Switching to Besifovir in Patients with Chronic Hepatitis B Receiving Tenofovir Disoproxil Fumarate: A Randomized Trial

Running title: Switching tenofovir DF to besifovir

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Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Ethic approval statement: This study was approved by the institutional review board of all site sites and performed in accordance with the declaration of Helsinki. Informed consent was obtained from all study participants.

Patient consent statement

Informed consent was obtained from all patients for being included in the study.

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None to declare

Clinical trial registration

ClinicalTrials.gov Identifier **NCT04202536 (https://clinicaltrials.gov/ct2/show/NCT04202536)**

Abstract

Background/Aims

Besifovir (BSV) showed comparable antiviral activity superior safety profiles to tenofovir disoproxil fumarate (TDF) in treatment-naïve chronic hepatitis B (CHB). However, no data are available regarding the antiviral efficacy and safety of BSV in patients with CHB who switched from long-term TDF to BSV. This study aimed to evaluate the outcome of a 48-week BSV therapy in patients with CHB who switched from long-term TDF treatment.

Methods

In this non-inferiority trial, 153 CHB patients treated with TDF for ≥48 weeks who had hepatitis B virus (HBV) DNA <20 IU/mL were randomized to receive either BSV 150 mg or TDF 300 mg for 48 weeks.

Results

The per-protocol analysis included 130 patients (BSV group, 64; TDF group, 66). The median duration of TDF use before enrollment was 4.14 years. After 48 weeks, 100.0% and 98.5% patients in the BSV and TDF groups, respectively, met the primary endpoint (HBV DNA <20 IU/mL), demonstrating the non-inferior antiviral efficacy of BSV to TDF (95% CI -0.01 to 0.04; *P*=1.000), with a predefined margin of -0.18. The mean percentage changes in estimated glomerular filtration rates were slightly better in the BSV group (1.67±11.73%) than in the TDF group (-1.24±11.02%). The BSV group showed a significant improvement in bone turnover biomarkers compared to the TDF group; accordingly, hip and spine bone mineral density increased in the BSV group.

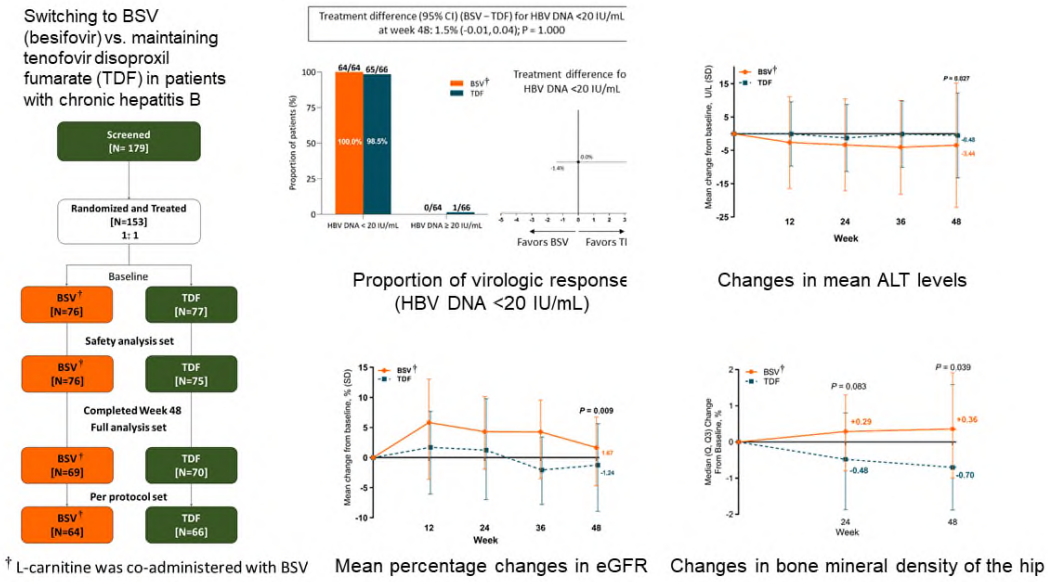
Conclusions

In patients with CHB receiving long-term TDF, switching to BSV may improve renal and bone safety with non-inferior antiviral efficacy compared to that of maintaining TDF.

ClinicalTrials.gov Identifier: **NCT04202536**

Keywords

Antiviral therapy; Hepatitis B; Bone mineral density; Nephrotoxicity; Sustained Virologic Response



Study highlights

- During a long-term treatment with tenofovir disoproxil fumarate (TDF), renal function and bone density decrease gradually.
- Switching TDF to BSV shows non-inferior antiviral efficacy to maintaining TDF in CHB patients receiving long-term TDF.
- The adverse effects of long-term TDF may be potentially reversible with improved renal function and bone density after switching to BSV.
- A significant decline in ALT levels and no antiviral resistance are noted after switching TDF to BSV although the change in ALT levels was clinically marginal.

Introduction

Chronic hepatitis B virus (HBV) infection, a common cause of chronic liver disease, is associated with the development of liver cirrhosis and hepatocellular carcinoma.[1] Hence, the aim of chronic hepatitis B (CHB) treatment is to prevent complications related to the progression of underlying liver disease via suppression of viral replication, normalization of the aminotransferases, and hepatitis B e antigen (HBeAg)/surface antigen (HBsAg) loss or seroconversion.[2-5] Of these, HBsAg loss is the optimal endpoint of treatment.

Currently, nucelos(t)ide analogues (NAs) are the mainstay of antiviral therapy; however, they produce HBsAg seroclearance in less than 1% of patients annually. Hence, most patients need long-term NA administration. Currently, the mean age and number of comorbidities are increasing in patients with CHB.[6-8] Therefore, minimizing adverse effects is highly important during prolonged NA therapies. Tenofovir disoproxil fumarate (TDF) is the most widely used potent antiviral agent for the treatment of CHB without the emergence of antiviral resistance.[9] However, the estimated glomerular filtration rates (eGFR) and bone mineral density (BMD) may decrease gradually during long-term therapy.[10-12] Considering that patients with CHB increasingly have diverse comorbid diseases,[6-8] such drug-related adverse events may hamper the benefit of antiviral therapy for CHB.

Besifovir dipivoxil maleate (BSV), previously known as LB80380, is a prodrug of a nucleotide analog of guanosine monophosphate with potent antiviral activity against HBV replication.[13] A phase 3 trial showed that the antiviral efficacy of BSV over 48 weeks was comparable to that of TDF, with improved renal and bone safety.[14,15] After 48 weeks, all patients received BSV only. During the extended period of the BSV therapy up to 196 weeks, virologic response, defined as undetectable HBV DNA by PCR assay, increased over 90% in patients with CHB.[16] No antiviral resistance was noted during the treatment. Notably, the decrease in eGFR and BMD was improved in the patients who received TDF during the first year after switching to BSV.[14] Hence, reversibility of such adverse events was demonstrated in patients who received 48 weeks of TDF therapy.[14] However, most patients in real-world practice have been already treated with TDF for several years, which suggests that improvement of renal and bone injury after switching to BSV should be evaluated further. More importantly, although BSV was comparable to TDF for achieving a virological response in treatment-naïve patients, maintenance of virological suppression should be confirmed in TDF-treated patients with CHB after the switching from TDF to BSV.

This study aimed to determine the efficacy and safety of switching from TDF to BSV in patients with CHB with suppressed viral replication.

