

CLINICAL TRIAL

Tenofovir disoproxil fumarate for multiple nucleos(t)ide analogues treatment failure hepatitis B: Is monotherapy enough?

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Key words

antiviral resistance, China, efficacy, hepatitis B e antigen, hepatitis B surface antigen, hepatitis B virus, hepatitis B virus resistance, multidrug-resistance, safety, virological response.

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Abstract

Background and Aim: Tenofovir disoproxil fumarate (TDF) is a first-line treatment for chronic hepatitis B virus (HBV) infection for its high potency and a low rate of drug resistance. This study investigated the efficacy and safety of TDF in Chinese patients with chronic hepatitis B (CHB) infection after treatment failure with multiple nucleos(t)ide analogues (NAs).

Methods: Patients included were aged 18–65 years, with treatment failure with multiple NAs (serum HBV DNA > 200 IU/mL after more than two different NA treatments). The primary endpoint was proportion of patients with serum HBV DNA < 20 IU/mL at Week 144 of TDF monotherapy. Secondary endpoints and safety were also assessed.

Results: Overall, 213 patients were enrolled. At Week 144, mean HBV DNA decreased significantly from baseline (4.4 vs 1.4 log₁₀ IU/mL), with 77.0% patients (95% confidence interval: 71.1, 82.9) achieving serum HBV DNA < 20 IU/mL. Three (1.4%) patients experienced virological breakthrough during TDF monotherapy, without hepatitis flare. At Week 144, 15.3% and 4.7% patients (hepatitis B e antigen [HBeAg]-positive at baseline) experienced HBeAg loss and HBeAg seroconversion, respectively; 68.3% patients achieved normalized alanine aminotransferase levels. Overall, 58.7% patients experienced more than one adverse event (AE). Most common AEs were upper respiratory tract infection and blood creatine phosphokinase increase; 8.5% patients experienced study drug-related AEs; 9.4% patients experienced serious AEs (none were TDF-related). Among renal safety parameters, overall trend of mean serum phosphorous level remained stable, while mean estimated glomerular filtration rate increased slightly.

Conclusions: Tenofovir disoproxil fumarate monotherapy is efficacious in CHB patients with multiple NAs treatment failure with no new safety findings.

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[Correction added on 12 January 2022, after first online publication: The copyright line was changed.]

Introduction

Chronic hepatitis B virus (HBV) infection remains a major health problem worldwide.¹ China is a high endemic region of chronic hepatitis B (CHB) with current prevalence estimated to be approximately 5% to 6% with 70 million hepatitis B surface antigen (HBsAg) carriers in China.^{2,3} This large pool of HBV in the Chinese population still poses a major challenge on the clinical practice.⁴

Nonpreferred antiviral treatment options with a low genetic barrier to resistance, such as lamivudine (LAM), adefovir dipivoxil (ADV), and telbivudine (LdT), have been widely used in some regions of China due to availability and reimbursement issues, which may result in the emergence of HBV resistance.^{5,6}

Tenofovir disoproxil fumarate (TDF), an oral nucleos(t)ide analogue (NA) and a DNA polymerase inhibitor, was approved by the US Food and Drug Administration in 2008 for the treatment of CHB in adults.⁷ TDF was efficacious and demonstrated a low rate of drug resistance in NA-naïve patients as well as NA-experienced patients in global and Chinese population.^{8–11}

The efficacy of TDF has been demonstrated in patients with CHB with suboptimal response to ADV¹¹ and in those with documented LAM-resistance¹²; however, the efficacy of TDF treatment after failure of multiple NAs has only been evaluated in a few previous studies with low sample sizes.^{13–17} Thus, the exposure of TDF in the Chinese population, especially in patients with failure of treatment with multiple NAs, is limited.¹⁷ In this study, the efficacy and safety of TDF in Chinese patients with CHB after treatment failure with multiple NAs was investigated.

Patients and methods

Ethics. The study protocol, any amendment, and the informed consent were reviewed and approved by an ethics committee of each study site. This study was conducted in accordance with the principle of Declaration of Helsinki, the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH), and Good Clinical Practice (GCP). All patients provided written informed consent at the time of entering this study.

Patients and study design. In this single-arm, open-label, multicenter study (NCT02195518), a total of 213 patients were enrolled between March 18, 2015 and August 14, 2018. The inclusion criteria were men and women aged between 18 and 65 years, who were positive for HBsAg for > 6 months and negative for HBs antibodies; had serum HBV DNA \geq 200 IU/mL at screening; and had treatment failure with multiple NAs (defined as serum HBV DNA > 200 IU/mL after \geq 2 different NA treatments [i.e. monotherapy followed by add-on/switch rescue therapy that was continued for \geq 6 months for each regimen for a total duration of > 12 months]).

The exclusion criteria were patients diagnosed with hepatocellular carcinoma or those having clinical signs of decompensated liver disease at baseline; creatinine clearance < 70 mL/min; alanine aminotransferase (ALT) > 10 times upper limit of normal at screening or history of acute exacerbation leading to transient decompensation; or documented coinfection with hepatitis A, C,

delta, E virus, or HIV; or evidence of active liver disease due to autoimmune hepatitis (antinuclear antibody titer > 1:160). Further, patients who underwent or planned to undergo liver transplantation were recipients of TDF within 6 months prior to screening or were treated with nephrotoxic drugs within 2 months before study screening were also excluded.

