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Randomised clinical trial: 48 weeks of treatment with tenofovir amibufenamide versus tenofovir disoproxil fumarate for patients with chronic hepatitis B

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Summary

Background: Tenofovir amibufenamide (TMF) can provide more efficient delivery than tenofovir disoproxil fumarate (TDF).

Aim: To compare the efficacy and safety of TMF and TDF for 48 weeks in patients with chronic hepatitis B (CHB).

Methods: We performed a randomised, double-blind, non-inferiority study at 49 sites in China. Patients with CHB were assigned (2:1) to receive either 25 mg TMF or 300 mg TDF with matching placebo. The primary efficacy endpoint was the proportion of patients with hepatitis B virus (HBV) DNA less than 20 IU/mL at week 48. We also assessed safety, particularly bone, renal and metabolic abnormalities.

Results: We randomised 1002 eligible patients. The baseline characteristics were well balanced between groups. After a median 48 weeks of treatment, the non-inferiority criterion was met in all analysis sets. In the HBeAg-positive population, 50.2% of patients receiving TMF and 53.7% receiving TDF achieved HBV DNA less than 20 IU/mL. In the HBeAg-negative population, 88.9% and 87.8%, respectively, achieved HBV DNA less than 20 IU/mL in the TMF and TDF groups. Patients receiving TMF had significantly less decrease in bone mineral density at both hip ($P < 0.001$) and spine ($P < 0.001$), and a smaller increase in serum creatinine at week 48 ($P < 0.05$). Other safety results were similar between groups.

Conclusion: TMF was non-inferior to TDF in terms of anti-HBV efficacy and showed better bone and renal safety. (NCT03903796).

Zhihong Liu and Qinglong Jin should be considered joint first authors.

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1 | INTRODUCTION

Hepatitis B virus (HBV) infection is a global health problem. The World Health Organization estimates that approximately 257 million people, 3.5% of the global population, live with chronic HBV infection.[1] Although the immune tolerance phase of chronic HBV infection usually lasts 10-30 years, chronic hepatitis B (CHB) can cause progressive liver fibrosis, cirrhosis and hepatocellular carcinoma once immune activation occurs.[2] In 2015, there were 0.9 million deaths due to hepatitis B globally.[3] In China, the mortality from HBV-related cirrhosis has recently decreased to 3.9/100 000 patient-years, but HBV-related liver cancer is still progressively increasing to 16.42/100 000 patient-years.[4,5]

Anti-viral treatment for HBV has been shown to halt or even reverse disease progression.[6,7] Up to date, eight drugs are licensed for the treatment of CHB to prevent disease progression and within which, entecavir, tenofovir disoproxil fumarate (TDF) and tenofovir alafenamide are recommended by most regional guidelines as first-line therapies.[8-10] Due to the persistency of HBV covalently closed circular DNA, the cure of CHB can rarely be achieved and the anti-HBV treatment with nucleos(t)ide analogues is generally life-long.[2] For entecavir, the 5-year resistance mutation rate is about 1.5% in treatment naïve patients and it is less potent in lamivudine-resistance patients.[11-13] The safety concern of TDF, mainly about renal toxicity and decreases in bone mineral density was a consistent focus of attention since it went on the market.[14-16] Tenofovir alafenamide, as a new formulation of tenofovir, has demonstrated its superior renal and boned safety in its registrational studies.[17,18] However, numerically lower proportion of virological response was observed in HBeAg-positive patients for the first 57 weeks of treatment.[18] In addition, some observational studies have raised new safety concerns that regimens with tenofovir alafenamide may lead to weight gain and dyslipidaemia in HIV or CHB patients.[19-22]