Methods

Study Design

This randomized, open-label, active-controlled, non-inferiority phase 4 clinical trial was performed at 22 tertiary hospitals in South Korea. We compared the efficacy and safety of switching to BSV (Ildong Pharmaceutical Co. Ltd, Seoul, Korea) with that of maintaining TDF (Gilead Sciences, Foster City, CA) after 48 weeks of randomization in patients with CHB exhibiting HBV DNA <20 IU/mL by TDF therapy. The patients were randomly assigned to either the BSV or TDF group in a ratio of 1:1 using a centralized interactive web response system. The random table was generated by a statistician using SAS (version 9.1.3, SAS Institute, Cary, NC) and stratified according to HBeAg status. TDF was administered until randomization was performed. Patients assigned to the BSV switch group were administered BSV 150 mg with carnitine 660 mg (Ildong Pharmaceutical Co. Ltd., Seoul, Korea) as a supplement. Patients assigned to the TDF maintenance group continued to receive TDF 300 mg.

Using PASS (version 2015, NCSS LLC, Kaysville, UT), we determined that 76 patients are needed in each group for demonstrating the non-inferiority of BSV to TDF in terms of virologic response (HBV DNA <20 IU/mL) at week 48, considering 80% of statistical power, 2.5% one-sided significance level, 18% of non-inferiority margin, and 10% of drop-out rate. The response rates were assumed as 0.95 for TDF and 0.91 for BSV based on previous clinical trials.[14]

Subjects

The key inclusion criteria were as follows: 1) history of CHB or positive HBsAg for more than 6 months before screening, 2) TDF monotherapy for ≥48 weeks and TDF use at the time of clinical screening, and 3) HBV DNA <20 IU/mL.

Key exclusion criteria were as follows: 1) history of (peg)interferon treatment for CHB, 2) previous antiviral resistance during NA therapy, 3) any malignant tumors within 5 years before screening, or 4) eGFR less than 50 mL/min by Modification of Diet in Renal Disease (MDRD). Detailed enrollment criteria and monitoring procedures are described in Supp. Table 1 and Supp. Methods, respectively.

Informed consent was obtained from all study participants, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Prior approval was granted by the Institutional Committee on

Human Research at all sites (ClinicalTrials.gov Identifier: **NCT04202536**).

Efficacy and safety measurements

The primary endpoint was the proportion of patients with HBV DNA <20 IU/mL at week 48. The secondary endpoints included the proportion of patients with undetectable HBV DNA at week 24 and normalization of alanine aminotransferase (ALT), HBeAg loss/seroconversion, and HBsAg loss/seroconversion at week 48. Changes in ALT levels, incidence of virological breakthrough and antiviral resistance mutations, and changes in the Fib-4 score during 48 weeks were also assessed.

Safety endpoints included incidence of any adverse events; CTCAE grade 3 adverse events; and changes in BMD, eGFR by MDRD, urine protein to creatinine ratio (P/C ratio), urine albumin to creatinine ratio (UACR), urine β2 microglobulin to creatinine ratio, bone turnover markers, and serum carnitine and vitamin D levels. The incidence of adverse events was collected through self-report by patients, laboratory tests, imaging studies, or medical chart review.

Definitions and laboratory tests

Virologic response was defined as HBV DNA <20 IU/mL by real-time PCR during antiviral therapy. Other definitions are described in the Supp. Methods section.

Laboratory tests were performed at the central laboratory (GC Labs, Yongin, South Korea). HBV DNA was quantified using the COBAS AmpliPrep-COBAS TaqMan™ HBV test (v.2.0; Roche Diagnostics, Branchburg, NJ). HBsAg, antibody to HBsAg (anti-HBs), HBeAg, and antibodies to HBeAg (anti-HBe) were assayed using qualitative chemiluminescence microparticle immunoassays (Abbott Diagnostics, Abbott Park, IL). HBsAg levels were quantified using the Architect HBsAg QT assay (Abbott Diagnostics, Abbott Park, IL). Genotypic resistance tests were performed using direct sequencing. An antiviral resistance test was indicated in cases of virological breakthrough at any time point or HBV DNA >69 IU/mL at week 48. The Fib-4 score was calculated using the following formula: age (years)×AST (U/L)/platelet count ($\times 10^9$/L)×√ALT (U/L). Other routine laboratory tests, such as biochemical, hematological, coagulation, and urinary tests, were performed according to standard methods. Urinary β2-microglobulin levels were determined by chemiluminescence immunoassay using the IMMULITE™ 2000 Beta-2 Microglobulin test (Siemens Healthineers, Erlangen, Germany). BMD was measured using dual-

energy X-ray absorptiometry, such as Lunar Prodigy Advance™ (GE Healthcare Inc., Chicago, IL), according to the availability of each site. Bone turnover markers and serum carnitine and vitamin D levels were assayed in the central laboratory.

Statistical Analysis

Data were analyzed using a per-protocol set (PPS) and full analysis set (FAS). The per-protocol (PP) analysis included only patients who had completed the treatment schedule according to the study protocol. Patients who discontinued study participation, showed poor adherence to taking study medicine (<80%), or violated important protocols of the study (e.g., visit window ≤ 2 weeks) were excluded from the analysis. The FAS included all randomized patients who had taken the study medication at least once or more, and they were subjected to modified intention-to-treat (mITT) analysis if they fulfilled the enrollment criteria and maintained the consent to participating the study. The efficacy analysis was performed primarily based on PP analysis, and mITT using FAS was additionally performed. The safety analysis was performed using a safety dataset, which included all available safety data for patients who had taken the study medication at least once or more. Categorical variables were analyzed using chi-square or Fisher's exact tests, as appropriate. Continuous variables were compared between the groups using Student's *t*-test or the Mann–Whitney U-test, as appropriate. Proportions are reported as the percentage of the number of patients. Continuous variables with normal distribution are presented as the mean ± standard deviation, and non-normal variables are presented as the median and interquartile ranges (Q1, Q3). Overall eGFR changes between the groups during the study period were compared using repeated-measures analysis of variance (ANOVA). SAS (version 9.4, SAS Institute, Cary, NC) was used for the statistical analyses. *P* values <0.05 were considered to indicate statistical significance.

Results

Characteristics of the patients

Between December 2019 and June 2020, 179 patients were screened and 153 were enrolled. Figure 1 presents the reasons for exclusion after screening. Seventy-six patients were randomized to the BSV group and seventy-seven were assigned to the TDF group. Sixty-nine and seventy patients completed 48 weeks of treatment, respectively; however, five and four patients, respectively, were excluded from the PPS because of protocol violation. Finally, 64 and 66 patients were included in the PP analysis, respectively.

Table 1 presents the baseline characteristics of patients in the PPS without intergroup differences. In the BSV and TDF groups, the mean prior-exposure durations to TDF before enrollment in the present study were 4.26 and 4.33 years, respectively, which are fairly long periods.

The baseline characteristics of patients in the FAS were similar to those in the PPS (Supp. Table 2)

Antiviral responses

In the PP analysis, the proportion of patients with a virological response (HBV DNA <20 IU/mL) at week 48, the primary endpoint, was 100.0% (64/64) and 98.5% (65/66) in the BSV and TDF groups, respectively (P=1.000), exhibiting a 1.5% difference between the groups (95% CI for difference, -0.01 to 0.04). As the predefined non-inferiority margin (-0.18) was not included in the 95% CI, switching to BSV was considered non-inferior to continuing TDF for maintaining a virologic response (Figure 2A). No virological breakthrough or antiviral resistance mutation was observed in either group. The virological response rate was not significantly different between the groups at week 24 (98.4% and 100.0%, respectively; P=0.492; 95% CI, -0.05 to 0.01). The mean HBV DNA level at 48 weeks did not differ between the groups (Figure 2B).