Treatment and follow-up. Patients received TDF 300-mg tablets once a day (QD) during the study period. The patients were explained the importance of compliance at each visit, and they were required to return all unused medicines so that it could be recorded. Patients who did not achieve a satisfactory response to TDF were required to include another drug without cross-resistance to TDF, including LAM (100 mg QD), entecavir (ETV) (0.5 mg QD), and LdT (600 mg QD). Drugs were selected based on the investigator's discretion. The efficacy and safety assessments were performed every 12 weeks for a total of 14 visits (144 weeks). Patients with treatment interruptions of \geq 28 days were required to withdraw from the study.

Study assessments. The primary efficacy endpoint was the proportion of patients with serum HBV DNA < 20 IU/mL at Week 144 of TDF treatment. The secondary efficacy endpoints included the proportion of patients with serum HBV DNA < 20 IU/mL at Weeks 48 and 96 and serum HBV DNA < 69 IU/mL (virological suppression) at Weeks 48, 96, and 144. The proportion of patients with serum HBV DNA < 20 and < 69 IU/mL in the subgroup with confirmed multidrug-resistance (MDR, resistant to more than two types of NAs with no cross-resistance) at baseline; reduction in serum HBV DNA (\log_{10} IU/mL); the proportion of patients achieving hepatitis B e antigen (HBeAg) and HBsAg loss and seroconversion; the proportion of patients achieving HBsAg loss and HBsAg seroconversion in HBeAg-negative patients at baseline; the proportion of patients with normalized ALT in patients who had abnormal ALT at baseline; the proportion of patients who experienced virological breakthrough (defined as \geq 1 \log_{10} IU/mL increase in serum HBV DNA from nadir, determined by two sequential serum HBV DNA measurements). All the endpoints were assessed at Weeks 48, 96, and 144. Overall unsatisfactory response (defined as HBV DNA \geq 200 IU/mL at Week 48 followed by \leq 1 \log_{10} IU/mL decrease in HBV DNA at two consecutive tests [confirmed by a third additional visit] \leq 1 month apart) was also recorded. Exploratory efficacy endpoints are given in the supporting information.

Resistance surveillance of the HBV polymerase gene was performed by direct sequencing. This was conducted for all the patients at baseline (Visit 2), patients with unsatisfactory response to TDF, and those with detectable HBV DNA at Weeks 48 and 96 or on their last visit.

Safety assessments included adverse events (AEs), serious AEs (SAEs), and abnormal laboratory parameters (including serum phosphorous level and estimated glomerular filtration rate).

Statistical analyses. It was hypothesized that 80% of the patients who had treatment failure with multiple NAs would have serum HBV DNA < 20 IU/mL at Week 144 after TDF treatment. A sample size of 170 patients was expected to allow us to estimate

the confidence interval (CI) of the incidence with a margin of error at $\pm 6\%$ (ranged from 74% to 86%). Assuming a dropout rate of 15% during the 144-week study period, the number of patients required was estimated to be 200. Additional details are given in the supporting information.

Results

Study population. Of the total 281 screened patients, 75.8% ($n = 213/281$) patients met the eligibility criteria and were included in the study as modified intent-to-treat (mITT) population. In total, 95.8% ($n = 204/213$) patients completed the study up to Week 144 (Fig. 1). The average treatment compliance (%) in this study was 99.33%.

Demographic and clinical characteristics. In this study, the mean (SD) age of the patients was 42.3 (10.3) years, and 87.3% ($n = 186/213$) patients were men. The mean (SD) duration of TDF exposure was 983 (143.07) days.

At baseline, the mean serum HBV DNA level was 4.4 \log_{10} IU/mL, and 89.2% ($n = 190$) patients were HBeAg-positive. The majority of the patients were resistant to LAM and LdT (71.8% each) and were earlier treated with ADV (93.4%) and LAM (71.4%). The most commonly observed HBV resistance mutation at baseline was M204I variant (28.6%) followed by the wild-type (28.2%) variant (Table 1).

Efficacy outcomes. The proportion of patients with serum HBV DNA < 20 IU/mL at Week 144 after TDF treatment was 77.0% (95% CI: 71.1, 82.9) in the mITT population. The proportion of patients who achieved serum HBV DNA < 20 IU/mL and serum HBV DNA < 69 IU/mL increased gradually up to Week 144 after TDF treatment (Table 2).

Serum HBV DNA < 20 IU/mL was achieved in 65.8% (95% CI: 49.4, 82.2) patients and serum HBV DNA < 69 IU/mL was achieved in 84.2% (95% CI: 71.3, 97.1) patients by Week 144 after TDF treatment in the subgroup of patients with confirmed MDR at baseline (Table 2). Serum HBV DNA < 20 IU/mL was achieved in 85.2% (95% CI: 78.3, 92.1) patients, and serum HBV DNA < 69 IU/mL was achieved in 90.4% (95% CI: 84.6,

96.2) patients by Week 144 after receiving TDF treatment in the subgroup of patients with non-MDR at baseline (Table 2).

