Tenofovir Monotherapy versus Tenofovir plus Lamivudine or Telbivudine Combination Therapy in Treatment of Lamivudine-Resistant Chronic Hepatitis B

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Tenofovir disoproxil fumarate (TDF) monotherapy is a therapeutic option for chronic hepatitis B (CHB) patients infected with hepatitis B virus (HBV) variants resistant to lamivudine (LAM). We evaluated the antiviral efficacy and safety of TDF alone compared to those of TDF plus LAM or telbivudine (LdT) combination in patients harboring HBV variants with genotypic resistance to LAM. This multicenter retrospective study included consecutive patients who had LAM-resistant HBV variants and were treated with TDF alone (monotherapy group) or TDF combined with LAM or LdT (combination therapy group) for at least 6 months. Inverse probability of treatment weighting (IPTW) for the entire cohort was applied to control for treatment selection bias. Overall, 153 patients (33 in the monotherapy group and 120 in the combination therapy group) were analyzed. The overall probability of achieving complete virologic suppression at month 12 was 91.6%: 88.6% in the monotherapy group and 92.6% in the combination therapy group. Combination therapy was not superior to monotherapy in viral suppression before and after IPTW ($P = 0.562$ and $P = 0.194$, respectively). Hepatitis B e antigen (HBeAg) loss, biochemical response, and virologic breakthrough did not differ between treatment groups. The probabilities of complete virologic suppression were comparable between treatment groups in the subsets according to HBeAg status and HBV DNA levels at baseline. No patient experienced any significant renal dysfunction during the treatment period. In conclusion, TDF monotherapy has antiviral efficacy comparable to that of TDF plus LAM or LdT combination therapy, with a favorable safety profile in CHB patients with LAM-resistant HBV variants.

Lamivudine (LAM), which is the first oral antiviral drug approved for chronic hepatitis B (CHB) treatment, has been widely prescribed for patients with chronic hepatitis B virus (HBV) infection (1). However, the major drawback of LAM is a low barrier to resistance; resistance to LAM occurs in approximately 24% of patients after 1 year of treatment and progressively increases, such that 70% of patients demonstrate resistance after 5 years of LAM therapy (2, 3).

Based on clinical evidence (4, 5), international guidelines recommend adding on adefovir (ADV) therapy rather than switching to ADV or entecavir (ETV) monotherapy as a treatment for CHB patients infected with HBV variants resistant to LAM (6, 7). However, because combination therapy with LAM and ADV has limited antiviral efficacy in LAM-resistant patients, a substantial number of patients show persistent viremia while on the rescue therapy with LAM plus ADV combination, which may then result in selection for multidrug-resistant HBV variants and progression of liver disease (8, 9). Other treatment options for patients harboring LAM-resistant HBV include tenofovir disoproxil fumarate (TDF) with or without other nucleoside analogues. Prolonged *in vitro* and clinical studies demonstrated that TDF has antiviral activity against LAM-resistant HBV isolates as well as wild-type isolates superior to that of ADV (10–14). In a previous retrospective cohort study, the virologic response to TDF monotherapy was not impaired by the presence of substitutions associated with LAM resistance (15). Moreover, a recent clinical trial demonstrated that TDF monotherapy has an efficacy comparable to that of TDF plus

emtricitabine (FTC) combination therapy, without resistance development in patients with LAM-resistant HBV variants (16, 17).

However, whether switching to TDF monotherapy is as effective as adding TDF to LAM in patients harboring HBV variants with genotypic resistance to LAM is still controversial (2, 18, 19). Therefore, we aimed to assess the antiviral efficacy and safety of TDF monotherapy compared to combination therapy with TDF plus LAM or telbivudine (LdT) in CHB patients with confirmed genotypic resistance to LAM.

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MATERIALS AND METHODS

Study population. This study was a multicenter retrospective cohort study and included CHB patients from three large-volume centers in Korea who developed LAM resistance and were treated with TDF monotherapy or combination therapy with TDF plus LAM or LdT as rescue therapy for at least 6 months. LAM resistance was defined as the presence of HBV variants with amino acid substitutions conferring resistance against LAM (rtM204V/I $\pm$ rtL180M). In total, 153 consecutive patients who started these therapeutic regimens from May 2012 to October 2013 were included. Patients with the following conditions at the initiation of rescue treatment were excluded: prior exposure to TDF; presence of genotypic resistance to ADV or ETV; a serum HBV DNA level of <50 IU/ml; a creatinine clearance of <50 ml/min, estimated by the Cockcroft-Gault formula; or a history of organ transplantation or coinfection with hepatitis C virus, hepatitis D virus, or human immunodeficiency virus (HIV).

This study was conducted in accordance with the ethical guidelines of the World Medical Association Declaration of Helsinki and approved by the Institutional Review Board of each investigational site.

