatments prior to the studies (Table 1). The mean age was 50 years, and males comprised 81.2% of the study population. Cirrhosis was present in 40 patients (20.8%), and 88.5% were HBeAg positive. All patients were being treated with various NUCs other than tenofovir at baseline. All had HBV genotype C. The median level of serum HBV DNA was 3.71 $\log_{10}$ IU/ml. Most patients had exposure to multiple NUCs. Most patients had resistance mutations to LAM (91.2%), ETV (67.7%), and ADV (53.1%) in diverse combinations.

Virologic response

In the full analysis set, the proportion of individuals with serum HBV DNA <15 IU/ml at 240-weeks was 78.6% (151/192), which was significantly increased from 67.2% at week 48 ($p = 0.003$), without significant differences between patients with ETV resistance only and those with ADV resistance (84.4% *vs.* 73.5%; $p = 0.07$; Table 2 and Fig. 2A).

By the on-treatment analysis, the proportion of patients with serum HBV DNA <15 IU/ml at week 240 was significantly higher in the ETV resistance group than in the ADV resistance group (92.7% *vs.* 79.8%, $p = 0.02$; Table 2 and Fig. 2B). The proportion of patients who had HBV DNA <60 IU/ml at week 240 was also significantly higher in the ETV resistance group than in the ADV resistance group (98.8% *vs.* 91.5%, $p = 0.03$; Table 2 and Fig. S1).

Among the 176 patients who completed treatment up to week 240, 25 had serum HBV DNA ≥15 IU/ml at week 240, with median levels of 41 IU/ml (range, 16–380 IU/ml) with no significant difference between ETV and ADV resistance groups ($p = 0.44$; Table 2).

Virologic response in the patient subgroups

Three groups were classified by baseline ADV-resistance mutations, namely no ADV-resistance mutation, single ADV-resistance mutation (rtA181T/V or rtN236T), and double ADV-resistance mutations (rtA181T/V and rtN236T). The 3 groups did not differ significantly in the proportion of patients achieving HBV DNA <15 IU/ml at 240-weeks in the full analysis set (84.4% [76/90] *vs.* 76.5% [52/68] *vs.* 67.6% [23/34], $p = 0.11$; Fig. 3A). The 3 groups also showed no significant difference in the proportion of patients with serum HBV DNA <60 IU/ml throughout the study (90.0% *vs.* 82.4% *vs.* 88.2% at week 240, $p = 0.36$; Fig. 3B).

Biochemical and serological responses

The proportion of patients with normal ALT levels at week 240 did not significantly differ between the ETV- and ADV-resistance groups by local laboratory criteria (84.1% *vs.* 87.2%; $p = 0.56$) and the AASLD criteria (70.7% *vs.* 77.7%, $p = 0.29$; Table 2).