In the subgroup analysis of patients previously treated with different NAs, serum HBV DNA < 20 IU/mL was achieved in 78% patients previously treated with ADV for > 6 months; in 67% patients previously treated with ADV for ≤ 6 months or with unknown time duration; in 71% patients previously treated with ETV for > 6 months and who developed ETV-R; in 74% patients previously treated with ETV for > 6 months and did not develop ETV-R; in 82% patients previously treated with ETV for ≤ 6 months or with unknown time duration by Week 144 after TDF treatment.

Mean (SD) serum HBV DNA (\log_{10} IU/mL) levels gradually decreased from baseline (4.4 [1.62]) to Week 48 (1.6 [0.55]), then stabilized up to Week 144 (1.4 [0.51]) after TDF treatment (Fig. 2a). The median (range) change from baseline in serum HBV DNA level was -2.4 ($-7.3, -0.0$) at Week 48, -2.6 ($-7.3, -0.4$) at Week 96, and -2.7 ($-7.3, 3.5$) \log_{10} IU/mL at Week 144. Reduction in serum HBV DNA by pattern of mutations Category 1 was significantly higher for ADV-R ($P = 0.002$), ETV-R ($P = 0.0007$), and LAM-R ($P = 0.006$) at Week 144 as compared with wild-type mutation (Fig. 2b). Contrastingly, in Category 2, the reduction in serum HBV DNA was not significant between ADV-R double mutations and ADV-R single mutation at Week 144 (Fig. 2c).

Among the patients who were HBeAg-positive at baseline, there was an increase in the proportion of patients experiencing HBeAg loss at Weeks 48 (5.3%), 96 (10%), and 144 (15.3%). Further, 2.6% patients experienced HBeAg seroconversion at Weeks 48 and 96, and it increased to 4.7% at Week 144 (Table 2).

None of the patients who were HBeAg-negative at baseline experienced HBsAg loss or seroconversion at Weeks 48, 96, or 144. Following TDF treatment, a low proportion of patients who were HBeAg-positive at baseline experienced HBsAg loss (Week 48: 0.5%, Week 96: 0.5%, and Week 144: 1.1%) and HBsAg seroconversion (Week 48: 0.0%, Week 96: 0.5%, and Week 144: 0.5%) (Table 2).

In patients who had abnormal ALT at baseline, ALT normalization was achieved in 52.5% ($n = 32/61$) patients at Week 48, in 60.7% ($n = 37/61$) patients at Week 96, and in 68.3% ($n = 41/61$) patients at Week 144 with TDF treatment (Table 2). Overall virological breakthrough was observed in 1.4% ($n = 3$) patients, with

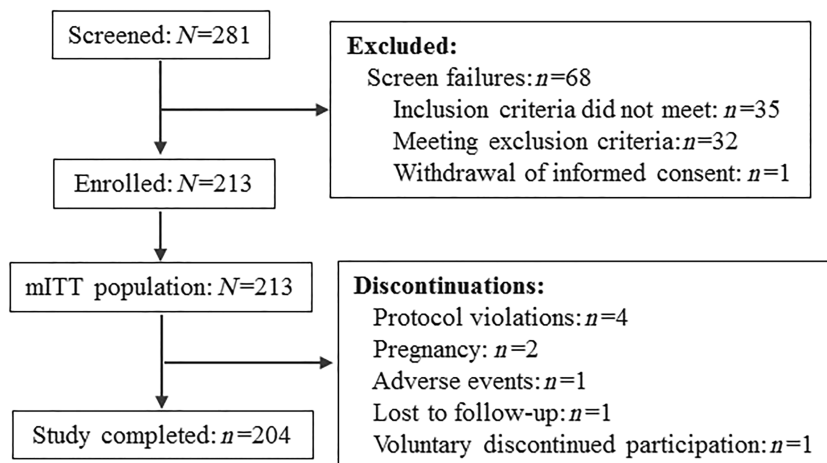


Figure 1 Patients disposition. mITT, modified intent-to-treat; n , number of patients.

Table 1 Demographics and clinical characteristics (mITT population)

Characteristics	Total population (n = 213)
Age, years, mean (SD)	42.3 (10.3)
Gender, men, n (%)	186 (87.3)
BMI, kg/m ²	23.7 (3.1)
Serum HBV DNA (log ₁₀ IU/mL), mean (SD)	4.4 (1.6)
HBeAg, n (%)	190 (89.2)
ALT, U/L, mean (SD)	46.9 (49.8)
NAs resistance, n (%)	
LAM	153 (71.8)
LdT	153 (71.8)
ADV	38 (17.8)
ETV	33 (15.5)
Multidrug	38 (17.8)
Previous exposure to medications for > 6 months, n (%)	
ADV	199 (93.4)
LAM	152 (71.4)
ETV	124 (58.2)
LdT	59 (27.7)
IFN	14 (6.6)
eGFR, mL/min/1.73 m ² , mean (SD)	105.2 (13.4)
Creatinine clearance, mL/min, mean (SD)	103.4 (23.0)
Serum phosphorous, mmol/L, mean (SD)	1.0 (0.2)
HBV resistance mutations, n (%)	
Wild type	60 (28.2)
M204I	61 (28.6)
M204V	2 (0.9)
L180M + M204V	38 (17.8)
A181T/V	27 (12.7)
N236T	1 (0.5)
A181T/V + N236T	10 (4.7)
L180M + M204V/I ± I169T ± V173L ± M250V	11 (5.2)
L180M + M204V/I ± T184G ± S202I/G	21 (9.9)
Other mutations	3 (1.6)
I233V	3 (1.6)
L180M	13 (76.5)
V173L	1 (5.9)
Patterns of mutation	
Category 1	
Wild type	60 (26.7)
ADV-R	38 (16.9)
ETV-R	32 (14.2)
LAM-R	95 (42.2)
Category 2	
Wild type	60 (28.2)
ADV-R single	28 (13.1)
ADV-R double	10 (4.7)
Other	115 (54.0)