Definitions and study endpoints. The primary endpoint was the achievement of complete virologic suppression, which was defined as an HBV DNA level of <20 IU/ml by quantitative PCR assay. Secondary endpoints were the reduction in serum HBV DNA level from baseline, biochemical response (normalization of serum alanine aminotransferase [ALT]), serologic response (loss of hepatitis B e antigen [HBeAg]), and virologic breakthrough during the treatment period. Virologic breakthrough was defined as an increase of serum HBV DNA level of >1 log₁₀ IU/ml above the lowest level achieved during rescue treatment (2, 18).

Study measurements. Serum levels of ALT, creatinine, phosphorus, HBV DNA, HBeAg, and anti-hepatitis B e antibody were assessed for all patients every 2 to 3 months. Serum HBV DNA levels were measured at baseline and each follow-up visit using either Roche COBAS TaqMan (lower limit of detection, 20 IU/ml; Roche Molecular Systems, Branchburg, NJ) or Abbott m2000 (lower limit of detection, 15 IU/ml; Abbott Diagnostics, Chicago, IL) (20). At baseline, testing for genotypic resistance, which was defined as the detection of HBV variants with substitutions conferring antiviral drug resistance, was performed for all study patients and was repeated for patients who experienced virologic breakthrough during the treatment period. Amino acid substitutions conferring resistance to LAM (rtM204V/I/S and rtL180M), ADV (rtA181T/V and rtN236T), and ETV (rtL180M + rtM204V/I $\pm$ rtI169T $\pm$ rtV173L $\pm$ rtM250V/I/L/M $\pm$ rtT184S/A/I/L/G/C/M $\pm$ rtS202I/G) were analyzed (2, 19). Direct PCR-based DNA sequencing using the BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA) with an ABI Prism 3730 genetic analyzer (Perkin-Elmer, Foster City, CA) or matrix-assisted laser desorption/ionization–time of flight mass spectrometry using an ABI Prism 3500 genetic analyzer (Applied Biosystems) was performed for genotypic testing as previously described (21, 22).

Statistical analysis. Group-wise comparisons were performed using the Mann-Whitney *U* test for continuous variables and using χ^2 test or Fisher's exact test for categorical variables. Times to events and cumulative probabilities were estimated with the Kaplan-Meier method and compared using the log rank test. The Cox proportional hazards model was applied to identify independent factors associated with complete virologic suppression. Subgroup analyses were also conducted according to HBV DNA levels and the status of HBeAg at baseline.

Inverse probability of treatment weighting (IPTW) based on the propensity score was applied to adjust for differences in baseline characteristics between treatment groups (23, 24). Propensity scores were generated using a logistic regression model in which baseline clinical and demographic characteristics were included and treatment with TDF monotherapy was deemed an outcome. The calculated propensity score represents the probability of being treated with TDF monotherapy. This propensity model yielded a c-statistic of 0.822. Each patient was then weighted by the inverse of the probability of receiving the treatment that

the patient received in reality. After IPTW, the balance of baseline characteristics between treatment groups was verified, and weighted Cox models were fitted thereafter. All tests were conducted as 2-sided tests, and a *P* value of <0.05 was considered statistically significant. SAS version 9.3 (SAS Inc., Cary, NC) and PASW version 18.0 (IBM, Chicago, IL) were used for all statistical analyses.

RESULTS

Characteristics of the study population. Thirty-three patients were treated with TDF (300 mg once daily) monotherapy (monotherapy group), and 120 patients were treated with TDF plus an L-nucleoside (LAM [100 mg once daily] or LdT [600 mg once daily]) combination therapy (combination therapy group; 58 patients received the TDF-LAM combination and 62 patients received the TDF-LdT combination). Of the 153 study patients, 141 were directly switched from their previous antiviral treatment to the current rescue treatment. Genotypic resistance to LAM but not to ETV or ADV was confirmed in all study patients at baseline of rescue treatment. The median duration of the rescue therapy was 15.6 (range, 7.2 to 25.0) months. Table 1 summarizes baseline clinical and demographic characteristics of the 153 patients. The baseline characteristics, including age, gender, serum levels of HBV DNA, ALT, and creatinine, liver cirrhosis, and duration of prior antiviral treatment, were not significantly different between treatment groups. However, patients in the combination therapy group had experienced more lines of prior antiviral treatment and had a higher proportion of prior ADV exposure ($P = 0.016$ and $P = 0.037$, respectively). The proportion of HBeAg-positive patients in the combination therapy group was higher than in the monotherapy group; however, the difference was not statistically significant ($P = 0.056$).