The HBeAg seroconversion rates at week 48 were 14.8% (4/27) and 10.7% (3/28) in the BSV and TDF groups, respectively (P=0.705), among the baseline HBeAg-positive subjects (Table 2). HBsAg loss and HBsAg to anti-HBs seroconversion occurred in only one patient in the BSV group (1.6%) and none in the TDF group (0.0%) over 48 weeks (P=0.488). Mean changes in HBsAg levels during 48 weeks were -0.11±0.34 log IU/mL (P<0.001,

within the group) and -0.05±0.08 IU/mL (P<0.001, within the group) in the BSV and TDF groups, respectively (P=0.455, between the groups).

The proportion of patients with normal ALT, according to the American Association for the Study of the Liver criteria, increased from 78.1% (50/64) to 82.8% (53/64) in the BSV group, while it did not change in 83.3% (55/66) patients in the TDF group over 48 weeks (Figure 2C). The mean ALT reduction (-3.44 U/L, P<0.001, within the group) was significantly greater in the BSV group compared to that (-0.48 U/L, P=0.371, within the group) in the TDF group during 48 weeks (P=0.027, between the groups) (Figure 2D). Biochemical breakthrough was observed in two and three patients in each group, respectively; however, none of them discontinued the study medications. Fib-4 score decreased from the baseline to week 48 in both groups without an intergroup difference (-0.10±0.45 and -0.04±0.44, respectively, P=0.451).

Antiviral efficacy results from mITT analyses using FAS were consistent with PP analysis (Supp. Table 3)

Adverse events

Both groups showed similar rates of adverse events (28 patients, 36.8%, BSV group; 29 patients, 38.7%, TDF group) over 48 weeks (Table 3). In the BSV and TDF groups, grade 3–4 adverse events occurred in one and three patients, respectively, and a serious adverse event (SAE) in one and four patients, respectively; none of them were related to the study medication. One patient in the TDF group developed hepatocellular carcinoma.

Renal and bone safety

The mean percentage changes in eGFR during 48 weeks were persistently higher in the BSV group than in the TDF group, as analyzed by repeated measures ANOVA (P=0.009) (Figure 3A). The urine P/C ratio and UACR did not change in the BSV group; however, they were significantly increased in the TDF group. The median changes in urine P/C ratio were significantly different between the groups (-1.48 vs. 13.2, respectively, P=0.027), whereas median changes in UACR were not (14.3 vs. 22.13, respectively, P=0.548). The urine β2-microglobulin-to-creatinine ratio significantly decreased in the BSV group compared to that in the TDF group (-7.89 vs. 11.85, respectively, P=0.003) over 48 weeks (Figure 3B).

The hip and spine BMD increased after the switch to BSV (Figure 4A, 4B). The hip %BMD changes were +0.36 (BSV) and -0.70 (TDF) at week 48 (P=0.039). The spine %BMD changes were +1.89 (BSV) and +0.28 (TDF) at

week 24 (P=0.172).

After the switch to BSV, the BSV group showed greater improvement in bone turnover markers than the TDF group (Figure 4C). The C-type collagen sequence, which is a bone-resorption marker, significantly decreased in the BSV group but increased in the TDF group (median percentage changes; -17.99 vs. 9.15, respectively, P<0.001). The following bone-formation markers significantly decreased in the BSV group but not in the TDF group, as indicated by their median percentage changes: bone-specific alkaline phosphatase (-16.33 vs. -1.59, respectively, P=0.001), procollagen type 1 N-terminal propeptide (-22.07 vs. 1.08, respectively, P<0.001), and osteocalcin (-13.85 vs. 2.90, respectively, P<0.001). The mean serum carnitine and vitamin D levels did not significantly change in either group.

Discussion

We have previously demonstrated the efficacy and safety of BSV in phase 2 and 3 trials in the treatment naïve patients with CHB.[14,17] Herein, we report results from a phase 4 clinical trial in patients treated with TDF for several years. The mean duration of the prior treatment was 4.3 years. The antiviral efficacy of switching to BSV was not inferior to that of maintaining TDF therapy in patients who had been treated with TDF. Interestingly, the mean ALT level decreased more in the BSV-switch group than in the TDF-maintenance group. Although the mechanism is unclear, a similar finding was observed in a phase 3 trial comparing tenofovir alafenamide (TAF) and TDF. Additionally, in a previous study on histologic changes after BSV and TDF treatment, patients in the BSV group showed better improvement of necroinflammation than those in the TDF group, which may be attributed to ALT changes, as observed in the present study.[18] Regarding renal and bone safety, although renal damage or decrease in BMD could have worsened during long-term TDF administration, the BSV-switch group showed a significant improvement in e-GFR and spine BMD compared with the TDF-maintenance group. This result suggests that the renal and bone damage caused by TDF may be potentially reversible despite the relatively long-term exposure to the drug.

In the earlier phase study, administration of BSV was associated with a decrease in serum L-carnitine levels.[17] Although clinical symptoms of L-carnitine deficiency were not observed in the previous trials, supplementation of L-carnitine was recommended during the treatment with BSV for chronic hepatitis B.

As L-carnitine is a naturally occurring substance that is required to generate adenosine triphosphate, it contains antioxidant effects and protects numerous tissues from oxidative stress including the liver, kidney, heart, bone, and muscles.[19,20] So, L-carnitine may have influenced improved renal function and bone mineral density in the BSV group.

Renal tubular damage has been suggested to be the main cause of renal function decline in patients receiving TDF.[10,12] Occasionally, short-term use of TDF may cause Fanconi's syndrome and nephrotic syndrome.[21] In addition, a recent animal study showed an increase in proportion of pyknotic epithelial cells and acidophilic cytoplasm in renal tubules and congestion and hemorrhage with increasing dose and duration of TDF administration; urinary protein and albumin and serum creatinine increased with renal tubular damage and dysfunction.[22] Therefore, caution should be exerted on changes in renal function during TDF therapy.

In the present study, switching from TDF to BSV was associated with improved e-GRF, probably due to less

induction of the tubulopathic effect of BSV. Indeed, in previous in vivo studies, the renal uptake of BSV was only one-third that of TDF, while the hepatic uptake of BSV was twice that of TDF. Additionally, the active metabolite of BSV has been shown to be associated with less systemic exposure and a high hepatic concentration.[14] This could be attributed to the improved renal safety of BSV in this study. However, the improvement of eGFR in the BSV group was marginal with a converging tendency over time. Hence, longer-term study is needed to confirm that the benefit of BSV can be maintained over time.

In this study, we further evaluated renal functional abnormalities using the urine P/C ratio, UACR, and urine β2-macroglobulin-to-creatinine ratio. These are useful markers of kidney damage, and all markers showed lower levels in the BSV group than in the TDF group. In particular, the urine β2-microglobulin-to-creatinine ratio, which represents renal tubular damage,[23] significantly decreased in the BSV group; this finding suggests recovery from the TDF-induced tubular injury. In addition, the urine P/C ratio decreased significantly in the BSV group compared to that in the TDF group, while the UACR did not significantly differ between the groups. This finding suggests that non-albumin proteinuria, which predominantly increases in tubulointerstitial kidney disease, improved after the switch from TDF to BSV.