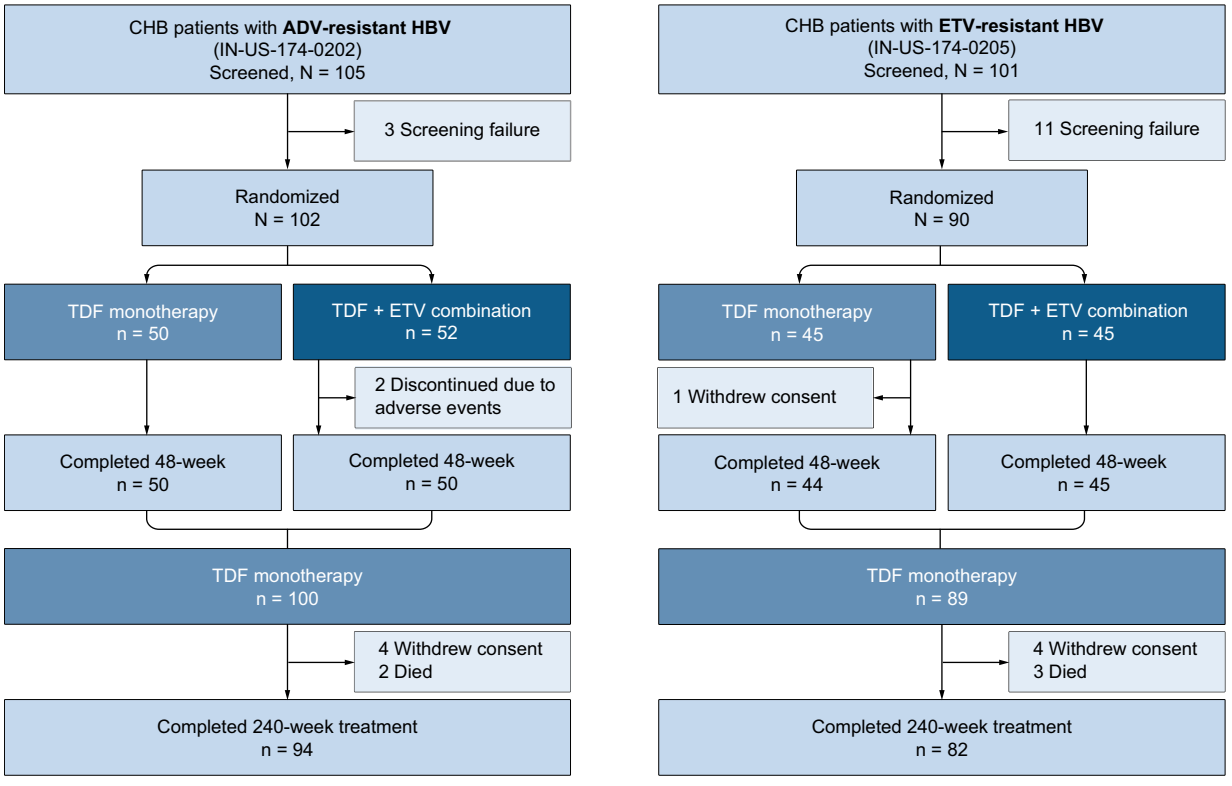

Fig. 1. Patient flow diagram. ADV, adefovir dipivoxil; CHB, chronic hepatitis B; ETV, entecavir; TDF, tenofovir disoproxil fumarate.

Table 1. Baseline characteristics of the study patients.

Characteristics	Total	ETV resistance (no ADV resistance)	ADV resistance	*p* value
N	192	90	102	
Age*, years	50 ± 10	51 ± 9	50 ± 10	0.28
Male, n (%)	156 (81.2%)	68 (75.6%)	88 (86.3%)	0.06
ALT†, IU/L	32 (11–275)	33 (20–44)	32 (12–275)	0.58
Normal ALT, n (%)	135 (70.3%)	62 (68.9%)	73 (71.6%)	0.69
Bilirubin†, mg/dl	0.9 (0.3–2.5)	0.8 (0.6–1.0)	0.9 (0.3–2.5)	0.18
Albumin†, g/dl	4.4 (2.8–5.2)	4.4 (4.2–4.6)	4.4 (3.4–5.2)	0.51
INR†	0.99 (0.85–1.40)	1.00 (0.93–1.04)	0.99 (0.85–1.40)	0.82
Creatinine†, mg/dl	0.9 (0.5–1.4)	0.9 (0.8–1.0)	0.9 (0.5–1.4)	0.93
eGFR by CKD-EPI equation*, ml/min/1.73 m^2	93 ± 15	91 ± 15	95 ± 15	0.12
Phosphate†, mg/dl	3.2 (1.7–5.0)	3.2 (2.8–3.6)	3.2 (1.7–4.5)	0.95
Cirrhosis‡, n (%)	40 (20.8%)	21 (23.3%)	19 (18.6%)	0.42
Diabetes Mellitus, n (%)	20 (10.4%)	9 (10.0%)	11 (10.8%)	0.86
Hypertension, n (%)	38 (19.8%)	22 (24.4%)	16 (15.7%)	0.13
BMI ≥25 kg/m^2, n (%)	88 (45.8%)	43 (47.8%)	45 (44.1%)	0.61
HBsAg*, log_{10} IU/ml	3.53 ± 0.52	3.51 ± 0.60	3.55 ± 0.43	0.60
HBeAg-positivity, n (%)	170 (88.5%)	80 (88.9%)	90 (88.2%)	0.89
HBV DNA†, log_{10} IU/ml	3.71 (1.78–9.00)	4.02 (3.08–5.24)	3.38 (1.78–9.00)	0.10
HBV genotype C, n (%)	192 (100%)	90 (100%)	102 (100%)	>0.99
LAM-resistance mutations§, n (%)	175 (91.2%)	90 (100%)	85 (83.3%)	<0.01
ETV-resistance mutations§, n (%)	130 (67.7%)	90 (100%)	40 (39.2%)	<0.01
ADV-resistance mutations§, n (%)	102 (53.1%)	0	102 (100%)	<0.01
Single (rtA181T/V or rtN236T)	68 (35.4%)	0	68 (66.7%)	
Double (rtA181T/V and rtN236T)	34 (17.7%)	0	34 (33.3%)	
Treatment at baseline, n (%)				<0.01
ADV	11 (5.7%)	1 (1.1%)	10 (9.8%)	
ETV	34 (17.7%)	29 (32.2%)	5 (4.9%)	
ADV + LAM	23 (12.0%)	3 (3.3%)	20 (19.6%)	
ADV + LdT	15 (7.8%)	8 (8.9%)	7 (6.9%)	
ADV + ETV	109 (56.8%)	49 (54.4%)	60 (58.8%)	
Response to treatments at baseline, n (%)				0.33
Partial virologic response	165 (85.9%)	75 (83.3%)	90 (88.2%)	
Virologic breakthrough	27 (14.1%)	15 (16.7%)	12 (11.8%)	
Previously exposed NUC¶, n (%)				N/A
ADV, LAM, and ETV	112 (58.3%)	51 (56.7%)	61 (59.8%)	
ADV, LAM, LdT, and ETV	24 (12.5%)	9 (10%)	15 (14.7%)	
LAM and ETV	16 (8.3%)	16 (17.8%)	0	
ADV and LAM	15 (7.8%)	1 (1.1%)	14 (13.7%)	
ADV and ETV	11 (5.7%)	7 (7.8%)	4 (3.9%)	
ADV, LAM, and LdT	6 (3.1%)	0	6 (5.9%)	
ETV only	5 (2.6%)	5 (5.6%)	0	
ADV only	2 (1.0%)	0	2 (2%)	
ETV and LdT	1 (0.5%)	1 (1.1%)	0	
Prior treatment with interferon, n (%)	8 (4.2%)	1 (1.1%)	7 (6.9%)	0.07
Overall duration of prior NUC treatment†, months	95 (11–273)	78 (55–117)	109 (11–194)	0.03