Virologic responses. The overall mean changes in serum HBV DNA levels at months 3, 6, and 9 were -2.67 log₁₀ IU/ml, -3.01 log₁₀ IU/ml, and -3.31 log₁₀ IU/ml, respectively, and the decline in HBV DNA levels was not significantly different between treatment groups at any time point ($P = 0.450$, $P = 0.135$, and $P = 0.087$, respectively) (Table 2). Overall, 140 out of 153 patients (91.5%) achieved complete virologic suppression during the complete observation period: 29 of 33 (87.9%) in the monotherapy group and 111 of 120 (92.5%) in the combination therapy group. The median times required to reach an HBV DNA level of <20 IU/ml were 3.6 (95% confidence interval [CI], 1.7 to 5.4) months in the monotherapy group and 3.2 (95% CI, 2.8 to 3.7) months in the combination therapy group. The probabilities of complete virologic suppression after 12 months of rescue treatment were 88.6% in the monotherapy group and 92.6% in the combination therapy group (Table 2). There was no significant difference between treatment groups during the treatment period ($P = 0.562$) (Fig. 1). Among the pretreatment clinical factors, rescue therapy regimen was not an independent predictor for complete virologic suppression (monotherapy versus combination therapy; hazard ratio [HR], 1.007; 95% CI, 0.661 to 1.535; $P = 0.974$) after adjustment for HBV DNA level, HBeAg status, the presence of cirrhosis, and biochemical breakthrough at baseline in the multivariate Cox regression analysis (Table 3).

The cumulative probabilities of achieving complete virologic suppression were comparable between treatment groups among both HBeAg-positive patients (monotherapy versus combination therapy; HR, 0.699; 95% CI, 0.378 to 1.294; $P = 0.249$) (see Fig. S1A in the supplemental material) and HBeAg-negative patients

TABLE 1 Baseline demographics and disease characteristics by treatment group

Characteristic	Monotherapy (<i>n</i> = 33)	Combination therapy (<i>n</i> = 120)	<i>P</i> value ^a
Age (yrs) ^b	54 (31–74)	54 (25–75)	0.875
Gender, male, <i>n</i> (%)	22 (66.7)	84 (70.0)	0.832
Serum HBV DNA (log ₁₀ IU/ml) ^b	2.83 (1.75–8.23)	2.77 (1.71–8.23)	0.238
Serum ALT (IU/liter) ^b	29 (9–522)	26 (9–1,103)	0.283
>ULN, ^c <i>n</i> (%)	12 (36.4)	31 (25.8)	0.275
Serum creatinine (mg/dl) ^b	0.90 (0.49–1.30)	0.90 (0.50–1.40)	0.552
HBeAg, positive, <i>n</i> (%)	16 (48.5)	80 (66.7)	0.056
Liver cirrhosis, ^d <i>n</i> (%)	8 (24.2)	27 (22.5)	0.833
Lines of prior antiviral treatment	2 (1–5)	3 (1–5)	0.016
Duration of prior antiviral treatment (mo) ^b	74.2 (18.0–155.7)	79.3 (7.0–171.9)	0.761
Prior or current ADV, <i>n</i> (%)	21 (63.6)	97 (80.8)	0.037
Time point of rescue therapy			
Virologic breakthrough, <i>n</i> (%)	16 (48.5)	56 (46.7)	0.853
Biochemical breakthrough, <i>n</i> (%)	12 (36.4)	31 (25.8)	0.233

^a Mann-Whitney *U* test and χ^2 test (or Fisher's exact test) were used to analyze the differences between treatment groups.

^b Data are medians, and data in parentheses are ranges.

^c ULN, upper limit of normal.

^d Liver cirrhosis was diagnosed when the platelet count was below 100,000/mm³ and associated splenomegaly or esophageal-gastric varices were detected.

(monotherapy versus combination therapy; HR, 0.837; 95% CI, 0.467 to 1.499; *P* = 0.548) (see Fig. S1B in the supplemental material). In the entire cohort, the probability of complete virologic suppression was significantly influenced by HBV DNA level at the

initiation of rescue treatment (HBV DNA level of <10³ IU/ml versus >10³ IU/ml; HR, 2.212; 95% CI, 1.559 to 3.139; *P* < 0.001) (see Fig. S2 in the supplemental material). The probabilities of complete virologic suppression were comparable between the

TABLE 2 Virologic, biochemical, and serologic responses by treatment group^a

Outcome	Monotherapy (<i>n</i> = 33)	Combination therapy (<i>n</i> = 120)	<i>P</i> value
Virologic response			
Change in HBV DNA (log ₁₀ IU/ml), median (range)			
3 mo	−2.47 (−8.23 to −0.27)	−2.39 (−7.93 to 0.51)	0.450
6 mo	−2.83 (−8.23 to −0.91)	−2.58 (−8.23 to 0.86)	0.135
9 mo	−3.70 (−8.23 to −1.29)	−2.77 (−8.23 to 0.52)	0.087
Complete virologic suppression			0.562 ^b
3 mo	42.4 (33)	35 (120)	
6 mo	72.7 (33)	75.2 (120)	
9 mo	84.8 (31)	87.9 (116)	
12 mo	88.6 (25)	92.6 (103)	
Virologic breakthrough			0.761 ^b
3 mo	0 (33)	0 (120)	
6 mo	0 (33)	1.7 (120)	
9 mo	0 (31)	1.7 (116)	
12 mo	5.6 (25)	1.7 (103)	
15 mo	5.6 (18)	3.4 (68)	
Biochemical response (normalization of serum ALT)			0.222 ^b
3 mo	33.3 (12)	29 (31)	
6 mo	66.7 (12)	45.2 (31)	
9 mo	66.7 (11)	58.1 (29)	
12 mo	83.3 (9)	66.5 (26)	
15 mo	83.3 (6)	72 (19)	
Serologic response (HBeAg loss)			0.115 ^b
3 mo	15.4 (16)	4.5 (80)	
6 mo	30.8 (16)	7.6 (80)	
9 mo	30.8 (15)	12.6 (77)	
12 mo	30.8 (12)	16.4 (68)	
15 mo	30.8 (10)	20.1 (45)	

^a Unless otherwise indicated, data are cumulative probabilities, of the response at the indicated time points, based on the Kaplan-Meier method (no. of patients under observation).