Some novel treatments, for example RNA interference, capsid inhibitors or toll-like receptors are going into phase II studies. Among them, RNA interference therapy has shown an excellent effect on reducing quantitative HBsAg,[23] but one should also be noted that no drug seems to be able to cure CHB alone at present.[24] Hence, nucleos(t)ide analogues are still the most effective and reliable treatment options for inhibiting disease progression in the next 5 years. Additionally, the availability of anti-viral treatments is still a global issue. Safer and more reliable treatment options for CHB patients are still of great importance to the goal of the World Health Organization to eliminate viral hepatitis in the year 2030.[25]

Tenofovir amibufenamide (TMF; codename: HS-10234), another formulation of tenofovir, shared the same ProTide technology as tenofovir alafenamide, which can provide more efficient intracellular delivery than TDF.[26] Structurally, it has one more methyl group and provides a lower median effective concentration than tenofovir alafenamide. A Phase 1b study has already provided the efficacy and tolerability of TMF for 28 days of treatment in CHB patients.[27] Hence, we conducted this randomised control trial to compare the efficacy and safety of TMF vs TDF in treatment-naive or treatment-experienced CHB patients.

Currently, TMF is approved in mainland China and planning to submit for registration in the United States.

2 | METHODS

2.1 | Study design and participants

Before enrolment began and any study procedures were performed, written informed consent was obtained from all patients. The study was approved by the Institutional Review Board or independent ethics committees at all participating sites and it was performed in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice. This study is registered with clinicaltrials.gov, number NCT03903796.

This was a phase 3, randomised, double-blind, non-inferiority study conducted at 49 centres across 15 provinces in mainland China. Patients aged 18–65 years with CHB infection (HBV DNA concentrations of at least 20 000 IU/mL with positive or negative HBeAg) were enrolled, with ALT concentrations between one and 10 times the upper limit of normal (ULN), as measured by a local laboratory, and an estimated creatinine clearance of at least 50 mL/min (according to the Cockcroft-Gault method) were enrolled. We excluded patients with platelet counts of 50×10^9 cells per L or less, haemoglobin of less than 10 g/dL, albumin of less than 3 g/dL and total bilirubin of more than 2.5 times the ULN. Patients with evident decompensation (ie clinical ascites, encephalopathy or variceal haemorrhage) and those with HCC were also excluded. Patients who have received <12 weeks of treatment with any nucleos(t)ide analogues were defined as treatment naïve; otherwise, patients were defined as treatment-experienced. Any interferon therapy (both

pegylated and standard interferons) must be completed at least 6 months prior to the baseline visit (Inclusion & exclusion criteria were provided in supplementary appendix).

2.2 | Randomisation and masking

Patients who were HBeAg positive and those who were HBeAg negative were studied as two separate cohorts, and they were randomly assigned (at a 2:1 ratio) to receive TMF or TDF within 45 days of screening. All patients received placebo tablets that matched the alternative treatment (i.e., patients assigned to receive TMF also received a matching TDF placebo tablet and vice versa). Patients and investigators were all blinded to the treatment assignment throughout the study. Pre-specified members from the statistics departments of the sponsoring institution were unblinded at the 48-week timepoint to perform the assessments related to the primary endpoint analysis.

The study investigators determined eligibility, obtained participant numbers and received automated treatment assignments via an interactive voice and web response system. The randomisation schedule was generated by an independent third party. Each patient received a unique patient number during randomisation. Randomisation was stratified by screening HBV DNA concentrations (≥8 $\log_{10}$ IU/mL vs < 8 $\log_{10}$ IU/mL) and previous oral anti-viral treatment (treatment naive *vs* treatment experienced).