ADV, adefovir dipivoxil (10 mg/day); ALT, alanine aminotransferase; BMI, body mass index; ETV, entecavir (1 mg/day); eGFR, glomerular filtration rate estimated by the CKD-EPI equation; HBeAg, HBV e antigen; HBV, hepatitis B virus; INR, international normalized ratio; IQR, interquartile range; LAM, lamivudine (100 mg/day); LdT, telbivudine; NUC, nucleos(t)ide analogue; TDF, tenofovir disoproxil fumarate (300 mg/day).

Between-group comparisons were conducted using the chi-square test for categorical or continuous variables and independent samples *t* test for continuous variables.

*Mean ± SD.

†median (IQR).

‡Cirrhosis was diagnosed using ultrasonography with identification of liver surface nodularity and splenomegaly.

§Genotypic resistance test was performed by both direct sequencing and RFMP analysis. HBV resistance mutation to LAM was defined as rtM204V/I ± rtL180M. HBV resistance mutation to ETV was defined as rtT184A/C/F/G/I/L/S, rtS202G, or rtM250L/V, in addition to rtM204V/I

¶NUCs that were used >6 months. Normal ALT was defined as <40 IU/L.

Among HBeAg-positive patients, the rate of HBeAg seroconversion at week 240 was low (7.1%), with no significant difference between the 2 groups (7.5% *vs.* 6.7%; $p = 0.83$; Table 2).

There was no patient who achieved HBsAg seroclearance. The mean change in HBsAg levels from baseline was −0.45 log_{10} IU/ml in the ETV resistance group and −0.42 log_{10} IU/ml in the ADV resistance group at week 240, without significant between-group difference ($p = 0.33$; Table 2 and Fig. S2). The number of patients with HBsAg level decline ≥1 log_{10} IU/ml from baseline was 15 (16.7%) in the ETV resistance group and 12 (11.8%) in the ADV resistance group ($p = 0.33$; Table 2).

Virologic breakthrough and resistance surveillance

During the 240-week study period, 3 and 4 patients in the ETV resistance and the ADV resistance groups, respectively, showed virologic breakthrough (Table 2), which was related to low medication adherence (<80%) and was transient. No modification of treatment was required. Some of the resistance mutations of

Table 2. Virologic, biochemical, and serologic responses at week 240 (year 5).

Endpoint	All	HBV resistance mutation profiles at baseline		
		ETV resistance (No ADV resistance)	ADV resistance	*p* value
HBV DNA <15 IU/ml, % (n/N)				
Full analysis	78.6 (151/192)	84.4 (76/90)	73.5 (75/102)	0.07
On-treatment analysis	85.8 (151/176)	92.7 (76/82)	79.8 (75/94)	0.02
HBV DNA <60 IU/ml, % (n/N)				
Full analysis	87.0 (167/192)	90.0 (81/90)	84.3 (86/102)	0.24
On-treatment analysis	94.9 (167/176)	98.8 (81/82)	91.5 (86/94)	0.03
HBV DNA change from baseline, $\log_{10}$ IU/ml, mean ± SD	−3.85 ± 1.62	−4.18 ± 1.52	−3.56 ± 1.66	0.01
Residual HBV DNA level[*], IU/ml, median (range)	41 (16–380)	33.5 (20–100)	41 (16–380)	0.44
Virologic breakthrough[†], % (n/N), Full analysis	3.6 (7/192)	3.3 (3/90)	3.9 (4/102)	>0.99
ALT, IU/L, median (range), median (range)	25 (10–137)	26 (11–76)	25 (10–137)	0.98
ALT change from baseline, IU/L, median (range)	−4 (-250–108)	−2 (-216–40)	−4 (-250–108)	0.39
ALT normal[‡], % (n/N), On-treatment analysis	85.8 (151/176)	84.1 (69/82)	87.2 (82/94)	0.56
ALT normal by AASLD criteria[§], % (n/N), On-treatment analysis	74.4 (131/176)	70.7 (58/82)	77.7 (73/94)	0.29
HBeAg seroclearance[¶], % (n/N), Full analysis	27.1 (46/170)	28.8 (23/80)	25.6 (23/90)	0.64
HBeAg seroconversion[¶], % (n/N), Full analysis	7.1 (12/170)	7.5 (6/80)	6.7 (6/90)	0.83
HBeAg seroclearance[¶], % (n/N), On-treatment analysis	29.5 (46/156)	31.5 (23/73)	27.7 (23/83)	0.60
HBeAg seroconversion[¶], % (n/N), On-treatment analysis	7.7 (12/156)	8.2 (6/73)	7.2 (6/83)	0.82
HBsAg seroclearance, % (n/N), On-treatment analysis	0	0	0	–
HBsAg change from baseline, $\log_{10}$ IU/ml, mean ± SD	−0.43 ± 0.49	−0.45 ± 0.47	−0.42 ± 0.50	0.33
HBsAg level decrease from baseline ≥1 $\log_{10}$ IU/ml, % (n/N)	14.1 (27/192)	16.7 (15/90)	11.8 (12/102)	0.33