^b Log rank test was used to compare the hazard rates between treatment groups.

FIG 1 Probability of complete virologic suppression by treatment group. Cumulative probabilities of complete virologic suppression, HBV DNA levels of <20 IU/ml, during the treatment period are shown for each group. TDF, tenofovir disoproxil fumarate; LAM, lamivudine; LdT, telbivudine.

monotherapy group and the combination therapy group within the subgroups of both patients with baseline HBV DNA levels of <10³ IU/ml (monotherapy versus combination therapy; HR, 0.958; 95% CI, 0.552 to 1.662; *P* = 0.877) (see Fig. S3A in the supplemental material) and those with >10³ IU/ml (HR, 0.852; 95% CI, 0.458 to 1.584; *P* = 0.610) (see Fig. S3B in the supplemental material).

Biochemical and serologic responses. Thirty-one out of 43 patients (72.1%) who had elevated ALT levels above the upper limits of the normal range achieved biochemical response during the treatment period. The cumulative probabilities of biochemical response after 12 months of treatment were 83.3% in the mono-

therapy group and 66.5% in the combination therapy group, which were comparable between treatment groups (monotherapy versus combination therapy; HR, 1.594; 95% CI, 0.748 to 3.396; *P* = 0.222) (Table 2).

HBeAg loss was observed in 15 patients (15.6%) in the subset of 96 HBeAg-positive patients, with comparable results observed between treatment groups. At month 12, the probabilities of HBeAg loss were 30.8% in the monotherapy group and 20.1% in the combination therapy group (monotherapy versus combination therapy; HR, 2.447; 95% CI, 0.776 to 7.717; *P* = 0.115) (Table 2).

Virologic breakthrough. Four patients developed virologic breakthrough during the observation period: 1 patient in the monotherapy group and 3 patients in the combination therapy group. The probability of developing virologic breakthrough after 12 months of treatment was 2.3% overall: 5.6% in the monotherapy group and 1.7% in the combination therapy group (Table 2). There was no significant difference between treatment groups during the observation period (monotherapy versus combination therapy; HR, 1.419; 95% CI, 0.147 to 13.731; *P* = 0.761) (Fig. 2). For the 4 patients who experienced virologic breakthrough, rtA181T/V, rtN236T, and rtM250L substitutions were newly detected at the time of virologic breakthrough in 1 patient who exhibited rtM204V and rtL180M substitutions at baseline and received TDF alone. However, no additional substitution, other than substitutions detected at baseline, was detected in the other 3 patients in the combination therapy group.

Treatment response analysis after inverse probability of treatment weighting. After adjusting for treatment selection bias by means of IPTW, baseline characteristics, including the status of HBeAg, the number of lines of prior antivirals, and prior ADV exposure, became well balanced between treatment groups (see Table S1 in the supplemental material).

Weighted cumulative probabilities of achieving complete virologic suppression after 12 months of treatment were 94.2% in the monotherapy group and 92.3% in the combination therapy group, which were still comparable between treatment groups (*P* = 0.194) (Fig. 3). When a weighted Cox proportional hazards model was fitted, rescue therapy regimen was not independently associated with complete virologic suppression (monotherapy versus combination therapy; HR, 1.266; 95% CI, 0.832 to 1.926;

TABLE 3 Univariate and multivariate analysis of the factors predictive of complete virologic suppression

Variable	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	<i>P</i> value ^a	Adjusted hazard ratio (95% CI)	<i>P</i> value ^a
Age (per year increase)	1.006 (0.991–1.021)	0.452		
Baseline HBV DNA (per 1 log ₁₀ IU/ml increase)	0.762 (0.679–0.856)	<0.001	0.816 (0.720–0.924)	0.001
ALT (per IU/liter increase)	0.998 (0.995–1.001)	0.138		
HBeAg (positive vs. negative)	0.598 (0.424–0.844)	0.003	0.547 (0.376–0.797)	0.002
Liver cirrhosis (yes vs. no) ^b	0.613 (0.407–0.925)	0.020	0.534 (0.342–0.834)	0.006
Time point of rescue therapy				
Virologic breakthrough (yes vs. no)	0.970 (0.695–1.353)	0.856		
Biochemical breakthrough (yes vs. no)	0.485 (0.326–0.721)	<0.001	0.713 (0.453–1.120)	0.142
Prior or current ADV (yes vs. no)	0.940 (0.630–1.403)	0.761		
Rescue therapy regimen (TDF vs. TDF-LAM/LdT)	0.887 (0.588–1.336)	0.565	1.007 (0.661–1.535)	0.974

^a *P* values were determined using Cox proportional hazards regression models. A *P* value of <0.05 indicated a significant difference.