The mechanism of TDF-induced bone loss is multifactorial. TDF produces renal tubular toxicity, inducing excessive renal phosphate wasting and osteomalacia, which leads to a decrease in BMD and an increase in bone turnover marker levels.[24] Additionally, its direct effect on bone metabolism may be associated with bone loss. TDF has been reported to negatively modulate the Wnt/β-catenin pathway, which is essential for osteoblast differentiation and osteogenesis, thereby leading to decreased bone formation.[25] Hence, monitoring bone homeostasis with bone turnover markers would provide information regarding fracture risk and need for therapies against osteopenia/osteoporosis.[26] In this study, we assessed the BMD and bone turnover markers during BSV and TDF treatment. BMD improved, and bone turnover markers representing bone resorption and bone formation decreased significantly in the BSV group compared to in the TDF group. During treatment with anti-osteoporosis therapies such as bisphosphonate, levels of bone resorption markers including C-type collagen sequence fall in the first 3 months, following which they decrease over the next 6–12 months.[27] Among the bone formation markers, alkaline phosphatase is the earliest marker of bone turnover, while osteocalcin is a late marker of bone formation.[27] All these markers significantly decreased during 48 weeks after switching TDF to BSV.

Recently, tenofovir alafenamide (TAF), a newly developed oral phosphoramidate prodrug of tenofovir, exhibited greater stability in the plasma than TDF.[28,29] As TAF can provide a high intracellular concentration of tenofovir diphosphate, an active metabolite in the hepatocytes, only a 25 mg dose was approved for CHB treatment showing non-inferior antiviral efficacy and better safety compared with TDF 300 mg.[28,29] More recently, a non-inferiority randomized trial compared a switch to TAF and TDF continuation in TDF-treated virologically suppressed patients with CHB.[30] The results were similar to that of the present study, showing non-inferior antiviral efficacy and improved renal and bone safety of TAF compared to that of TDF. Till date, no data has compared the efficacy or safety of BSV and TAF in patients with CHB administered TDF. However, we previously performed a retrospective analysis to compare the two drugs in treatment-naïve patients with CHB; the clinical outcomes, including the virological response and incidence of liver-related complications, were not significantly different.[31] As these two drugs exhibit similar antiviral activity and safety, they are expected to serve as successors of TDF in the near future.

This study has several limitations. First, the number of patients was not sufficiently large. The number of patients required was calculated on the basis of the primary end point. Hence, we were able to demonstrate the non-inferiority of BSV to TDF; however, the results of the secondary endpoints, such as changes in spine BMD, were slightly suboptimal. Second, the follow-up duration of this study was relatively short. However, we found significant changes in renal and bone health indices and maintenance of virological response after the switch from TDF to BSV. To extend the observation period, we are performing additional follow-up for up to 96 weeks after switching therapies according to the clinical situation. Third, although the mean ALT reduction was significantly greater in the BSV group compared to that in the TDF group during 48 weeks, the improvement was clinically marginal considering the initial levels were slightly higher in the BSV group.

To summarize, we demonstrated the non-inferior antiviral efficacy of switching to BSV compared to that of maintaining TDF in virologically suppressed CHB patients using TDF. The mean duration of previous TDF therapy was over 4 years, which is a substantially long-term duration. The adverse effects of TDF were readily reversible with improved e-GFR and BMD after switching to BSV, which was supported by significant changes in the various markers of renal injury and bone turnover. Ancillary findings included a decline in ALT levels, no incidence of antiviral resistance, and HBsAg loss in the BSV group. No significant changes were noted in the lipid profile, serum carnitine level, or vitamin D levels.

In conclusion, switching to BSV is effective and safe for patients with CHB receiving long-term TDF.

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Table 1. Baseline characteristics of the patients

	Overall (n=130)	BSV (n=64)	TDF (n=66)	*P* value
Mean age, y (SD)	49.9 (9.8)	50.8 (9.6)	49.1 (10.0)	0.304†
Male, n (%)	84 (64.6)	43 (67.2)	41 (62.1)	0.546‡
HBeAg negative, n (%)	75 (57.7)	37 (57.8)	38 (57.6)	0.978‡
Years positive for HBV (SD)	10.01 (9.45)	9.62 (8.52)	10.39 (10.34)	0.988§
Mean HBV, IU/mL (SD)	10.1 (0.5)	10.0 (0.4)	10.1 (0.7)	0.585§
Mean ALT, U/L (SD)	26.4 (14.9)	27.6 (16.0)	25.2 (13.7)	0.375§
ALT ≤ULN (AASLD 2018 criteria), n (%)	105 (80.8)	50 (78.1)	55 (83.3)	0.451‡
Mean AST, U/L (SD)	26.8 (17.4)	27.9 (22.4)	25.8 (10.6)	0.405§
Mean HBsAg, $\log_{10}$ IU/mL (SD)	3.21 (0.77)	3.14 (0.89)	3.28 (0.63)	0.736§
Median duration of prior TDF use, y (Q1, Q3)	4.14 (2.78, 5.83)	4.33 (2.91, 5.68)	3.85 (2.70, 6.12)	0.800§
FIB-4 score (SD)	1.43 (0.73)	1.39 (0.74)	1.48 (0.72)	0.357§
Liver cirrhosis, n (%)	37 (28.5)	16 (25.0)	21 (31.8)	0.389‡
Mean albumin, g/dL (SD)	4.72 (0.27)	4.72 (0.26)	4.72 (0.28)	0.793§
Mean hemoglobin, g/dL (SD)	14.50 (1.43)	14.59 (1.54)	14.42 (1.32)	0.490¶
Mean platelet, 10^3/μL (SD)	210.7 (66.4)	222.3 (70.0)	199.4 (61.2)	0.049¶
Mean AFP, ng/mL (SD)	2.71 (1.20)	2.85 (1.41)	2.58 (0.94)	0.493§
Median creatinine, mg/dL	0.88	0.87	0.90	0.916†

(Q1, Q3)	(0.77, 0.98)	(0.77, 0.97)	(0.77, 0.98)	
Median e-GFR, mL/min	84.0	84.0	84.5	0.871§
(Q1, Q3)	(74.0, 95.0)	(72.5, 96.0)	(76.0, 94.0)	
Hip BMD, n (%)				0.626¶
Normal (T-score≥-1.0)	85 (75.9)	42 (76.4)	43 (75.4)	
Osteopenia (-2.5<T-score<-1.0)	25 (22.3)	13 (23.6)	12 (21.1)	
Osteoporosis (T-score <-2.5)	2 (1.8)	0 (0.0)	2 (3.5)	
Spine BMD, n (%)				0.994‡
Normal (T-score≥-1.0)	87 (66.9)	43 (67.2)	44 (66.7)	
Osteopenia (-2.5<T-score<-1.0)	33 (25.4)	16 (25.0)	17 (25.8)	
Osteoporosis (T-score<-2.5)	10 (7.7)	5 (7.8)	5 (7.6)	

HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; ALT, alanine aminotransferase; AASLD, American Association for the Study of Liver Disease; ULN, upper limit of normal; AST, aspartate aminotransferase; TDF, tenofovir disoproxil fumarate; FIB-4, fibrosis-4; AFP, alpha-fetoprotein; BMD, bone mineral density.