In the full analysis, patients with missing data were counted as failures. In the on-treatment analysis, patients with missing data were excluded.
AASLD, American Association for the Study of Liver Diseases; ADV, adefovir dipivoxil; ALT, alanine aminotransferase; ETV, entecavir; HBeAg, HBV e antigen; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen.
Between-group comparisons were conducted using the chi-square test, Fisher exact test, or independent samples t test for categorical or continuous variables as appropriate.
[*]In patients who had HBV DNA detectable at week 240.
[†]Cumulative number of patients with virologic breakthrough. Virologic breakthrough was defined as an increase in HBV DNA ≥1 $\log_{10}$ IU/ml from nadir.
[‡]Normal ALT was defined as <40 IU/L by local laboratory criteria.
[§]Normal ALT was defined as ≤25 IU/L for females and ≤35 IU/L for males.
[¶]Among HBeAg-positive patients at baseline.

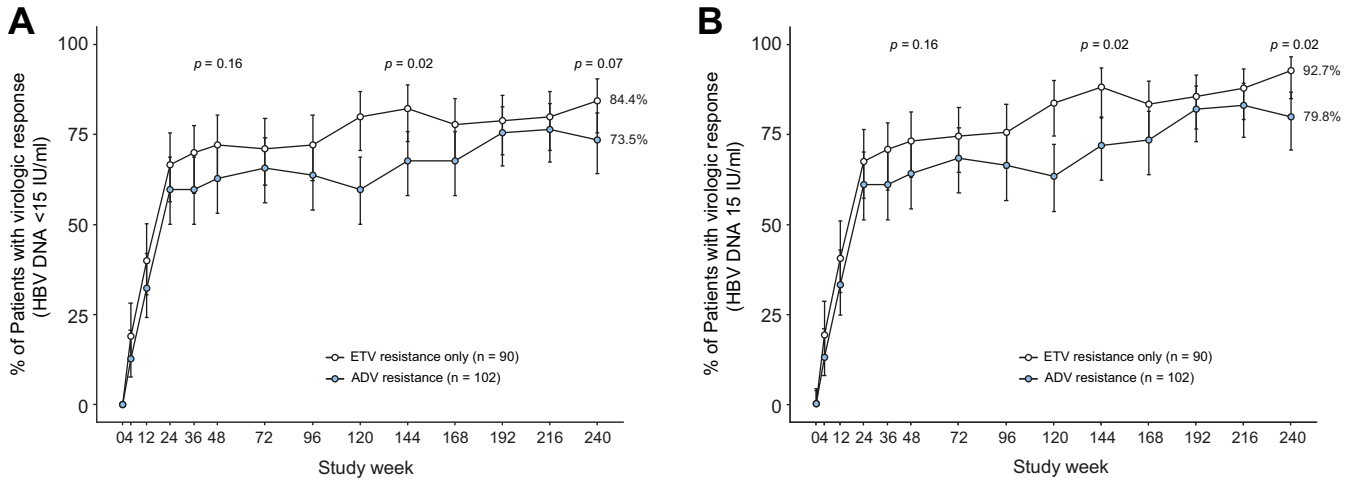

Fig. 2. Proportion of patients with serum HBV DNA levels of <15 IU/ml according to the presence of ADV resistance at baseline. (A) Full analysis set. All the patients randomized were included in the analysis. Patients who discontinued the study prior to week 240 for any reason were considered failures for all end points after the time of discontinuation, regardless of HBV DNA suppression. (B) On-treatment analysis set. All the study patients with no missing data were included in the analysis. Error bars indicate 95% CI. The *p* values are for the between-group comparisons at week 48, 144, and 240 by chi-square test. ADV, adefovir dipivoxil; ETV, entecavir; HBV, hepatitis B virus.

HBV that were present at baseline were detected during the virologic breakthrough without emergence of additional resistance substitutions (Table 3).

Of 12 patients who stopped the study early, none experienced virologic breakthrough at dropout, but 2 had serum HBV DNA >60 IU/ml at the last time point in the study and were assessed for genotypic resistance analysis; no additional resistance substitutions were identified compared with the baseline (Table 3).