^b Liver cirrhosis was diagnosed when the platelet count was below 100,000/mm³ and associated splenomegaly or esophageal-gastric varices were detected.

FIG 2 Probability of virologic breakthrough by treatment group. Cumulative probabilities of virologic breakthrough, increase of HBV DNA levels of $>1 \log_{10}$ IU/ml, during the treatment period are shown for each group. TDF, tenofovir disoproxil fumarate; LAM, lamivudine; LdT, telbivudine.

$P = 0.271$) after adjustment for HBV DNA level, HBeAg status, presence of cirrhosis, and biochemical breakthrough at baseline (see Table S2 in the supplemental material). Weighted probabilities of developing virologic breakthrough were not significantly different between treatment groups (monotherapy versus combination therapy; HR, 1.391; 95% CI, 0.115 to 16.869; $P = 0.795$).

Adverse events. During the treatment period, no patient experienced significant deterioration of renal function, increase in serum creatinine of ≥ 0.5 mg/dl from baseline, or decrease in creatinine clearance to <50 ml/min. The median changes in serum creatinine levels at months 3, 6, and 12 from baseline were not significantly different between treatment groups ($P = 0.568$, $P = 1.000$, and $P = 0.086$, respectively). Two patients (one patient from each group) had transient hypophosphatemia, a decrease in serum phosphorus to <2 mg/dl, which resolved without treatment interruption or dose reduction. No patient experienced muscle-related symptoms such as muscle pain or weakness.

DISCUSSION

In this study, we investigated the antiviral efficacy of TDF monotherapy versus combination therapy with TDF plus LAM or LdT in patients infected with HBV variants resistant to LAM using IPTW. The probability of achieving complete virologic suppression was 89% after 12 months of TDF monotherapy, indicating that TDF alone has excellent antiviral efficacy in LAM-resistant patients. TDF plus LAM or LdT combination therapy did not demonstrate superior efficacy over TDF monotherapy in viral suppression before and after adjustment for treatment selection bias using IPTW. Overall, TDF monotherapy and TDF plus LAM or LdT combination therapy were safe and well tolerated, without significant renal events or myopathy.

FIG 3 Weighted probability of complete virologic suppression by treatment group. Weighted cumulative probabilities of complete virologic suppression during the treatment period are shown for each group. TDF, tenofovir disoproxil fumarate; LAM, lamivudine; LdT, telbivudine.

This is the largest study to compare the efficacy and safety of TDF monotherapy with TDF plus LAM or LdT combination therapy in CHB patients with documented LAM resistance. In a small prospective open-label study that assessed the efficacy of TDF monotherapy or TDF plus LAM combination therapy in 60 patients with prior treatment failure of both LAM and ADV, combination therapy with TDF and LAM did not significantly enhance virologic response compared to TDF monotherapy. However, that study included only 20 patients with confirmed LAM resistance, and 17 of 60 study patients (28%) had substitutions conferring ADV resistance at baseline. Moreover, patients who had persistent viremia after 24 weeks of TDF monotherapy received LAM in addition to TDF thereafter; thus, a direct comparison of antiviral efficacy of TDF monotherapy versus TDF plus LAM combination therapy was limited (25).

This study was a retrospective observational study. Although we acknowledge that conducting a randomized controlled trial is the best way to exactly compare different treatments under control for treatment selection bias, the results of such trials may not reflect real-world practice, since patients enrolled in clinical trials are often highly selected (26). Therefore, we aimed to reduce treatment selection bias by means of IPTW. Before and after IPTW, adding LAM or LdT to TDF did not strengthen the antiviral efficacy of TDF in patients with genotypic resistance to LAM. This finding is consistent with the results of a recent clinical trial comparing TDF monotherapy with TDF plus FTC combination therapy in CHB patients with genotypic resistance to LAM; the study demonstrated a similar proportion of patients who achieved an HBV DNA level of <29 IU/ml between treatment groups (16). However, taking into account the point that LAM or LdT used in

our study is a widely prescribed antiviral, while neither FTC alone nor FTC in combination with TDF has been approved for the treatment of chronic HBV infection in many countries, the present study gives strong evidence for clinical practice (2, 18).

Pretreatment HBeAg negativity was determined to independently predict favorable virologic response in this LAM-resistant population. The reason why HBeAg status was an independent predictive factor for virologic response in the present study could be explained by impaired host immune response, which might be involved in an attenuated virologic response in patients who cannot clear HBeAg over a long period on antiviral treatment (27). Nevertheless, in the present study, TDF alone and TDF combined with LAM or LdT were comparably effective within the subgroups of both HBeAg-positive and HBeAg-negative patients. When the impact of the baseline HBV DNA level on viral suppression was evaluated, we found that a lower baseline HBV DNA level was also independently predictive of a favorable virologic response, and viral suppression was impaired in patients with higher baseline HBV DNA levels, as expected. However, viral suppressive activity of TDF monotherapy was comparable to that of combination therapy with TDF plus LAM or LdT, regardless of the baseline HBV DNA levels. These findings indicate that close monitoring for virologic response is needed for patients on TDF rescue treatment, specifically patients who are positive for HBeAg or have high HBV DNA levels at baseline.