† Student's t-test

‡ chi-square test

§ Wilcoxon rank-sum test

¶ Fisher's exact test

Table 2. Serological responses in the per protocol at week 48

		BSV	TDF	*P*-value
HBeAg	Loss, n/N (%)	4/27 (14.8%)	3/28 (10.7%)	0.705†
	Seroconversion, n/N (%)	4/27 (14.8%)	3/28 (10.7%)	0.705†
HBsAg	Loss, n (%)	1/63 (1.6%)	0/66 (0.0%)	0.488†
	Seroconversion, n/N (%)	1/63 (1.6%)	0/66 (0.0%)	0.488†
	Mean change at week 48, log_{10} IU/mL (SD)	-0.11 (0.34)	-0.05 (0.08)	0.455‡

† Fisher's exact test

‡ Wilcoxon rank-sum test

Table 3. Adverse events during study

	BSV (n=76)	TDF (n=75)	Proportional difference (95% CI)[†]
Any adverse event	27 (35.5)	29 (38.7)	-3.14% (-18.54 to 12.26)
Study drug-related adverse events	7 (9.2)	4 (5.3)	3.88% (-4.38 to 12.13)
Grade 3 adverse events[‡]	1 (1.3)	3 (4.0)	-2.68% (-7.81 to 2.44)
Diverticulitis	1 (1.3)	0	1.32% (-1.25 to 3.88)
Hepatocellular carcinoma[§]	0	1 (1.3)	-1.33% (-3.93 to 1.26)
Alveolar proteinosis[§]	0	1 (1.3)	-1.33% (-3.93 to 1.26)
Cartilage injury	0	1 (1.3)	-1.33% (-3.93 to 1.26)
Meniscus injury	0	1 (1.3)	-1.33% (-3.93 to 1.26)
Serious adverse event[¶]	1 (1.3)	4 (5.3)	-4.02% (-9.71 to 1.68)
Study drug-related serious adverse event	1 (1.3)	0 (0.0)	1.32% (-1.25 to 3.89)
Premature study drug discontinuation due to adverse events	2 (2.6)	1 (1.3)	1.30% (-3.14 to 5.74)
Most common treatment-emergent adverse events[#]			
Nasopharyngitis	3 (3.9)	5 (6.7)	-2.72% (-9.86 to 4.42)
Osteoporosis	3 (3.9)	4 (5.3)	-1.39% (-8.10 to 5.32)
Hypertension	3 (3.9)	0	3.95% (-0.43 to 8.33)
Rhinitis	0	3 (4.0)	-4.00% (-8.43 to 0.43)
Dyspepsia	0	3 (4.0)	-4.00% (-8.43 to 0.43)
Grade 3 or 4 laboratory abnormalities			
Grade 3 or 4 laboratory abnormalities in ≥1% of patients in either group	2 (2.6)	12 (16.0)	-13.37% (-22.41 to -4.32)
Potassium, grade 3 >6.0–7.0 mmol/L	1 (1.3)	5 (6.7)	-5.35% (-11.55 to 0.85)
PT(INR), grade 3 >2.5	0	2 (2.7)	-2.67% (-6.31 to 0.98)

ANC, grade 3 <1000/mm^3	0	3 (4.0)	-4.00% (-8.43 to 0.43)
Creatine phosphokinase, grade 3 >5 to 10 × upper limit of normal	0	1 (1.3)	-1.33% (-3.93 to 1.26)
Uric acid, grade 3, >ULN with physiologic consequences	0	1 (1.3)	-1.33% (-3.93 to 1.26)
Sodium, grade 3, 120–124 mmol/L§	0	1 (1.3)	-1.33% (-3.93 to 1.26)
Sodium, grade 4, <120 mmol/L§	0	1 (1.3)	-1.33% (-3.93 to 1.26)
Triglycerides, grade 3 >500 mg/dL–1000 mg/dL;	1 (1.3)	0	1.32% (-1.25 to 3.88)

Data are n (%).

† Normal approximation for the difference between two proportions.

‡ No grade 4 adverse events.

§ These events occurred in the same patient.

¶ One patient receiving besifovir dipivoxil maleate experienced the following serious adverse event: diverticulitis. Three patients receiving tenofovir disoproxil fumarate experienced the following serious adverse events: hepatocellular carcinoma, pulmonary tuberculoma, ligament sprain, cholelithiasis, cartilage injury, and meniscus injury.

diverse events occurring in at least 3% of patients in any arm.

Figure Legends

Figure 1. Disposition of the study subjects

†L-carnitine was co-administered with BSV.

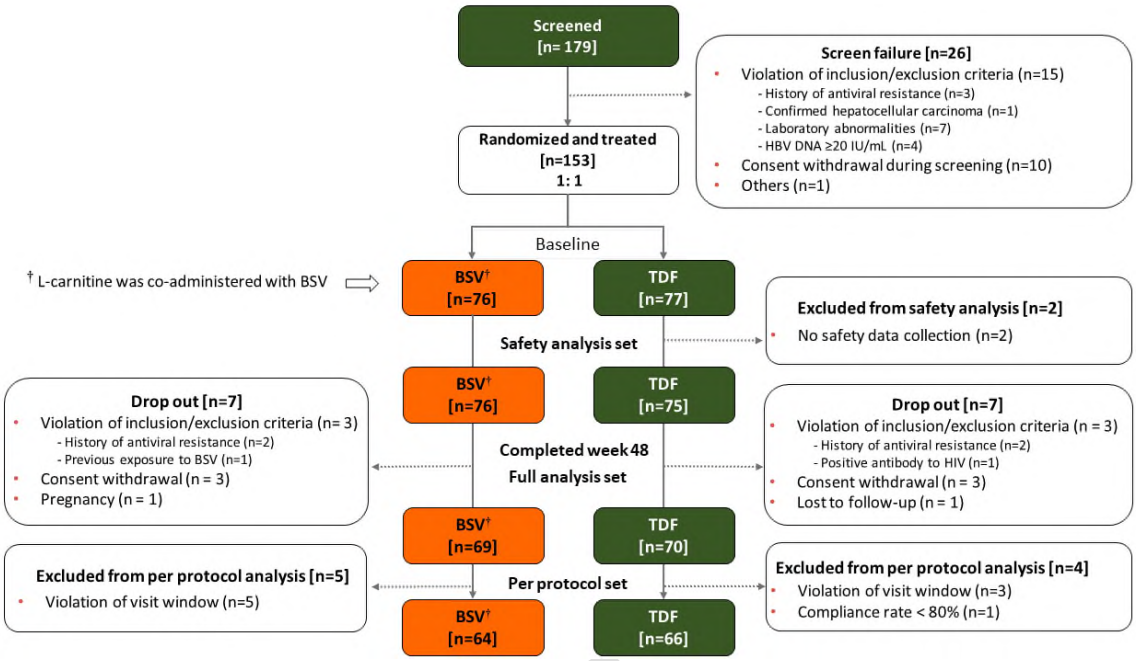
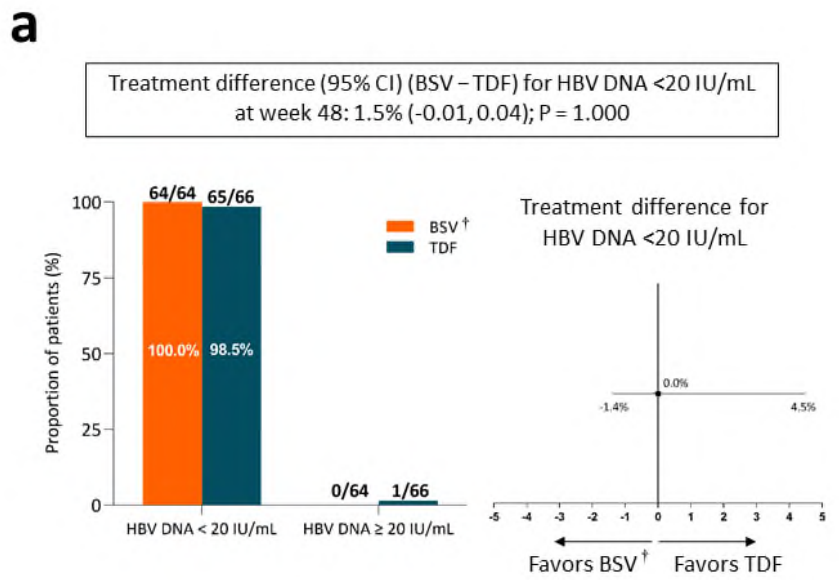
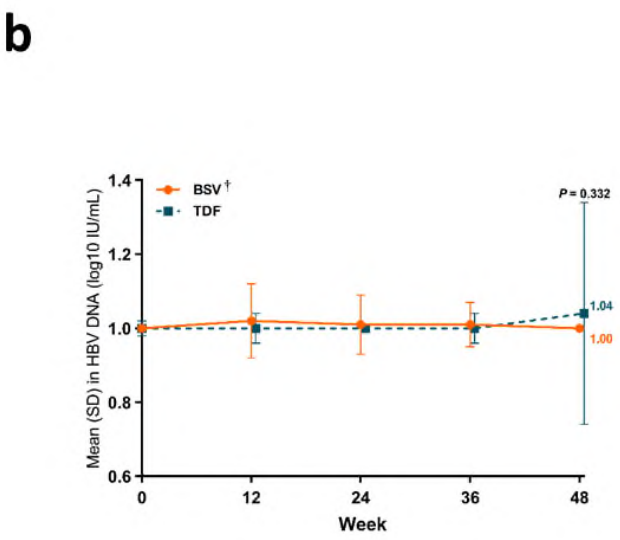