2.3 | Procedures

The patients received TMF 25 mg orally once daily or TDF 300 mg orally once daily. Study visits occurred every 4 weeks starting at treatment week 4 until treatment week 12, after which study visits occurred every 12 weeks. Study drugs were counted by research stuffs every 12 weeks to assess adherence. Laboratory assessments included haematological analysis, serum chemistry tests, fasting lipid parameters and measures of renal function (serum creatinine, estimated glomerular filtration rate, proteinuria). Serum samples were collected for backup at each visit. In case of virological breakthrough (HBV DNA increased more than 1 $\log_{10}$ IU/mL from nadir or became detectable if once undetected), a genotypic resistance test would be carried out on the backup serum samples collected at baseline, and the visit period at the viral breakthrough occurred. The percentage change in bone mineral density was assessed in all patients by dual-energy X-ray absorptiometry (DXA) scans of the lumbar spine and hip at screening and every 24 weeks thereafter. The DXA scans were evaluated by a centralised quality control team to ensure that each examination met the requirements; in particular, they ensured that the patient's body position was consistent with the baseline (slight rotation of the hip or lumbar spine will lead to false differences).[28,29]

Biomarkers of bone turnover were also assessed, including C-type collagen sequence (CTX, associated with bone resorption) and procollagen type 1 N-terminal propeptide (P1NP, associated with bone formation). An optional pharmacokinetics sub-study was performed at the week 36 and 48 visits, opens to all enrolled patients who were willing to provide informed consent.

2.4 | Outcomes

The primary efficacy endpoint was the proportion of patients with HBV DNA less than 20 IU/mL at week 48 of treatment, as determined by PCR (COBAS TaqMan HBV Test for use with the High Pure System; Roche Diagnostics), which was assessed at the central laboratory. The lower limit of quantitation of this PCR assay was 20 IU/mL, and the lower limit of detection was 10 IU/mL. Key pre-specified secondary safety endpoints at week 48 included the percentage change in the hip bone mineral density, percentage change in spine bone mineral density and the serum creatinine change from baseline.

Other pre-specified efficacy endpoints were the proportion of ALT normalisation, the proportion of patients with HBsAg seroconversion to anti-HBs at week 48, the proportion of patients with HBeAg loss and with HBeAg seroconversion to anti-HBe at week 48 (HBeAg-positive patients), the incidence of drug-resistant mutations in patients who had virological breakthrough within week 48, and the change in fibrosis, as assessed by the Fibro-4 score (FIB-4; age (years) × AST (IU/L)/[PLT] ($\times 10^9$/L) × $\sqrt{\text{ALT (IU/L)}}$) and liver stiffness measurements (LSM; measured by transient elastography) at week 48. The ALT normalisation was assessed by two sets of criteria, one based on the ULN of each local laboratory and the other was based on the criteria recommended by the American Association for Study of Liver Diseases (AASLD). Adverse events were also assessed.

2.5 | Statistical analysis

Considering a 20% drop-out rate, we calculated a total sample size of 963 subjects, with 696 HBeAg-positive patients (464 in the TMF group and 232 in the TDF group) and 267 HBeAg-negative patients (178 in the TMF group and 89 in the TDF group), providing at least 82% power for both subgroups to rule out non-inferiority with a margin of 12% at a one-sided significance level of 2.5%. It assumes that the expected difference in the proportion of patients with HBV DNA <20 IU/mL is zero and that the proportion of patients with HBV DNA <20 IU/mL in the TDF group is 66% for HBeAg-positive patients and 90% for HBeAg-negative patients.

The 12% non-inferiority margin, accepted by regulatory authorities (Center for Drug Evaluation, National Medical Products Administration, China), was a comprehensive consideration based on the necessary sample size, clinical experts' opinion and the results of TDF and tenofovir alafenamide registrational trials.[17,18,30] Nevertheless, none of these trials used HBV DNA <20 IU/mL as a primary endpoint standard; instead, they used 29 or 69 IU/mL. Even under a lower 29 IU/mL standard, the 12% non-inferiority margin could preserve at least

76% of the additional efficacy of TDF over adefovir in HBeAg-positive patients and 53% in HBeAg-negative patients, which still satisfied the FDA guidance for the non-inferiority margin setting.[31]

Efficacy was assessed in the per-protocol analysis set and the full analysis set. The full analysis set refers to all patients who were randomly assigned and received at least one dose of the study drug. The per-protocol set refers to all patients in the full analysis set except those who did not have week 48 HBV DNA data for any reason other than discontinuation due to lack of efficacy, those who received ongoing therapy with any of the prohibited medications that had a direct impact on the primary efficacy endpoint and those with an adherence rate below 90% for the active study drug as of the week 48 visit. Safety was assessed in the safety set, which was defined as all randomly assigned patients, received at least one dose of the study drug, and had post-drug safety records.