HBV resistance mutations were LAM-R: M204I, M204V, L180M + M204V; ADV-R: A181T/V, N236T, A181T/V + N236T; ETV-R: L180M + M204V/I ± I169T ± V173L ± M250V, L180M + M204V/I ± T184G ± S202I/G; ADV-R single: A181T/V, N236T; ADV-R double: A181T/V + N236T.

ADV, adefovir dipivoxil; ADV-R, ADV-resistant; ALT, alanine aminotransferase; BMI, body mass index; ETV, entecavir; ETV-R, ETV-resistant; eGFR, estimated glomerular filtration rate; HBeAg, hepatitis B e antigen; HBV DNA, hepatitis B virus deoxyribonucleic acid; IFN, interferon; LAM, lamivudine; LAM-R, LAM-resistant; LdT, telbivudine; mITT, modified intent-to-treat; NAs, nucleotide analogues; SD, standard deviation.

HBV DNA levels in all patients lowered to < 20 IU/mL by 96 weeks of TDF therapy without any rescue treatment. Unsatisfactory response was observed in 7.5% (n = 16) patients (Table 2), and seven of the 16 patients added ETV therapy as per investigator's decision.

The impact of the baseline variables (patients and virological) on early serum HBV DNA suppression in response to TDF was evaluated at Week 144. Among all the factors analyzed, body mass index (BMI, normal vs overweight) was statistically significant; the odds ratio for BMI was 5.42 (95% CI: 1.14, 25.73; P = 0.03) in multivariate analysis.

The virological response events were observed in 85% patients with a median time of 11.1 months (95% CI: 8.3, 11.3). As per the Cox regression analysis, serum HBV DNA, HBeAg status, early response (P < 0.001 for all three parameters) and r-GT (P = 0.04) significantly affected the time to virological response (Table 3).

Safety outcomes. Adverse events were reported in 58.7% (n = 125/213) patients, including SAEs in 9.4% (n = 20) patients and study drug-related AEs in 8.5% (n = 18) patients. One death, which was not related to the study drug, was reported.

The most frequently reported AEs (occurring in more than three patients) were upper respiratory tract infection (13.1%, n = 28) followed by blood creatine phosphokinase increased (5.6%, n = 12) (Table 4). One AE led to a permanent discontinuation of TDF, and the patient was withdrawn from the study due to sudden cardiac death (not related to the study drug).

The most frequently reported SAE (occurring in more than two patients) was hepatocellular carcinoma (1.9%, n = 4) (Table 4); none of the SAEs were related to the study drug. The most common study drug-related AEs were blood creatine phosphokinase increased (2.8%, n = 6) and transient hypophosphatemia (2.3%, n = 5).

Any grade 3/4 laboratory abnormalities were reported in 21 (9.9%) patients; none of them were related to the study drug; decrease in serum phosphorus was the most common AE occurring in 11 (5.2%) patients (Table 4). None of the patients experienced a confirmed increase in serum creatinine (0.5 mg/dL) above baseline or creatinine clearance (< 50 mL/min).

Among the renal safety parameters, the overall trend of mean serum phosphorous level remained stable, while mean estimated glomerular filtration rate showed a slight increase up to Week 144 of TDF treatment, despite a transient slight decrease in these two variables at Week 12 (Fig. 3).

Discussion

There is limited data to demonstrate the effect of TDF treatment in patients with CHB infection who have failed prior treatment with multiple NAs (> 2). In this study, the efficacy and safety of TDF treatment in Chinese patients with CHB who have failed prior treatment with multiple NAs were evaluated.

The results from this study demonstrate that the majority of the patients (77%) who have failed prior treatment with multiple NAs achieved HBV DNA < 20 IU/mL at Week 144 after TDF treatment. These results are in consonance with a prospective, open-label, multicenter study conducted in patients with CHB from

Table 2 Summary of efficacy assessments at Weeks 48, 96, and 144 (mITT population)