Virologic breakthrough was noted for 4 patients during the observation period. Notably, in one patient who developed virologic breakthrough after 11.6 months of TDF monotherapy, amino acid substitutions conferring multidrug resistance were newly demonstrated by genotypic analysis. However, because this patient had been treated with LAM plus ADV combination therapy and 1 mg of ETV monotherapy sequentially before TDF treatment, the possibility of the existence of multidrug-resistant HBV variants at baseline of TDF treatment cannot be excluded, considering the limited sensitivity of genotypic testing used for this patient (19). Virologic breakthrough in 3 other patients was determined to be associated with nonadherence to antiviral medication. The probability of developing virologic breakthrough was not significantly different according to the treatment regimens. Regular investigation of adherence to antiviral medication and close HBV DNA monitoring with genotypic testing are necessary to avoid subsequent treatment failure, especially in patients who fail to achieve early viral suppression with the rescue treatment (2, 19). The rates of developing virologic breakthrough in both the monotherapy group and the combination therapy group were higher than that in the treatment-naïve patients who received TDF treatment in the controlled environment of randomized clinical trials (12). The main cause leading to virologic breakthrough in our study was nonadherence to antiviral medication, which might result from the nature of this real-life clinical study, as indicated in a previous review article (28). In addition, all patients included in our study had LAM-resistant HBV variants at baseline, and the majority of them were treated with multiple lines of antivirals. These critical differences between our study and the aforementioned randomized trial with treatment-naïve patients made it unsuitable to compare the results directly. Of course, since the follow-up period of the current study was relatively short, further long-term follow-up study for resistance surveillance is warranted.

TDF treatment was well tolerated, without confirmed deteriora-

tion of renal function in this study population, which included patients with decompensated cirrhosis. Although two of the study patients experienced hypophosphatemia, the episodes were transient and resolved without treatment intervention, such as treatment interruption or dose modification. These results are consistent with those of recently reported studies of TDF (16, 29, 30). TDF in the treatment of HBV-monoinfected patients is thought to be generally safe, whereas TDF-associated nephrotoxicity and declines in bone mineral density have been reported for patients with HIV infection (31–33). Regarding safety issues, additional long-term studies to evaluate the safety of TDF for the treatment of CHB, especially in cirrhotic patients, are needed.

In conclusion, the results of our study demonstrate that TDF monotherapy is as effective and safe as combination therapy with TDF plus LAM or LdT in CHB patients infected with LAM-resistant HBV variants. TDF monotherapy may be a favorable therapeutic option with regard to antiviral efficacy as well as cost in these patients. Further long-term follow-up studies are warranted to determine whether there is an additional treatment benefit of adding LAM or LdT to TDF, particularly to prevent subsequent development of resistance.

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REFERENCES

- Liaw YF, Sung JJ, Chow WC, Farrell G, Lee CZ, Yuen H, Tanwandee T, Tao QM, Shue K, Keene ON, Dixon JS, Gray DF, Sabbat J. 2004. Lamivudine for patients with chronic hepatitis B and advanced liver disease. *N Engl J Med* 351:1521–1531. <http://dx.doi.org/10.1056/NEJMoa033364>.
- European Association for the Study of the Liver 2012. EASL clinical practice guidelines: management of chronic hepatitis B virus infection. *J Hepatol* 57:167–185.
- Lai CL, Dienstag J, Schiff E, Leung NW, Atkins M, Hunt C, Brown N, Woessner M, Boehme R, Condreay L. 2003. Prevalence and clinical correlates of YMDD variants during lamivudine therapy for patients with chronic hepatitis B. *Clin Infect Dis* 36:687–696. <http://dx.doi.org/10.1086/368083>.
- Lampertico P, Vigano M, Manenti E, Iavarone M, Sablon E, Colombo M. 2007. Low resistance to adefovir combined with lamivudine: a 3-year study of 145 lamivudine-resistant hepatitis B patients. *Gastroenterology* 133:1445–1451. <http://dx.doi.org/10.1053/j.gastro.2007.08.079>.
- Rapti I, Dimou E, Mitsoula P, Hadziyannis SJ. 2007. Adding-on versus switching to adefovir therapy in lamivudine-resistant HBeAg-negative chronic hepatitis B. *Hepatology* 45:307–313. <http://dx.doi.org/10.1002/hep.21534>.
- Lok AS, McMahon BJ. 2007. Chronic hepatitis B. *Hepatology* 45:507–539. <http://dx.doi.org/10.1002/hep.21513>.
- Liaw YF, Leung N, Kao JH, Piratvisuth T, Gane E, Han KH, Guan R, Lau GK, Locarnini S. 2008. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2008 update. *Hepatol Int* 2:263–283. <http://dx.doi.org/10.1007/s12072-008-9080-3>.
- Seto WK, Liu K, Fung J, Wong DK, Yuen JC, Hung IF, Lai CL, Yuen MF. 2012. Outcome of lamivudine-resistant chronic hepatitis B after up to 5 years of combination therapy with adefovir. *Antivir Ther* 17:1255–1262. <http://dx.doi.org/10.3851/IMP2335>.
- Zoulim F, Locarnini S. 2009. Hepatitis B virus resistance to nucleos(t)ide analogues. *Gastroenterology* 137:1593–1608, e1591–e1592. <http://dx.doi.org/10.1053/j.gastro.2009.08.063>.
- Delaney WE, IV, Ray AS, Yang H, Qi X, Xiong S, Zhu Y, Miller MD. 2006. Intracellular metabolism and in vitro activity of tenofovir against