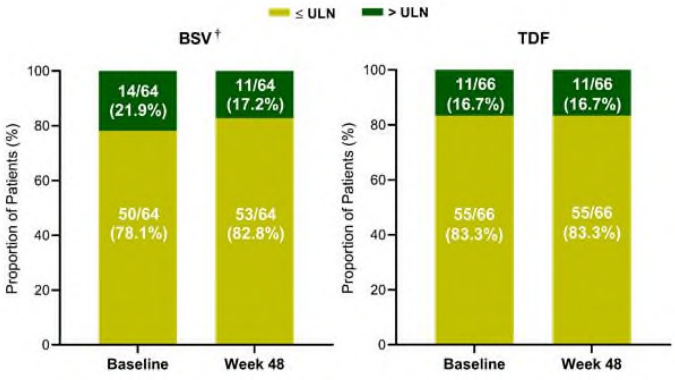
Figure 2. Comparison of treatment responses. (a) Proportion of virologic response (HBV DNA <20 IU/mL) at week 48, (b) Mean HBV DNA levels, (c) Proportion of biochemical response at week 48, (d) Changes in mean ALT levels.

†L-carnitine was co-administered with BSV.





c


≤ ULN
> ULN
BSV†
TDF
Proportion of Patients (%)
100
80
60
40
20
0
14/64 (21.9%)
50/64 (78.1%)
11/64 (17.2%)
53/64 (82.8%)
11/66 (16.7%)
55/66 (83.3%)
11/66 (16.7%)
55/66 (83.3%)
Baseline
Week 48

d

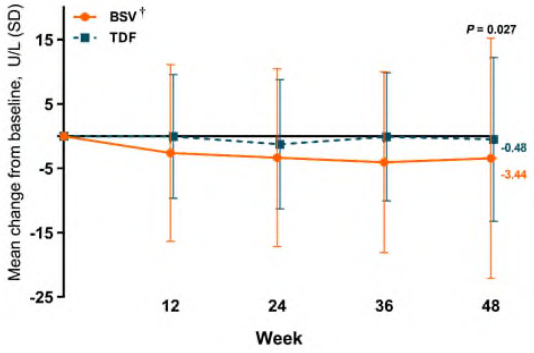

BSV†
TDF
P = 0.027
Mean change from baseline, U/L (SD)
15
5
-5
-15
-25
-0.48
-3.44
12
24
36
48
Week

Figure 3. Comparison of renal safety. (a) Mean percentage changes in eGFR through 48 weeks,* (b) Changes in urine biomarkers for renal injury.

*Repeated measures ANOVA

†L-carnitine was co-administered with BSV.