At week 240, 1 patient in the ETV resistance group and 8 patients in the ADV resistance group had HBV DNA >60 IU/ml ($p = 0.04$) and were assessed for genotypic resistance. Two patients in the ADV resistance group had at least 1 detectable HBV resistance mutation at week 240 (Table 3), which were

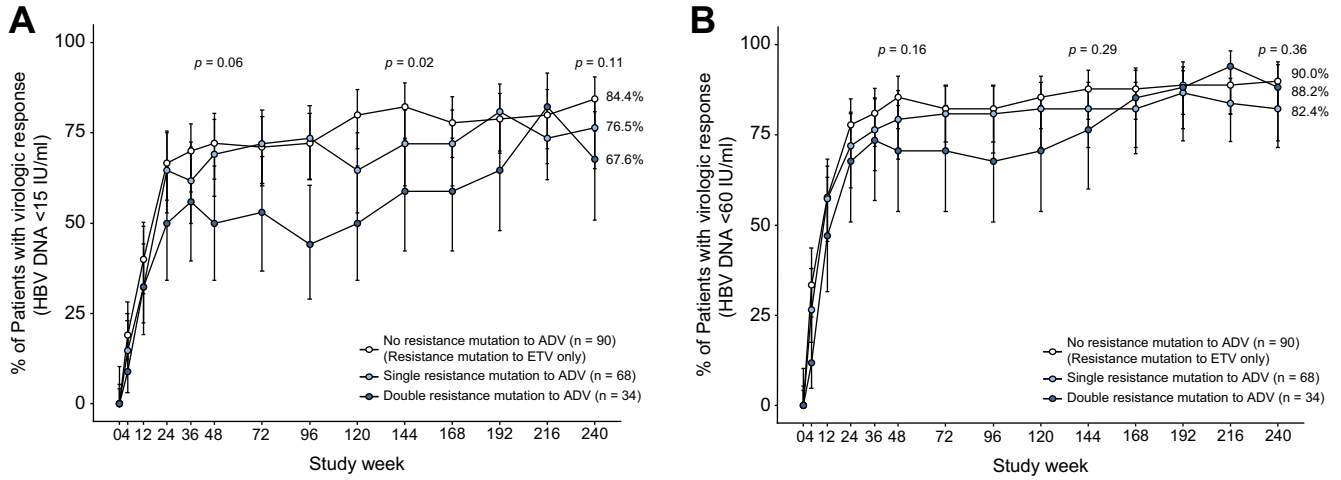

Fig. 3. Proportion of patients with virologic response according to ADV-resistance mutation profiles at baseline in the full analysis set. (A) Proportion of patients with serum HBV DNA <15 IU/ml. (B) Proportion of patients with serum HBV DNA <60 IU/ml. All the patients randomized were included in the analysis. Patients who discontinued the study prior to week 240 for any reason were considered failures for all end points after the time of discontinuation, regardless of HBV DNA suppression. Error bars indicate 95% confidence intervals. The *p* values are for the between-group comparisons at weeks 48, 144, and 240 by chi-square test. ADV, adefovir dipivoxil; ETV, entecavir; HBV, hepatitis B virus.

Table 3. Resistance surveillance in patients with a virologic breakthrough or HBV DNA >60 IU/ml at dropout and week 240.

Study group	Patient No.	HBV DNA level at test (log_{10} IU/ml)	Week at test	Treatment at test	HBV mutation at baseline	HBV mutation at test
Patients with virologic breakthrough						
ETV resistance	1,001	7.99	48	TDF	rtM204V + rtL180M + rtM250V	rtM204V + rtL180M + rtM250V
ETV resistance	1,012	2.90	192	TDF	rtM204V/I + rtL180M + rtM202G	not detected
ETV resistance	1,062	2.62	120	TDF	rtM204V + rtL180M + rtM250V	rtM204V + rtL180M + rtM250V
ADV resistance	2,064	8.04	36	TDF	rtM204V/I + rtL180M + rtA181T	rtA181T
ADV resistance	2,014	5.36	120	TDF	rtM204V/I + rtL180M + rtA181T/V + rtM202G	rtA181T
ADV resistance	2,015	2.59	96	TDF	rtM204V/I + rtL180M + rtA181T/V + rtN236T	not detected
ADV resistance	2,045	2.00	36	TDF/ETV	rtM204V/I + rtL180M + rtA181T/V + rtN236T + rtT184L	rtM204V/I + rtL180M + rtT184L
Patients with HBV DNA >60 IU/ml at discontinuation						
ETV resistance	1,001	2.68	96	TDF	rtM204V + rtL180M + rtM250V	rtM204V + rtL180M + rtM250V
ADV resistance	2,098	2.84	60	TDF	rtM204V/I + rtL180M + rtA181T/V + rtM202G	rtL180M + rtM202G
Patients with HBV DNA >60 IU/ml at week 240						
ETV resistance	1,012	2.00	240	TDF	rtM204V/I + rtL180M + rtM202G	not detected
ADV resistance	2,064	2.58	240	TDF	rtM204VI + rtL180M + rtA181T	A181T
ADV resistance	2,014	2.20	240	TDF	rtL180M + rtM204VI + rtA181T + rtS202G	not detected
ADV resistance	2,100	2.13	240	TDF	rtA181T/V	not detected
ADV resistance	2,050	2.00	240	TDF	rtA181TV + rtN236T + rtM250V	A181T + rtN236T
ADV resistance	2,030	1.99	240	TDF	rtM204V/I + rtL180M + rtA181T/V + rtN236T	not detected
ADV resistance	2,036	1.90	240	TDF	rtM204V/I + rtL180M + rtA181T/V + rtT184L + rtS202G	not detected
ADV resistance	2,005	1.82	240	TDF	rtM204V/I + rtL180M + rtA181T	not detected
ADV resistance	2,020	1.80	240	TDF	rtM204V/I + rtL180M + rtA181T/V + rtN236T + rtM202G + rtM250V	not detected