	Patient proportion, <i>n</i> (%)	95% CI
Primary efficacy endpoint, <i>N</i> = 213		
HBV DNA < 20 IU/mL		
Week 144	164 (77.0)	71.1, 82.9
Secondary efficacy endpoints, <i>N</i> = 213		
HBV DNA < 20 IU/mL		
Week 48	100 (46.9)	40.0, 53.9
Week 96	130 (61.0)	54.2, 67.8
HBV DNA < 69 IU/mL		
Week 48	163 (76.5)	70.6, 82.5
Week 96	179 (84.0)	78.9, 89.2
Week 144	188 (88.3)	83.7, 92.8
In patients with confirmed multidrug-resistance at baseline, <i>n</i> = 38		
HBV DNA < 20 IU/mL		
Week 48	13 (34.2)	17.8, 50.6
Week 96	21 (55.3)	38.1, 72.4
Week 144	25 (65.8)	49.4, 82.2
HBV DNA < 69 IU/mL		
Week 48	23 (60.5)	43.7, 77.4
Week 96	29 (76.3)	61.5, 91.1
Week 144	32 (84.2)	71.3, 97.1
In patients with non-multidrug-resistance at baseline, <i>n</i> = 115		
HBV DNA < 20 IU/mL		
Week 48	66 (57.4)	47.9, 66.9
Week 96	82 (71.3)	62.6, 80.0
Week 144	98 (85.2)	78.3, 92.1
HBV DNA < 69 IU/mL		
Week 48	98 (85.2)	78.3, 92.1
Week 96	103 (89.6)	83.5, 95.6
Week 144	104 (90.4)	84.6, 96.2
In patients with HBeAg-positive at baseline, <i>n</i> = 190		
HBeAg loss		
Week 48	10 (5.3)	1.8, 8.7
Week 96	19 (10.0)	5.5, 14.5
Week 144	29 (15.3)	9.9, 20.6
HBeAg seroconversion		
Week 48	5 (2.6)	0.1, 5.2
Week 96	5 (2.6)	0.1, 5.2
Week 144	9 (4.7)	1.5, 8.0
HBsAg loss		
Week 48	1 (0.5)	0.0, 1.8
Week 96	1 (0.5)	0.0, 1.8
Week 144	2 (1.1)	0.0, 2.8
HBsAg seroconversion		
Week 48	0 (0.0)	0.0, 0.3
Week 96	1 (0.5)	0.0, 1.8
Week 144	1 (0.5)	0.0, 1.8
ALT normalization in patients with abnormal ALT at baseline [†] , <i>n</i> = 61		
Week 48	32 (52.5)	39.1, 65.8
Week 96	37 (60.7)	47.6, 73.7
Week 144	41 (68.3)	55.7, 80.9
Overall virological breakthrough, <i>N</i> = 213	3 (1.4)	0, 3.2
Overall unsatisfactory response, <i>N</i> = 213	16 (7.5)	3.7, 11.3

(1) 95% CI was constructed by normal approximation and continuity correction method. (2) Multidrug-resistance = resistant to more than two types of NAs that have no cross-resistance. (3) Non-multidrug-resistance = not resistance or resistant to only one type of NA. This group includes mITT population (*n* = 213)—(MDR [*n* = 38] + WT [*n* = 60]). [†]Upper limit of normal (ULN) for ALT [U/L]: For all the centers [except for centers 8 and 9], 50 for men and 40 for women; for center 8: 64 each for men and women both; for center 9: 40 for men and 35 for women). ADV, adefovir dipivoxil; CI, confidence interval; ETV, entecavir; HBV DNA, hepatitis B virus DNA; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; LAM, lamivudine; mITT, modified intent-to-treat; TDF, tenofovir disoproxil fumarate.

Australia who received TDF treatment after failure of LAM/ADV therapy due to the emergence of drug resistance. After TDF treatment, 64% (*n* = 38/59) patients achieved serum HBV DNA < 15 IU/mL by Week 96.¹⁴

In the current study, the proportion of patients achieving serum HBV DNA < 20 IU/mL at Week 144 with TDF treatment was higher in the mITT population compared with the subgroup with confirmed MDR at baseline (77% vs 65.8%). A multicenter study conducted in Korean patients (*n* = 292) with CHB and MDR HBV after exposure to multiple NAs demonstrated a gradual increase in the virological response rate (serum HBV DNA < 15 IU/mL) from 67.2% at Week 48 to 78.6% at Week 240 with TDF treatment.¹⁸ This implies that the virological response rate of TDF was more pronounced in the overall population than the MDR population. Moreover, as shown by multivariate analysis, ADV resistance mutation was not a significant factor for virological response in this study. The presence of adefovir (ADV) resistance has been shown to impair TDF efficacy in a retrospective cohort study on HBV-infected patients (*n* = 168) from Germany.¹³ However, there are other studies that show virological response is not influenced by ADV resistance mutations, which is in corroboration with findings of the current study.^{14,19}

The proportion of patients achieving serum HBV DNA < 20 IU/mL in the overall population was almost similar to the subgroup population who were previously treated with ADV for > 6 months, ETV for > 6 months with or without ETV-R, and ETV for ≤ 6 months. This suggests that treatment history of the patients with respect to different NAs did not impact the virological suppression effect of TDF, which was also comparable among patients with different medical histories. This is in contrast with the findings from a retrospective cohort study by Chung *et al.* in Korean patients with CHB (*n* = 252) that demonstrated inferior efficacy of TDF in adefovir (ADV)-experienced CHB patients compared with ADV-naïve CHB patients.²⁰

Suboptimal administration of TDF, which is defined as TDF being taken on empty stomach instead of after a high-fat meal, has been shown to result in partial virological response in a recent study in Chinese CHB patients (*n* = 892).²¹ As part of the current study, we did not look at this factor; however, we would like to investigate this in future studies aiming at improving TDF efficacy in this patient population.