- hepatitis B virus. *Antimicrob Agents Chemother* 50:2471–2477. <http://dx.doi.org/10.1128/AAC.00138-06>.
11. Lada O, Benhamou Y, Cahour A, Katlama C, Poynard T, Thibault V. 2004. In vitro susceptibility of lamivudine-resistant hepatitis B virus to adefovir and tenofovir. *Antivir Ther* 9:353–363.
 12. Marcellin P, Heathcote EJ, Buti M, Gane E, de Man RA, Krastev Z, Germanidis G, Lee SS, Flisiak R, Kaita K, Manns M, Kotzev I, Tchernev K, Buggisch P, Weilert F, Kuras OO, Shiffman ML, Trinh H, Washington MK, Sorbel J, Anderson J, Snow-Lampart A, Mondou E, Quinn J, Rousseau F. 2008. Tenofovir disoproxil fumarate versus adefovir dipivoxil for chronic hepatitis B. *N Engl J Med* 359:2442–2455. <http://dx.doi.org/10.1056/NEJMoa0802878>.
 13. Heathcote EJ, Marcellin P, Buti M, Gane E, De Man RA, Krastev Z, Germanidis G, Lee SS, Flisiak R, Kaita K, Manns M, Kotzev I, Tchernev K, Buggisch P, Weilert F, Kuras OO, Shiffman ML, Trinh H, Gurel S, Snow-Lampart A, Borroto-Esoda K, Mondou E, Anderson J, Sorbel J, Rousseau F. 2011. Three-year efficacy and safety of tenofovir disoproxil fumarate treatment for chronic hepatitis B. *Gastroenterology* 140:132–143. <http://dx.doi.org/10.1053/j.gastro.2010.10.011>.
 14. Snow-Lampart A, Chappell B, Curtis M, Zhu Y, Myrick F, Schwalder J, Kitrinos K, Svarovskaia ES, Miller MD, Sorbel J, Heathcote EJ, Marcellin P, Borroto-Esoda K. 2011. No resistance to tenofovir disoproxil fumarate detected after up to 144 weeks of therapy in patients mono-infected with chronic hepatitis B virus. *Hepatology* 53:763–773. <http://dx.doi.org/10.1002/hep.24078>.
 15. van Bömmel F, de Man RA, Wedemeyer H, Deterding K, Petersen J, Buggisch P, Erhardt A, Huppe D, Stein K, Trojan J, Sarrazin C, Bocher WO, Spengler U, Wasmuth HE, Reinders JG, Moller B, Rhode P, Feucht HH, Wiedenmann B, Berg T. 2010. Long-term efficacy of tenofovir monotherapy for hepatitis B virus-monoinfected patients after failure of nucleoside/nucleotide analogues. *Hepatology* 51:73–80. <http://dx.doi.org/10.1002/hep.23246>.
 16. Fung S, Kwan P, Fabri M, Horban A, Pelemis M, Hann HW, Gurel S, Caruntu FA, Flaherty JF, Massetto B, Dinh P, Corsa A, Subramanian GM, McHutchison JG, Hsu P, Gane E. 2014. Randomized comparison of tenofovir disoproxil fumarate vs emtricitabine and tenofovir disoproxil fumarate in patients with lamivudine-resistant chronic hepatitis B. *Gastroenterology* 146:980–988. <http://dx.doi.org/10.1053/j.gastro.2013.12.028>.
 17. Corsa AC, Liu Y, Flaherty JF, Mitchell B, Fung SK, Gane E, Miller MD, Kitrinos KM. 2014. No resistance to tenofovir disoproxil fumarate through 96 weeks of treatment in patients with lamivudine-resistant chronic hepatitis B. *Clin Gastroenterol Hepatol* 12:2106–2112.e1. <http://dx.doi.org/10.1016/j.cgh.2014.05.024>.
 18. Lok AS, McMahon BJ. 2009. Chronic hepatitis B: update 2009. *Hepatology* 50:661–662. <http://dx.doi.org/10.1002/hep.23190>.
 19. Zoulim F, Locarnini S. 2012. Management of treatment failure in chronic hepatitis B. *J Hepatol* 56(Suppl 1):S112–S122.
 20. Chevaliez S, Bouvier-Alias M, Laperche S, Hezode C, Pawlotsky JM. 2010. Performance of version 2.0 of the Cobas AmpliPrep/Cobas TaqMan real-time PCR assay for hepatitis B virus DNA quantification. *J Clin Microbiol* 48:3641–3647. <http://dx.doi.org/10.1128/JCM.01306-10>.
 21. Allen MI, Deslauriers M, Andrews CW, Tipples GA, Walters KA, Tyrrell DL, Brown N, Condreay LD. 1998. Identification and characterization of mutations in hepatitis B virus resistant to lamivudine. *Lamivudine Clinical Investigation Group. Hepatology* 27:1670–1677.