Genotypic resistance test was performed by both direct sequencing and restriction fragment mass polymorphism analysis. ADV, adefovir dipivoxil; ETV, entecavir; HBV, hepatitis B virus.

present at baseline. None of the patients developed additional resistance substitutions compared with baseline.

Safety

Table 4 summarizes safety profiles during 240 weeks of treatment with TDF. Reasons for discontinuing the study before week 240 were withdrawal of consent for logistical reasons (n = 8), adverse events (n = 3), death by HCC (n = 3), and death by other causes (n = 2). Among 39 serious adverse events, none was considered to be related with the study drugs. HCC was diagnosed in 3 and 5 patients in the ETV resistance and ADV resistance groups, respectively. One patient in the ETV

Table 4. Safety profiles.

Adverse event category	Total	ETV resistance (No ADV resistance)	ADV resistance
N	192	90	102
Serious adverse events	39 (20.3%)	24	15
Hepatocellular carcinoma	8 (4.2%)	3	5
Deaths	5 (2.6%)	3	2
Discontinuation due to adverse event[†]	3 (1.6%)	0	3
Dose reduction due to adverse event[‡]	2 (1.0%)	0	2
Bone fracture[§]	3 (1.6%)	1	2
ALT flare[*]	9 (4.7%)	4	5
eGFR decrease ≥20%	10 (5.2%)	6	4
Serum phosphate <2.0 mg/dl	2 (1.0%)	2	0

ADV, adefovir dipivoxil; ALT, alanine aminotransferase; eGFR, estimated glomerular filtration rate by CKD-EPI equation; ETV, entecavir; TDF, tenofovir disoproxil fumarate.
[*]ALT flares (increase in ALT >5 times the upper limit of the normal range) were not associated with an increase in HBV DNA levels.
[†]Due to epigastric pain by TDF, headache by ETV, and ALT flare of uncertain cause.
[‡]TDF dose was reduced owing to transient elevation of creatinine level attributed to the use of non-steroidal anti-inflammatory drugs, which was not suspected to be associated with TDF treatment.
[§]Bone fractures were associated with trauma.

resistance group died of sudden cardiac arrest and 1 in the ADV resistance group died of sleep apnea.

ALT flares (rise of ALT >5 times the upper normal limit) were observed in 9 patients throughout the study period. Five patients had the flare within the first 12 weeks of treatment in association with a rapid reduction in serum HBV DNA levels of >2 $\log_{10}$ IU/ml, while 4 patients had the flare at a later period of the study without a preceding increase in HBV DNA levels (Fig. S3). No flares were associated with bilirubin increase or hepatic decompensation. ALT levels returned to baseline levels with continued treatment in all patients, except 1 patient who received herbal medication prior to the flare and withdrew from the study. All patients were HBeAg(+) at baseline, and 4 patients with the former pattern and 1 patient with the latter pattern of ALT flare experienced HBeAg seroclearance during the study. Concomitant medications and abrupt body weight gain were suspected as causes for the latter pattern of ALT flares.

The estimated GFR (eGFR) by CKD-EPI equation significantly decreased from baseline at 240-weeks (−3.21 ml/min/1.73 m^2, p <0.001) with no significant difference between the ETV and ADV resistance groups (p = 0.52; Fig. 4A).

The mean percent decrease in BMD (g/cm^2) at week 240 from baseline was significant at the femur (−2.48%, p <0.001) but not at the spine (−0.63%, p = 0.23; Fig. 4B). Baseline DXA scans for the lumbar spine and femur showed that 15 patients (7.8%) had osteoporosis (T scores, <−2.5) and 66 (34.4%) had evidence of osteopenia (T-score range, −1 to > − 2.5). At week 240, the numbers of patients with osteoporosis and osteopenia were 10 (5.8%) and 68 (39.5%), respectively, which did not differ significantly compared with the numbers at baseline (p = 0.26). Bone fractures associated with trauma occurred in 3 patients (at weeks 72, 112, and 136). Females experienced a greater decline in eGFR and femur BMD than males during the study period (Fig. S4).