Importantly, in this study, serum HBV DNA continued to decline until Week 48 and then remained stable up to Week 144 in

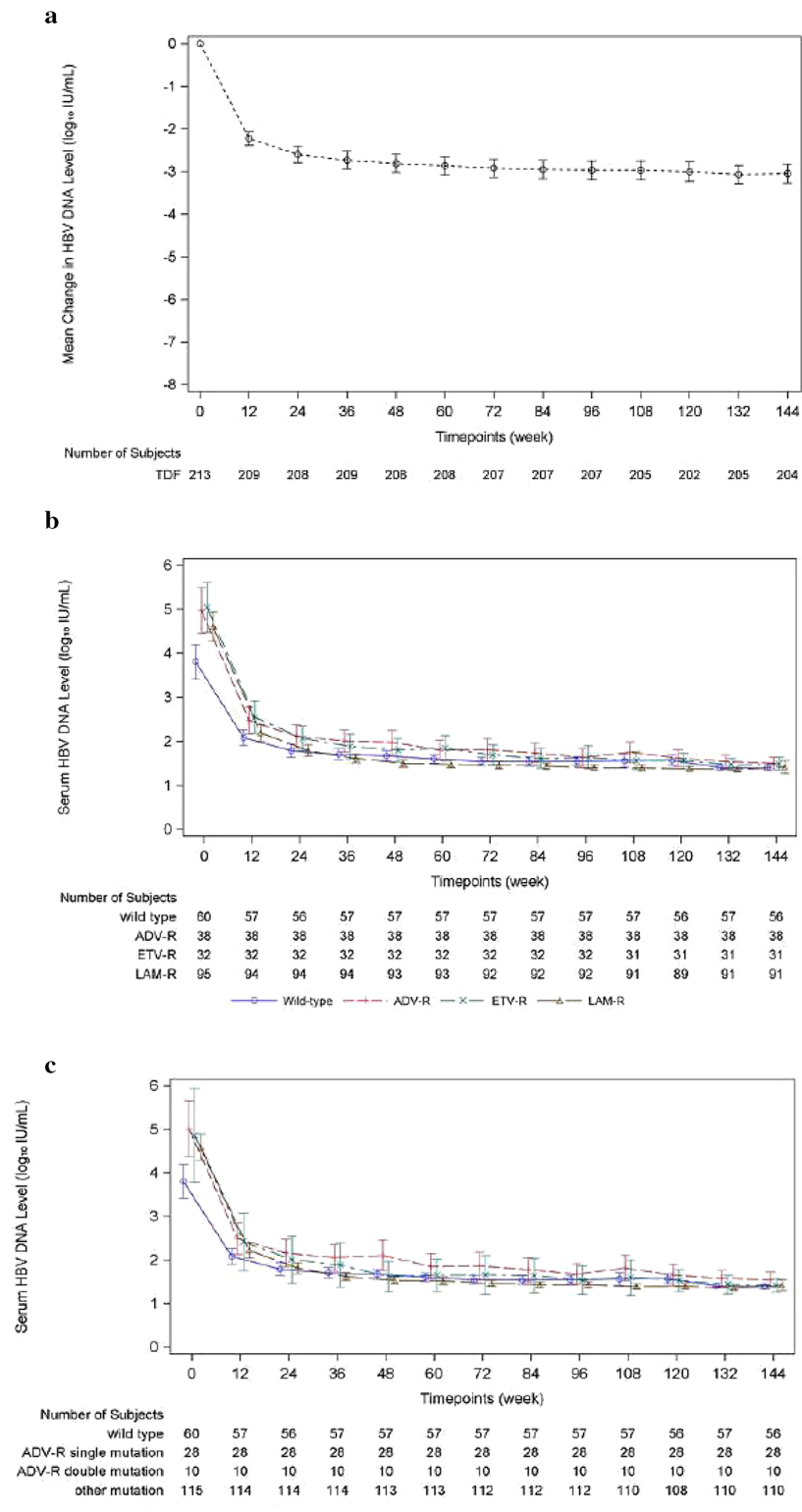


Figure 2 Mean serum HBV DNA (a) reduction from baseline, (b) grouped by mutation patterns Category 1 and (c) grouped by mutation patterns Category 2 over 144 weeks of treatment (mITT population). ADV-R, adefovir-resistant; ETV-R, entecavir-resistant; HBV DNA, hepatitis B virus DNA; LAM-R, lamivudine-resistant; mITT, modified intent-to-treat; TDF, tenofovir disoproxil fumarate. (a): $\text{---}\circ\text{---}$, TDF. (b): $\text{---}\square\text{---}$, wild type; $\text{---}\square\text{---}$, ADV-R; $\text{---}\times\text{---}$, ETV-R; $\text{---}\triangle\text{---}$, LAM-R. (c): $\text{---}\square\text{---}$, wild type; $\text{---}\square\text{---}$, ADV-R single mutation; $\text{---}\times\text{---}$, ADV-R double mutation; $\text{---}\triangle\text{---}$, other mutation.

the majority of the patients undergoing TDF treatment; this was similar to the findings reported by Patterson *et al.*¹⁴ Additionally, reduction in serum HBV DNA by pattern of mutations Category 1 indicate that TDF is efficacious in patients with MDR.