eGFR, estimated glomerular filtration rate

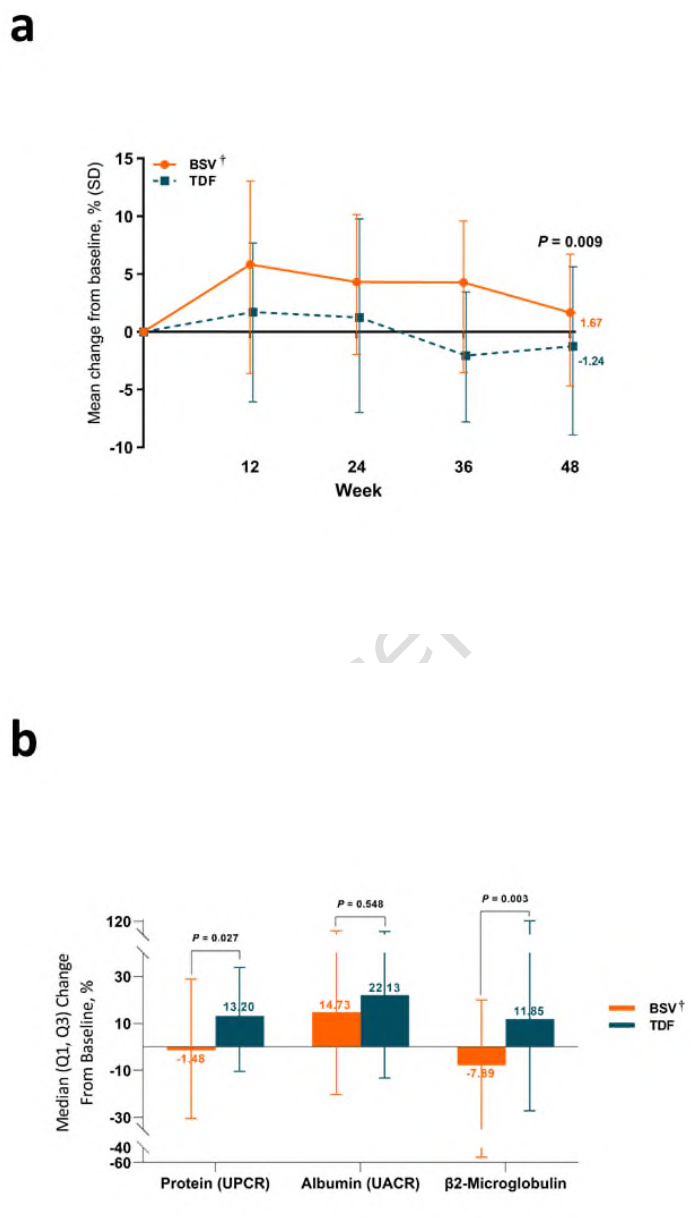
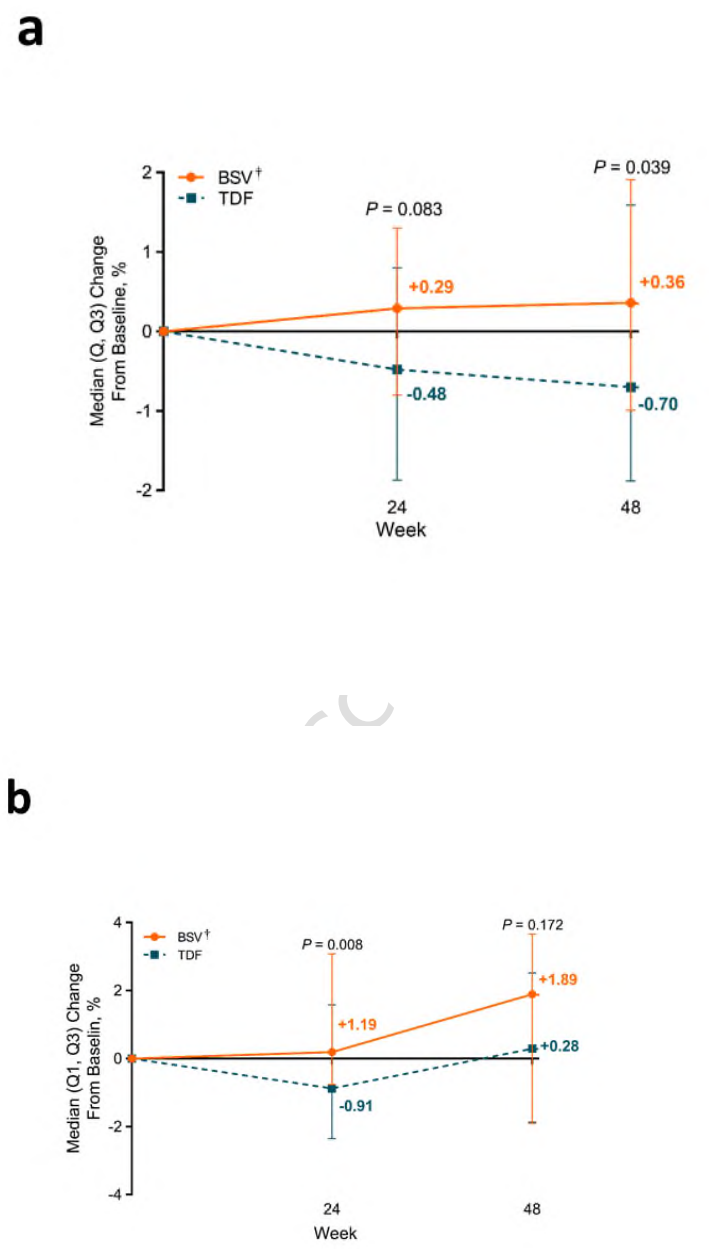


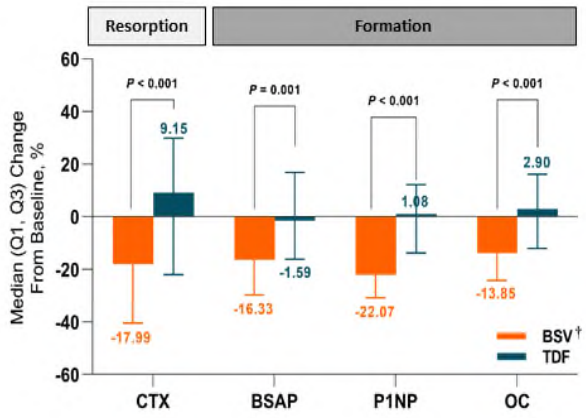
Figure 4. Comparison of bone safety. (a) Changes in bone mineral density of the hip over 48 weeks, (b) Changes in bone mineral density of the spine over 48 weeks, (c) Changes in bone turnover markers over 48 weeks.

†L-carnitine was co-administered with BSV.

CTX, C-type collagen sequence; BSAP, bone-specific alkaline phosphatase; P1NP, procollagen type 1 N-terminal propeptide; OC, osteocalcin.



C


Resorption
Formation
Median (Q1, Q3) Change From Baseline, %
60
40
20
0
-20
-40
-60
P < 0.001
P = 0.001
P < 0.001
P < 0.001
9.15
-1.59
1.08
2.90
-17.99
-16.33
-22.07
-13.85
BSV†
TDF
CTX
BSAP
P1NP
OC

Supplementary Material

Switching to Besifovir in Patients with Chronic Hepatitis B Receiving Tenofovir Disoproxil Fumarate: A Randomized Trial

Running title: Switching tenofovir DF to besifovir

Supplementary methods

1. Monitoring protocol during follow-up

After enrollment, patients were monitored every 12 weeks. Compliance with taking the study medications was assessed by counting the number of pills that were returned to the clinical pharmacy at each visit. Subjects were excluded from further study participation in case of withdrawal of consent to participate in the clinical trial, occurrence of any adverse event preventing further participation, detection of violation of the inclusion or exclusion criteria of the study, failure to follow-up, consumption of prohibited concomitant medication, or intercurrent illness or medical conditions determined by the clinical investigators. In addition, the occurrence of virologic and biochemical breakthroughs during the study period could be a reason for withdrawing study participation at the discretion of the investigators after performance of antiviral resistance tests. Patients who experienced any serious adverse events were excluded from the study. Any untoward medical events were recorded for addressing safety issues.

2. Definitions

Virologic breakthrough was defined as an increase in HBV DNA level over 1 log IU/mL from the nadir at two time points separated by more than 2 weeks during treatment. Antiviral resistance mutation was confirmed upon detection of new mutations that have been demonstrated by *in vitro* studies to confer resistance to the drug being administered. Biochemical breakthrough was defined as an increase in ALT level above the upper limit of normal after normalization with antiviral therapy.

Supp. Table 1. Detailed inclusion and exclusion criteria

Inclusion Criteria	
	1. 20 years of age and older, male or female patients 2. Patients who show positive HBsAg or have had a history of chronic hepatitis B (CHB) for more than six months before screening 3. Patients who have been on tenofovir disoproxil fumarate (TDF) monotherapy for ≥48 weeks and are taking TDF at the time of clinical screening 4. At screening, patients who have HBV DNA <20 IU/mL 5. Patients to whom the purpose, methods, and effects of the clinical trial were explained and then signed a written consent form
Exclusion Criteria:	
	1. Patients who have received interferon (including pegylation formulation) to treat CHB for more than 12 months. 2. Patients who have taken besifovir 3. Patients who have had a previous history of antiviral resistance during nucleos(t)ide analogue therapy 4. Patients diagnosed with a malignant tumor within 5 years before screening or patients with a relapse 5. Patients with history of organ transplantation

6. Patients who have received the following drugs for the last 2 months before screening (however, short-term use (less than consecutive 14 days) of these drugs and low-dose aspirin (100 mg, maximally, 300 mg/day) are allowed)
    - ✓ Nephrotoxic drugs (e.g., aminoglycosides, amphotericin B, and NSAIDs)
    - ✓ Hepatotoxic drugs (e.g., erythromycin, ketoconazole, rifampin, fluconazole, dapsone)
    - ✓ Anticoagulant (e.g., warfarin)
7. Patients administered immunosuppressants within 12 months before screening who are suspected by an investigator of having decreased immunity
8. Patients who have been administered long-term systemic corticosteroids (more than consecutive 14 days) at a high dose (greater than prednisolone 20 mg daily[a]) within 3 months before screening (In case of local corticosteroids, an investigator decides it.)
9. Patients with history of clinical alcohol or drug abuse within a year before screening or those who are currently abusers
10. Patients with hepatitis C virus, hepatitis D virus, or human immunodeficiency virus
11. Patients who have other hepatic diseases (hematochromatosis, Wilson's disease, alcoholic liver diseases, nonalcoholic steatohepatitis, and α1-antitrypsin deficiency), except hepatitis B