For the primary efficacy analysis in the full analysis set, the missing values were imputed using the last observation carried forward method. Unless otherwise specified, the per-protocol set analysis did not include any filling in of the missing data (the 48-week serum HBV DNA data of patients who withdrew from the trial early due to lack of efficacy were handled using the missing equals failed approach).

The rate difference and its two-sided 95% confidence interval were calculated from a CMH test adjusted with serum HBV DNA levels at screening (≥8 $\log_{10}$ IU/mL vs < 8 $\log_{10}$ IU/mL) and oral antiviral treatment status (treatment naive *vs* treatment experienced).

During the study, an independent data monitoring committee reviewed the safety results on five occasions (approximately every 6 months). SAS version 9.4 was used for all analyses.

2.6 | Post hoc safety analysis with special interests

Two post hoc safety analyses with special interests were conducted in the presented study, one focused on osteal and renal abnormalities and the other focused on metabolic abnormalities. Regarding osteal abnormalities, the occurrence of bone mineral deterioration was defined as a bone mineral density decrease in more than 5% from baseline in any one measuring point of the femoral neck, total hip or lumbar spine (L1-L4) at week 24 or week 48. This criterion was amended from the definition of osteoporosis treatment failure by the International Society for Clinical Densitometry. The occurrence of renal function deterioration was defined as an estimated glomerular filtration rate (eGFR) decrease in more than 10% once or 8% twice in a row from baseline. This criterion was adapted from DAIDS recommendations in assessing renal-related adverse events.[32] Beyond the primary safety outcomes, these post hoc analyses may further describe the risk of clinical significant events in the future.

For the post hoc analysis focused on metabolic abnormalities, the results of low-density lipoprotein cholesterol (LDL-C), total cholesterol, high-density lipoprotein cholesterol (HDL-C), total triglyceride, weight, BMI were analysed as continuous variables. It should be noticed that the actual fasting status was not assessed in this post hoc analysis as most patients obeyed the requirements of fasting; the effect of combination treatment of lipid-lowering drugs was not excluded because only 10 patients had received these drugs during the study and all of these drugs were prescribed for a medical history of dyslipidaemia from baseline. Meanwhile, all the related adverse events were carefully reviewed and reported separately.

3 | RESULTS

3.1 | Study population

Of the 1361 patients screened between August 11, 2018, and April 30, 2019, 1005 eligible patients underwent randomisation, and 1002 received at least one dose of the assigned treatment (three withdrew their consent after randomisation; Figure 1). Of the 732 HBeAg-positive patients, 486 were randomised to receive TMF, and 246 received TDF. Of the 270 HBeAg-negative patients, 180 were randomised to receive TMF, and 90 received TDF. All 1002 subjects were included in the full analysis set and safety set analysis. Most patients who did not meet the eligibility criteria had HBV DNA levels lower than 2×10^4 IU/mL, ALT more than 10 times the ULN, prior medical history and haematology or biochemistry parameter abnormalities (Table S1). At week 48, 38 subjects discontinued the study pre-maturely, which means that 964 (96.2%) subjects had completed 48 weeks of study drug treatment. Of these, one patient used forbidden drugs, and 18 patients missed the week 48 visit (See 'Patient management during the COVID-19 pandemic'. in supplementary appendix). None of them were included in the per-protocol set, which comprised 945 subjects.