 22. Kim HS, Han KH, Ahn SH, Kim EO, Chang HY, Moon MS, Chung HJ, Yoo W, Kim SO, Hong SP. 2005. Evaluation of methods for monitoring drug resistance in chronic hepatitis B patients during lamivudine therapy based on mass spectrometry and reverse hybridization. *Antivir Ther* 10:441–449.
 23. Rosenbaum PR, Rubin DB. 1983. The central role of the propensity score in observational studies for causal effects. *Biometrika* 70:41–55. <http://dx.doi.org/10.1093/biomet/70.1.41>.
 24. Curtis LH, Hammill BG, Eisenstein EL, Kramer JM, Anstrom KJ. 2007. Using inverse probability-weighted estimators in comparative effectiveness analyses with observational databases. *Med Care* 45:S103–S107. <http://dx.doi.org/10.1097/MLR.0b013e31806518ac>.
 25. Patterson SJ, George J, Strasser SI, Lee AU, Sievert W, Nicoll AJ, Desmond PV, Roberts SK, Locarnini S, Bowden S, Angus PW. 2011. Tenofovir disoproxil fumarate rescue therapy following failure of both lamivudine and adefovir dipivoxil in chronic hepatitis B. *Gut* 60:247–254. <http://dx.doi.org/10.1136/gut.2010.223206>.
 26. Weintraub WS, Grau-Sepulveda MV, Weiss JM, O'Brien SM, Peterson ED, Kolm P, Zhang Z, Klein LW, Shaw RE, McKay C, Ritzenthaler LL, Popma JJ, Messinger JC, Shahian DM, Grover FL, Mayer JE, Shewan CM, Garratt KN, Moussa ID, Dangas GD, Edwards FH. 2012. Comparative effectiveness of revascularization strategies. *N Engl J Med* 366:1467–1476. <http://dx.doi.org/10.1056/NEJMoa1110717>.
 27. Feld JJ, Heathcote EJ. 2006. Hepatitis B e antigen-positive chronic hepatitis B: natural history and treatment. *Semin Liver Dis* 26:116–129. <http://dx.doi.org/10.1055/s-2006-939750>.
 28. Pol S, Lampertico P. 2012. First-line treatment of chronic hepatitis B with entecavir or tenofovir in 'real-life' settings: from clinical trials to clinical practice. *J Viral Hepat* 19:377–386. <http://dx.doi.org/10.1111/j.1365-2893.2012.01602.x>.
 29. Berg T, Zoulim F, Moeller B, Trinh H, Marcellin P, Chan S, Kitrinos KM, Dinh P, Flaherty JF, Jr, McHutchison JG, Manns M. 2014. Long-term efficacy and safety of emtricitabine plus tenofovir DF vs. tenofovir DF monotherapy in adefovir-experienced chronic hepatitis B patients. *J Hepatol* 60:715–722. <http://dx.doi.org/10.1016/j.jhep.2013.11.024>.
 30. Marcellin P, Gane E, Buti M, Afdhal N, Sievert W, Jacobson IM, Washington MK, Germanidis G, Flaherty JF, Schall RA, Bornstein JD, Kitrinos KM, Subramanian GM, McHutchison JG, Heathcote EJ. 2013. Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study. *Lancet* 381:468–475. [http://dx.doi.org/10.1016/S0140-6736\(12\)61425-1](http://dx.doi.org/10.1016/S0140-6736(12)61425-1).
 31. Verhelst D, Monge M, Meynard JL, Fouqueray B, Mougenot B, Girard PM, Ronco P, Rossert J. 2002. Fanconi syndrome and renal failure induced by tenofovir: a first case report. *Am J Kidney Dis* 40:1331–1333. <http://dx.doi.org/10.1053/ajkd.2002.36924>.
 32. Woodward CL, Hall AM, Williams IG, Madge S, Copas A, Nair D, Edwards SG, Johnson MA, Connolly JO. 2009. Tenofovir-associated renal and bone toxicity. *HIV Med* 10:482–487. <http://dx.doi.org/10.1111/j.1468-1293.2009.00716.x>.
 33. Gallant JE, Staszewski S, Pozniak AL, DeJesus E, Suleiman JM, Miller MD, Coakley DF, Lu B, Toole JJ, Cheng AK. 2004. Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naïve patients: a 3-year randomized trial. *JAMA* 292:191–201. <http://dx.doi.org/10.1001/jama.292.2.191>.