12. Patient concerned about decline in daily activity or inability to understand the objectives and methods of the study because of psychiatric problems
13. Patients exhibiting glomerular filtration rate (GFR) less than 50 mL/min/1.73m^2 on calculating the Modification of Diet in Renal Disease (MDRD: 186 × serum creatinine in mg/dL $^{-1.154}$ × age $^{-0.203}$ (×0.742 for women)) during screening
14. Patients who show more than 50 ng/mL of alpha-fetoprotein (AFP) levels during screening and who are estimated to have hepatocellular carcinoma (HCC) according to liver/abdomen CT scans
15. At least one of the following laboratory values during screening
    a Hemoglobin <9.0 g/dL
    b Absolute neutrophil count (ANC) <1.0×10^9/L (1000 /mm^3)
    c Platelet count <75×10^9 /L
    d Serum creatinine >1.5 mg/dL
    e Serum amylase >2 × upper limit normal (ULN) and lipase >2 × ULN
    f Total bilirubin >2 × ULN
    g Serum albumin <28 g/L (2.8 g/dL)
16. Pregnant women, lactating women, or patients planning pregnancy during the trial period

	17. Patients who participate in other clinical trials or those who are supposed to do so during the study period 18. Patients who have hypersensitivity to the study drug in this clinical trial 19. Patients with genetic conditions such as galactose intolerance, Lapp lactase deficiency, or glucose–galactose malabsorption 20. Patients who are considered unacceptable in this study under the opinion of the investigator

a. It is equal to cortisone 125 mg, hydrocortisone 100 mg, prednisone 20 mg, methylprednisolone 16 mg, triamcinolone 16 mg, dexamethasone 3 mg, and betamethasone 2.4 mg

Supp. Table 2. Baseline characteristics of the patients in the full analysis set

	Overall n=139	BSV n=69	TDF n=70	*P*-value
Mean age, y (SD)	49.63 (9.91)	50.09 (9.90)	49.19 (9.97)	0.594†
Male, n (%)	90 (64.7)	47 (68.1)	43 (61.4)	0.409‡
HBeAg negative, n (%)	83 (59.7)	41 (59.4)	42 (60.0)	0.944‡
Years positive for HBV (SD)	10.02 (9.59)	9.30 (8.31)	10.73 (10.71)	0.771§
Mean HBV, IU/mL (SD)	10.07 (0.52)	10.04 (0.34)	10.09 (0.65)	0.575§
Mean ALT, U/L (SD)	26.35 (14.63)	27.71 (15.81)	25.01 (13.33)	0.289§
ALT ≤ULN (AASLD 2018 criteria), n (%)	111 (79.9)	53 (76.8)	58 (82.9)	0.374‡
Mean AST, U/L (SD)	26.6 (16.9)	27.8 (21.7)	25.5 (10.4)	0.225§
Mean HBsAg, $\log_{10}$ IU/mL (SD)	3.22 (0.76)	3.16 (0.87)	3.28 (0.63)	0.828§
Median duration of prior TDF use, y (Q1, Q3)	4.06 (2.78, 5.83)	4.31 (2.92, 5.56)	3.93 (2.72, 6.12)	0.903§
FIB-4 score (SD)	1.42 (0.74)	1.36 (0.72)	1.48 (0.76)	0.261§
Liver cirrhosis, n (%)	41 (29.5)	18 (26.1)	23 (32.9)	0.381‡
Mean albumin, g/dL (SD)	4.73 (0.27)	4.73 (0.27)	4.72 (0.27)	0.938§
Mean hemoglobin, g/dL (SD)	14.54 (1.44)	14.68 (1.53)	14.41 (1.33)	0.267†
Mean platelet, 10^3/μL (SD)	211.3 (66.3)	223.2 (69.1)	199.6 (61.8)	0.036†
Mean AFP, ng/mL (SD)	2.76 (1.21)	2.89 (1.43)	2.63 (0.95)	0.562§

Median creatinine, mg/dL (Q1, Q3)	0.87 (0.77, 0.97)	0.87 (0.77, 0.97)	0.88 (0.77, 0.98)	0.828†
Median e-GFR, mL/min (Q1, Q3)	85.0 (74.0, 96.0)	84.0 (73.0, 97.0)	85.0 (76.0, 95.0)	0.794§
Hip BMD, n (%)				0.592¶
Normal (T-score≥-1.0)	91 (75.8)	45 (76.3)	46 (75.4)	
Osteopenia (-2.5<T-score<-1.0)	27 (22.5)	14 (23.7)	13 (21.3)	
Osteoporosis (T-score<-2.5)	2 (1.7)	0 (0.0)	2 (3.3)	
Spine BMD, n (%)				0.941‡
Normal (T-score≥-1.0)	95 (68.3)	48 (69.6)	47 (67.1)	
Osteopenia (-2.5<T-score<-1.0)	34 (24.5)	16 (23.2)	18 (25.7)	
Osteoporosis (T-score<-2.5)	10 (7.2)	5 (7.2)	5 (7.1)	

HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; ALT, alanine aminotransferase; AASLD, American Association for the Study of Liver Disease; ULN, upper limit of normal; AST, aspartate aminotransferase; TDF, tenofovir disoproxil fumarate; FIB-4, fibrosis-4; AFP, alpha-fetoprotein; BMD, bone mineral density

† Student's t-test

‡ chi-square test

§ Wilcoxon rank-sum test

¶ Fisher's exact test

Supp. Table 3. Antiviral responses according to modified intention-to-treat analysis using the full analysis set at week 48

	BSV n=69	TDF n=70	*P*-value
HBV DNA <20 IU/mL	69 (100.0)	69 (98.6)	1.000¶
normal ALT (2018 AASLD)†	56 (81.2)	58 (82.9)	0.794#
normal ALT (central laboratory)†	62 (89.9)	60 (85.7)	0.456#
ALT normalization (2018 AASLD)‡	9/16 (56.3)	6/12 (50.0)	0.743#
ALT normalization (central laboratory)‡	8/12 (66.7)	4/8 (50.0)	0.648¶
HBeAg loss§	4/28 (14.3)	3/28 (10.7)	1.000¶
HBeAg seroconversion§	4/28 (14.3)	3/28 (10.7)	1.000¶
HBsAg loss	1/68 (1.5)	0/70 (0.0)	0.493¶
HBsAg seroconversion	1/68 (1.5)	0/70 (0.0)	0.493¶

Data n (%).

AASLD, American Association for the Study of Liver Diseases; ALT, alanine aminotransferase; HBV, hepatitis B virus.

† ALT normal is the proportion with ALT ≤ULN at week 48, regardless of the baseline ALT status.

‡ ALT normalization is the proportion of ALT >ULN at baseline and ALT ≤ULN at week 48.

§ Only for patients who were HBeAg-positive at baseline.

¶ Calculated using Fisher's exact test.

Calculated using Pearson's chi-square test.