Discussion

Our present 5-year trials in patients with CHB and multidrug-resistant HBV after exposure to multiple NUCs, showed that the virologic response rate gradually increased with TDF monotherapy, irrespective of the presence of ADV resistance at baseline. Although the virologic response rate in patients

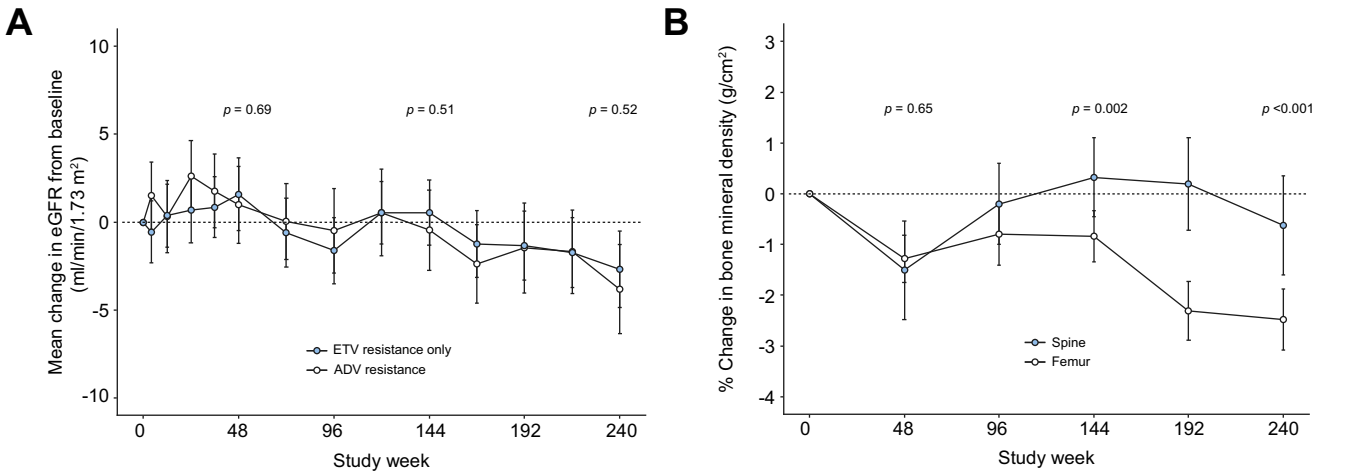

Fig. 4. Changes in eGFR and BMD from baseline. (A) eGFR by CKD-EPI equation. (B) Mean percent changes in BMD determined on dual-energy x-ray absorptiometry scan. All the study patients with non-missing data were included in the analysis. Error bars indicate 95% CI. The p values are for the between-group comparisons at week 48, 144, and 240 by Independent samples t test. ADV, adefovir dipivoxil; BMD, bone mineral density; eGFR, estimated glomerular filtration rate; ETV, entecavir.

with ADV resistance was slower than that in those with ETV resistance, most patients in both studies showed effective viral suppression with TDF monotherapy at week 240. TDF monotherapy was associated with a marked reduction and no additional emergence in detectable HBV resistance mutations. A few virologic blips occurred during the study period, which were related with low adherence to TDF. The overall retention rate of the study patients was high (91.7%) at week 240, and the treatment was well tolerated. However, the HBeAg seroconversion rate was only 7.1% and none achieved HBsAg seroclearance at week 240. Significant decreases in eGFR and femoral BMD were observed between weeks 144 and 240.

Previous *in vitro* studies showed that HBV mutants with ADV-resistance mutations, namely rtA181T/V and/or rtN236T, demonstrated reduced susceptibility to tenofovir, ranging from 2.9- to 10-fold of that of the wild-type HBV.[18] The presence of a double ADV-resistance mutation (rtA181T/V and rtN236T) was associated with a further 7- to 10-fold reduction in susceptibility to tenofovir, compared with that of the single rtA181T mutant.[18,19] Several cohort studies also showed low TDF efficacy in patients with ADV-resistant HBV.[20,21] The short-term probability of achieving HBV DNA <400 copies/ml with TDF monotherapy was significantly impaired in patients with ADV-resistant HBV compared to those without ADV-resistant HBV.[21] We also previously reported a significantly lower virologic response rate in patients with double ADV-resistance mutations than those with no or single resistance mutations to ADV, when receiving up to 144 weeks of TDF monotherapy.[12] However, our present study showed that most of the patients with double ADV-resistance mutations at baseline achieved virologic response or had extremely low levels of HBV DNA (median, 41 IU/ml), and there was a remarkable reduction in detectable resistance mutations at week 240. The increasing rate of virologic response without emergence of additional resistance mutations up to 240-weeks of TDF monotherapy was reassuring. These findings may be associated with the sufficiently high TDF concentrations *in vivo* to suppress the replication of HBV variants, which may prevent the accumulation of additional substitutions.[22]

Most patients without virologic response at week 240 had low-level viremia in the range between 16 IU/ml and 380 IU/ml. It was not within the scope of this study to analyze the clinical significance of this persistent low-level viremia. However, it is notable that many previous studies have shown that clinically significant level of HBV DNA is >2,000 IU/ml, and many previous pivotal clinical trials for ETV and TDF have used the definition of virologic response as HBV DNA <60 IU/ml.[17,23–26]

Although most patients achieved and maintained a virologic response, the HBeAg seroconversion rate and HBsAg level decrease during long-term TDF treatment were minimal in this study. Most patients (88.5%) were HBeAg positive at baseline, and the HBeAg seroconversion rate was only 7.1% at 240 weeks, which is a striking contrast to the HBeAg seroconversion rate of 39.6% reported with 336 weeks of TDF therapy in treatment-naive patients with CHB.[27] Our data are even lower than the HBeAg seroconversion rate of 11.3% achieved with 240 weeks of TDF therapy in patients with LAM resistance.[28] These findings suggest the influence of host immune factors in achieving serological responses and indicate that prolonged treatment is inevitable in patients with multiple drug-resistant HBV variants.