In the pooled population and the HBeAg-positive or the HBeAg-negative populations, two treatment groups were well balanced with respect to baseline demographic and clinical characteristics (Table 1). Generally, the median of subjects' age at baseline was 35 years old, and 72.1% of the subjects were male. The median levels of HBV DNA at baseline were approximately 7.92 (IQR: 6.68-8.23) $\log_{10}$ IU/mL and 5.78 (IQR: 5.06-6.58) $\log_{10}$ IU/mL for the HBeAg-positive patients and HBeAg-negative patients respectively. Thirty-eight percent of the patients had an HBV DNA level equal to or greater than 8 $\log_{10}$ IU/mL. The most common HBV genotype was genotype C (55.8%), followed by genotype B (42.7%) and others. The median ALT level at baseline was 103.35 (IQR:68-167) U/L for HBeAg-positive patients and 84.8 (IQR:58.3-148) U/L for HBeAg-negative patients. The percentage of previous cirrhosis was 19.5% for the HBeAg-positive population and 17.8% for the HBeAg-negative population. For HBV treatment history, 6.6% of the pooled population experienced interferon-based treatment before, and 28.5% of the patients had been previously treated with oral nucleos(t)ide analogues. Of these, entecavir was the most common previous regimen (55.2%), followed by adefovir, TDF and others. The median duration of previous nucleos(t)ide analogues exposure was 366 (IQR: 78,1097) days, while only 22 patients maintained these anti-viral treatments until baseline. For the renal and osteal function assessment, the median eGFR according to the Epidemiology Collaboration Equation (CKD-EPI_{scr}) was 112.88 (IQR:

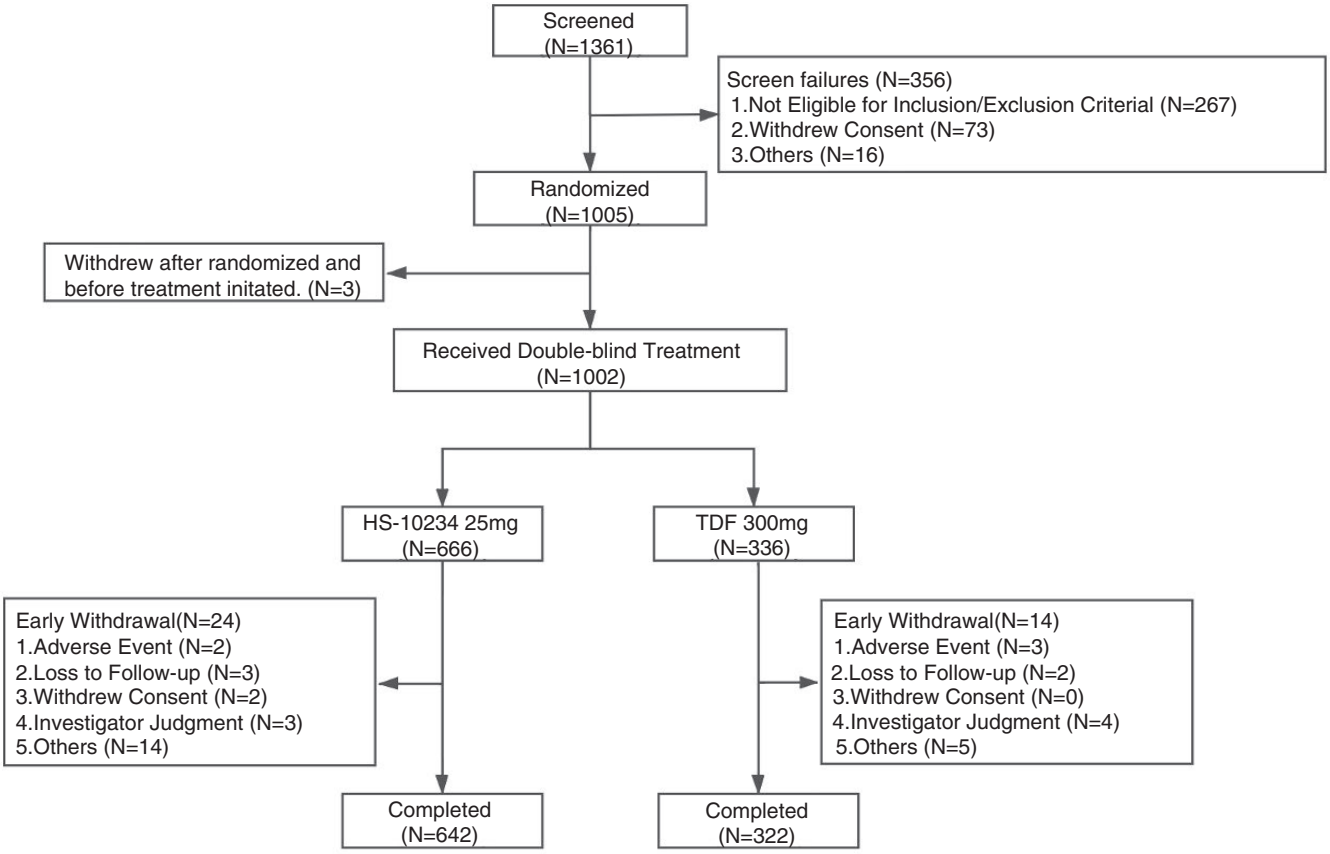

FIGURE 1 Trial profile. This flowchart presented the screening, randomisation and study drug exposure in our study

104.16-120.89) mL/min × 1.73 m^2, while 6.79% of the subjects had an eGFR less than 90 units. According to the WHO standard, 10.4% of subjects presented osteopenia at baseline, and very few patients had osteoporosis. Comorbidity distributions (including hypertension, diabetes mellitus, cardiovascular disease and dyslipidaemia) were also balanced between the TMF and TDF groups. The median duration of exposure to the masked study drug at the present analysis was 48 weeks (IQR 47-48) in both groups.

Of treatment naive patients enrolled in this study, 42 might be deemed immune-tolerant. At screening, all of them were HBeAg positive and had a HBV DNA level greater than 7 $\log_{10}$ IU/mL, a LSM less than 7.3 kPa and an ALT level lower than 2 × ULN. However, all the patients in our study were required to provide evidence of abnormal ALT from much earlier dates before screening. Moreover, only three out of these 42 patients had ALT level recovered to normal range at baseline. Hence, few of the included patients would be immune-tolerant in our study.

3.2 | Virological response

Compared with TDF, TMF was non-inferior in virological response as the lower bounds of the 95% CI of the between-group difference were greater than the pre-specified –12% margin, both in the HBeAg-positive or HBeAg-negative population. Specifically, in the HBeAg-positive population, 50.2% of patients achieved HBV DNA levels less than 20 IU/mL at week 48 in the TMF group, compared to 53.7% in the TDF group (Table 2; adjusted difference: −3.4% [95% CI −10.44 to 3.72]; $P = 0.353$). The mean (SD) decrease in HBV DNA level from baseline was 5.79 (1.089) $\log_{10}$ IU/mL and 5.89 (1.001) $\log_{10}$ IU/mL in TMF group and TDF group respectively (Table S3). In the HBeAg-negative population, 88.9% of patients had HBV DNA levels less than 20 IU/mL at week 48 in the TMF group, compared to 87.8% in the TDF group (Table 2; adjusted difference: 1.2% [95% CI −6.73 to 9.12]; $P = 0.767$). The mean (SD) decrease in HBV DNA level from baseline was 5.34 (1.774) $\log_{10}$ IU/mL and 5.36 (1.961) $\log_{10}$ IU/mL in TMF group and TDF group respectively. The results of the pre-specified per-protocol set were consistent with those of the primary analysis, showing that TMF was non-inferior to TDF in terms of anti-viral efficacy (Figure 2).