Three years of finite treatment of patients with CHB who have failed prior treatment with multiple NAs seems unpractical as only a very low proportion of patients achieved HBeAg loss, HBsAg loss, and seroconversion at Week 144. Similar results were

Table 3 Cox regression analysis for time to virological response (mITT population)

Variables	HR	95% CI	P value
Serum HBV DNA (> 10 ⁴ vs ≤ 10 ⁴ IU/mL)	0.50	0.33, 0.74	< 0.001
HBeAg status (positive vs negative)	0.33	0.17, 0.63	< 0.001
Early virological response [†] (yes vs no)	8.52	4.53, 16.02	< 0.001
Prior treatment with ADV (yes or no)	1.02	0.45, 2.32	0.97
Resistance type (ADV or not)	0.88	0.54, 1.44	0.62
Cirrhosis (yes vs no)	1.77	0.66, 4.77	0.26
Age (≥ 40 vs < 40 years)	1.22	0.79, 1.88	0.37
Sex (men vs women)	0.86	0.46, 1.64	0.65
BMI (< 25 vs ≥ 25)	1.00	0.62, 1.60	0.99
AST level (abnormal vs normal)	1.18	0.61, 2.27	0.62
ALT level (abnormal vs normal)	0.69	0.42, 1.13	0.14
r-GT level (abnormal vs normal)	0.47	0.23, 0.97	0.04
Platelet level (abnormal vs normal)	1.12	0.55, 2.30	0.75
INR (abnormal vs normal)	0.60	0.16, 2.20	0.44
Total bilirubin level (abnormal vs normal)	1.11	0.61, 2.00	0.74
Albumin level (abnormal vs normal)	2.16	0.80, 5.81	0.13

[†]Early virological response was defined as serum HBV DNA < 69 IU/mL at Week 48 after TDF treatment.

ADV, adefovir dipivoxil; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; HR, hazard ratio; HBV DNA, hepatitis B virus DNA; INR, international normalized ratio; mITT, modified intent-to-treat; r-GT, r-glutamyl transpeptidase; TDF, tenofovir disoproxil fumarate.

observed in the study conducted by Patterson *et al.* where 10% ($n = 6/60$) of patients achieved HBeAg loss and none of the patients achieved HBsAg loss at Week 96.¹⁴ Previously, the rate of HBeAg seroconversion at Week 240 was demonstrated to be 7.1% ($n = 12/170$) among HBeAg-positive patients; none of the patients achieved HBsAg seroclearance.¹⁸ In a retrospective study conducted in Korean patients with CHB after failure of treatment with multiple NAs, HBeAg loss, or seroconversion to anti-HBe occurred in 14.6% ($n = 7/48$) patients (HBeAg-positive at baseline) at Week 52 with TDF treatment.¹⁶ A possible explanation for low seroconversion obtained in the current study could be that the included patients represented a population that had previously failed to achieve serological endpoints after treatment with multiple NAs.

Elevation from the normal ALT levels indicates an irregular hepatic function and is associated with severe liver disease in patients with CHB.^{22–24} A considerable number of patients (68.3%, $n = 41/61$) who had abnormal ALT at baseline achieved ALT normalization in the present study at Week 144 with TDF treatment. TDF treatment of patients with CHB who have failed prior treatment with multiple NAs has been shown to result in ALT normalization in 51.5% ($n = 17/33$) patients at Week 96 and 87.1% ($n = 27/31$) patients at Week 208.^{14,16}

In this study, the BMI levels exhibited a significant odds ratio in favor of patients having body weight within the normal range for achieving serum HBV DNA < 20 IU/mL. The results implied that TDF treatment was more effective in patients having body weight within the normal weight range. It has been reported that a number of factors, including BMI, are responsible for influencing ALT

Table 4 Summary of adverse events (safety population)

	TDF, $n = 213$, n (%)
Patients with any AE	125 (58.7)
Patients with SAEs	20 (9.4)
Patients with drug-related AEs	18 (8.5)
Patients with treatment-related SAEs	0
Patients with AEs leading to study drug discontinuation	1 (0.5)
Patients with AEs leading to discontinuation from the study	1 (0.5)
Patients with AEs leading to a change in dose or a temporary interruption of the study drug	3 (1.4)
Death	1 (0.5)
AEs occurring in ≥ 3 patients	
Upper respiratory tract infection	28 (13.1)
Blood creatine phosphokinase increased	12 (5.6)
Hypophosphatemia	8 (3.8)
Hepatic steatosis	7 (3.3)
Nephrolithiasis	5 (2.3)
Urinary tract infection	4 (1.9)
Diarrhea	4 (1.9)
Hepatocellular carcinoma	4 (1.9)
Insomnia	4 (1.9)
Hypertension	4 (1.9)
Blood uric acid increased	3 (1.4)
Weight decreased	3 (1.4)
Chronic gastritis	3 (1.4)
Toothache	3 (1.4)
Cholelithiasis	3 (1.4)
Gallbladder polyp	3 (1.4)
Hypokalemia	3 (1.4)
Renal cyst	3 (1.4)
Pyrexia	3 (1.4)
SAEs occurring in ≥ 2 patients	
Hepatocellular carcinoma	4 (1.9)
Cholecystitis acute	2 (0.9)
Cholelithiasis	2 (0.9)
Fibula fracture	2 (0.9)
Tibia fracture	2 (0.9)
Study drug-related AEs occurring in ≥ 2 patients	
Blood creatine phosphokinase increased	6 (2.8)
Hypophosphatemia	5 (2.3)
Any grade [†] 3 or 4 laboratory abnormalities in ≥ 2 patients	21 (9.9)
Serum phosphorus decrease	11 (5.2)
Hemoglobin decrease	3 (1.4)
Alanine aminotransferase increase	3 (1.4)
Creatine phosphokinase increase	3 (1.4)
Hepatitis exacerbation	2 (0.9)

[†]The severity of these laboratory abnormalities were graded according to the criteria described in report analytic plan.