ALT flares occurred in 9 patients during the study, and showed 2 distinct patterns: first, those associated with a robust decline in serum HBV DNA levels within the first 12 weeks of TDF therapy (5 patients); second, those occurring at a later period of the study without a preceding increase in HBV DNA levels (4 patients). The first pattern of ALT flares has previously been reported to occur in up to 10% of patients during NUC therapy, and suggested to be associated with a transient restoration of HBV-specific T cell responses,[29] as supported by high rate of HBeAg seroclearance (4 of 5 patients) in our patients. Concomitant medications and abrupt body weight gain were suspected as possible causes for the latter pattern of ALT flares.

Although treatment with TDF was well tolerated, eGFR by the CKD-EPI equation began to decrease after week 144. The decrease in femoral BMD was also accelerated from week 144. Interestingly, the decrease in spine BMD was not significant. Although the reasons for the discrepancy in BMD changes between the femur and spine are not known, these findings are similar with those observed for 240 weeks of TDF therapy in patients with LAM-resistant HBV.[28] Clinically overt renal impairment was not observed, and bone fractures were rare in our study patients. However, the progressive decreases in eGFR and femoral BMD were worrisome, given the necessity of long-term TDF treatment in most patients with multidrug-resistant HBV.

This study has potential limitations. First, this was an extended open-label single-arm study. Original randomized comparisons between TDF monotherapy and TDF + ETV combination therapy for 48 weeks yielded no significant difference in antiviral efficacy.[10,11] Nevertheless, longer-term data were required to better evaluate the safety and efficacy of TDF monotherapy in a prospective manner. Second, all the patients in this study had genotype C HBV. Although this may limit the generalizability of the results, we did not find any evidence that the antiviral efficacy of TDF is influenced by HBV genotypes.[25,30] That said, because a broad range of inclusion criteria were applied in our trials, including cirrhotic patients and various degrees of HBV resistance, we believe that our results could be generalized to the real-world population of heavily pretreated patients with chronic HBV infection. Third, analysis of the markers for subclinical renal tubular injury, such as urine beta-2-microglobulin and urine retinol-binding protein, were not planned and could not be measured in this study. Of note, a randomized controlled trial to evaluate the safety and efficacy of switching to tenofovir alafenamide (TAF) and incorporating biomarkers for renal tubular dysfunction was recently initiated in patients with multidrug-resistant CHB receiving long-term TDF monotherapy (IN-US-320-4390; NCT03241641).

In conclusion, up to 240 weeks of TDF monotherapy provided a slowly increasing virologic response rate in patients chronically infected with multidrug-resistant HBV following previous exposure to multiple NUCs, regardless of the presence of ADV-resistant HBV mutants. Virologic breakthrough was rare and was not associated with the emergence of additional resistance mutations. Nonetheless, the HBeAg seroconversion rate was very low, and no patient achieved HBsAg seroclearance up to week 240, which suggests the necessity of prolonged continuous treatment to maintain viral suppression in these patients. However, the progressive and significant decrease in renal function and BMD after week 144 raises concerns about the safety of TDF therapy in a longer-term setting. Further studies to evaluate the safety and efficacy of TAF, which may provide improved renal and bone safety than TDF, are warranted in patients with multiple drug-resistant HBV.

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Conflict of interest

This work was financially supported by Gilead Sciences, who also provided the study drug (tenofovir disoproxil fumarate). Gilead Sciences was permitted to review the manuscript and suggest changes but had no role in the study design, data collection, analysis, decision to publish, or preparation of the manuscript. The final decision on content was exclusively retained by the authors. YS Lim is an advisory board member of Bayer Healthcare and Gilead Sciences, and receives research funding from Bayer Healthcare and Gilead Sciences. The remaining authors have nothing to disclose that would be relevant for the publication of this manuscript.

A part of this study (5-year results) was presented at the Liver Meeting of the American Association for the Study of Liver Diseases, San Francisco, on November 12, 2018.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

The principal investigator, YS Lim, was responsible for the conception and design of the study; the acquisition, analysis, and interpretation of the data; and the drafting of the manuscript. GY Gwak, HC Lee, J Choi, KS Byun, YJ Kim, BC Yoo, SY Kwon, and YS Lee helped with the design of the study, acquisition of data, and critical revision of the manuscript regarding intellectual content. YS Lee and HC Lee supervised the study.

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Supplementary data

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