The virological response rates according to HBV DNA levels less than 29 IU/mL, less than 69 IU/mL at week 48 were also obtained and no significant differences were observed between treatment groups (Figure S2 and Table S2). Among HBeAg-positive subjects, 55.3% of the TMF group and 57.3% of the TDF group achieved HBV DNA levels less than 29 IU/mL at week 48; 73.0% of the TMF group and 77.2% of the TDF group had HBV DNA levels less than 69 IU/mL at week 48. Among those HBeAg-negative subjects, more than 95% of patients in both groups achieved HBV DNA levels less than 69 IU/mL at week 48. For the 42 patients who seemed to be in immune-tolerant phase, the median (IQR) decline of HBV DNA level from

TABLE 1 Baseline characteristics

	TMF 25 mg (N = 666)	TDF 300 mg (N = 336)	Total (N = 1002)
Age (years)	35 (29-44)	35 (28-45)	35 (29-44)
Male (%)	480 (72.1)	243 (72.3)	723 (72.2)
Body mass index (kg/m^2)	23.2 (21.21-25.48)	23.1 (21.11-25.53)	23.2 (21.16-25.51)
HBeAg positive (%)	486 (73.0)	246 (73.2)	732 (73.0)
HBV-DNA (log_{10} IU/mL)			
Pooled	7.28 (5.86-8.23)	7.34 (5.86-8.23)	7.31 (5.86-8.23)
HBeAg positive	7.93 (6.61-8.23)	7.91 (6.75-8.23)	7.92 (6.68-8.23)
HBeAg negative	5.82 (5.06-6.58)	5.72 (5.08-6.54)	5.78 (5.06-6.58)
HBV-DNA ≥8 log_{10}IU/mL	253 (37.9%)	128 (38.1%)	381 (38.0%)
HBV genotype (%)			
B	285 (42.8)	143 (42.6)	428 (42.7)
C	372 (55.9)	187 (55.7)	559 (55.8)
Others	9 (1.4)	6 (1.8)	15 (1.5)
ALT (U/L)	98.6 (65-163)	99.95 (62.7-157.5)	99 (64-162)
HBeAg positive	104.5 (68-167.3)	101.45 (69-164)	103.35 (68-167)
HBeAg negative	87.5 (60.75-153.1)	82.5 (53-146.2)	84.8 (58.3-148)
Previous cirrhosis (%)[b]			
Pooled	125 (18.8)	66 (19.64)	191 (19.1)
HBeAg positive	95/486 (19.5)	48/246 (19.5)	143/732 (19.5)
HBeAg negative	30/180 (16.7)	18/90 (20.0)	48/270 (17.8)
Previously treated for HBV (%)[a]			
Any interferon	46 (6.9)	20 (6.0)	66 (6.6)
Any Nucleot(s)ide	190 (28.5)	96 (28.6)	286 (28.5)
Analogues (%)			
Entecavir	100 (15.0)	58 (17.3)	158 (15.8)
Adefovir	49 (7.4)	23 (6.8)	72 (7.2)
TDF	37 (5.6)	10 (3.0)	47 (4.7)
eGFR-EPIscr (mL/min × 1.73 m^2)	113.13 (104.75-121.65)	111.82 (102.99-120.13)	112.88 (104.16-120.89)
eGFR<90 mL/min × 1.73 m^2	44 (6.6%)	24 (7.14%)	68 (6.79%)
Bone mineral density by DXA (g/cm^2)			
Total hip	0.94 (0.86-1.02)	0.94 (0.86-1.02)	0.94 (0.86-1.02)
Femur neck	0.84(0.75-0.94)	0.84 (0.75-0.95)	0.84 (0.75-0.94)
Lumbar spine (L1-L4)	1(0.91-1.11)	1 (0.92-1.1)	1 (0.92-1.11)
Osteopenia by WHO standard (%)	77 (11.6)	27 (8.0)	104 (10.4)
Osteoporosis by WHO standard (%)	4 (0.6)	1 (0.3)	5 (0.5)
Comorbidities[c] (%)			
Diabetes mellitus	18 (2.7)	8 (2.4)	26 (2.6)
Dyslipidaemia	126 (18.9)	48 (14.3)	174(17.4)
Hypertension	38 (5.7)	21 (6.3)	59 (5.9)
Cardiovascular disease	54 (8.1)	27 (8.0)	81 (8.1)

Data are n (%), n/N (%) or median (IQR).