AEs, adverse events; SAEs, serious adverse events; TDF, tenofovir disoproxil fumarate.

normalization in patients with CHB.^{22–25} Previously, few studies have provided clinical evidence on the association of BMI improvement with a beneficial effect on ALT normalization and also with the reduced risk of development of hepatic sequelae in patients with HBV infection.^{26,27} The results obtained in the present

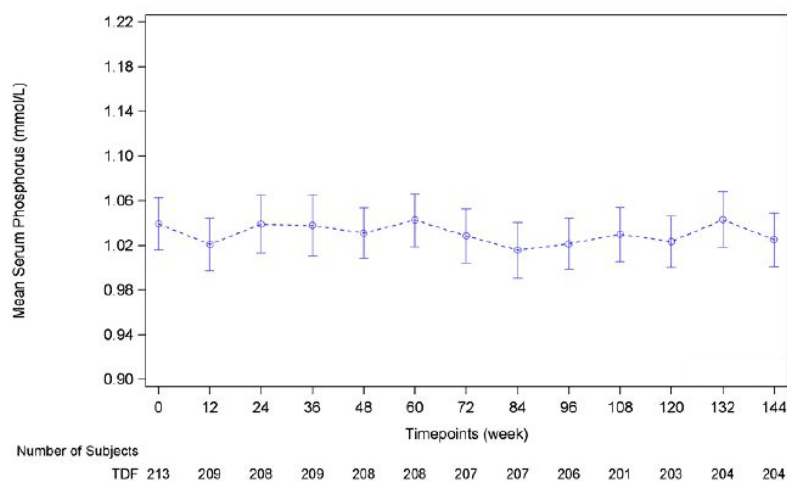
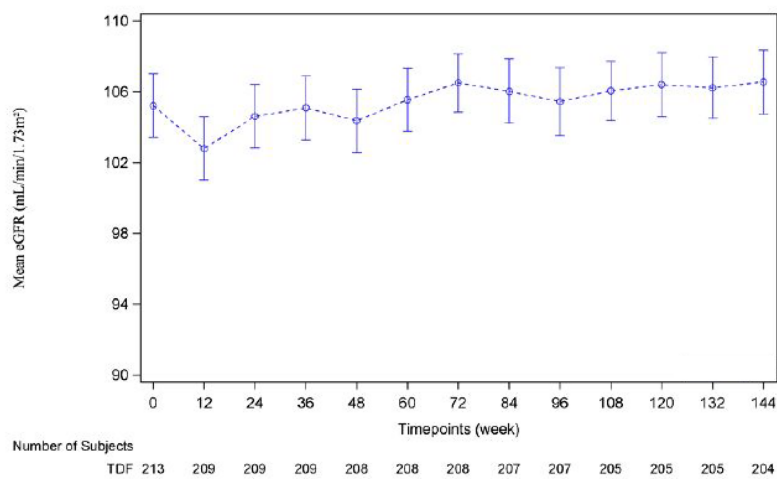
a**b**

Figure 3 Mean (a) serum phosphorous level and (b) estimated glomerular filtration rate up to 144 weeks of TDF treatment (mITT population). mITT, modified intent-to-treat; TDF, tenofovir disoproxil fumarate. ---◇---, TDF.

study suggest that BMI improvement may further help in achieving normalization of ALT in patients with CHB.

A new prodrug of tenofovir, tenofovir alafenamide (TAF), has been shown to be noninferior to TDF with respect to viral suppression. The findings from two randomized, double-blind, international phase 3 trials in patients with CHB have also shown that TAF has a better bone and renal safety profile compared with TDF. However, more studies investigating long-term safety and efficacy are needed in support of TAF for the treatment of CHB patients.²⁸

Previously published studies have also demonstrated that TDF was well tolerated in patients with CHB.^{12,14,16,18,29} In this patient population, there were no new safety findings, including renal safety parameters. Hypophosphatemia was transient and reversible. Despite a transient slight decrease, estimated glomerular filtration rate showed a slight increase up to Week 144 of TDF treatment in the present study.

This study also has a few limitations. First, the open-label design could have had an influence on the reporting of events. Second, because it was a single-arm study, there was no control group for

comparison. However, the multicenter, large cohort design of the present study could still provide informative evidence for clinical practice.

Conclusions

In patients with CHB from China who have failed prior treatment with multiple NAs, 144 weeks of TDF monotherapy is efficacious with 77% patients achieving sustained virological suppression (HBV DNA < 20 IU/mL) in the total population and 65.8% in patients with MDR mutations at baseline. There were no new safety findings in the studied patient population.

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Data availability statement. Anonymized individual participant data and study documents can be requested for further research from www.clinicalstudydatarequest.com.

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