Abbreviations: DXA, dual energy X-ray absorptiometry; eGFR, estimated glomerular filtration rate; EPIscr, chronic kidney disease epidemiology collaboration serum creatinine equation; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; LLN, lower limit of normal range; ULN, upper limit of normal range.

[a]The proportion of previously treated HBV patients were similar in HBeAg-positive and -negative population.

[b]Patients were diagnosed as cirrhosis by transient elastography under different ALT levels.

[c]Comorbidities were collected according to medical history.

TABLE 2 Primary and secondary efficacy endpoints

	TMF 25 mg	TDF 300 mg	Difference in proportions, (95% CI)	*P* value
HBV DNA <20 IU/mL				
HBeAg positive	244/486 (50.2%)	132/246 (53.7%)	−3.4 (−10.44,3.72)	0.353
HBeAg negative	160/180 (88.9%)	79/90 (87.8%)	1.2 (−6.73,9.12)	0.767
HBeAg loss[a]	82/478 (17.2%)	39/246 (15.9%)	1.4 (−4.15,6.99)	0.624
HBeAg seroconversion[b]	39/417 (9.4%)	17/206 (8.3%)	1.2 (−3.51,5.81)	0.637
HBsAg loss[c]				
HBeAg positive	0/486 (0)	0/246 (0)	—	—
HBeAg negative	1/180 (0.6%)	0/90 (0)	—	—
ALT normalisation				
Local laboratory normal range[d]				
Pooled population	514/613 (83.8%)	235/296 (79.4%)	4.3 (−1.10, 9.77)	0.108
HBeAg positive	370/450 (82.2%)	173/225 (76.9%)	5.3 (−1.23,11.85)	0.102
HBeAg negative	144/163 (88.3%)	62/71 (87.3%)	0.7 (−8.41,9.86)	0.877
AASLD normal range[e]				
Pooled population	470/652 (72.1%)	212/328 (64.6%)	7.4 (1.22,13.63)	0.017
HBeAg positive	341/479 (71.2%)	154/242 (63.6%)	7.5 (0.26,14.81)	0.039
HBeAg negative	129/173 (74.6%)	58/86 (67.4%)	7.2 (−4.79,19.12)	0.229

Data are n (%) or n/N (%) unless otherwise stated.

Abbreviations: AASLD, American Association for the Study of Liver Diseases; ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; ULN, upper limit of normal.

[a]Among patients who were seropositive for HBeAg.

[b]Among patients who were seropositive for HBeAg and negative for anti-HBe at baseline.

[c]Among patients who were seropositive for HBeAg.

[d]Among patients who were seropositive for HBeAg and negative for anti-HBe at baseline.

[e]The ALT ULN of each local laboratory ranged from 35 to 72 U/L for male and 35 to 69 U/L for female.

[f]ULN adopted by AASLD guidance 2018, which is 35 U/L for male and 25 U/L for female.

baseline was 5.87 (5.42, 6.36) $\log_{10}$ IU/mL and 16 patients achieved HBV DNA levels less than 69 IU/mL at week 48 (Table S14).

The change in HBV DNA levels by visit presented a continuous decline from weeks 4 to 48 and was similar for the two groups in each population (Table S3 and Figure S1). An evaluation of the treatment response in subgroups defined by baseline characteristics showed no significant interactions, including age (≥50 years vs < 50 years), sex, HBV genotype (B *vs* C), treatment status (naive *vs* experienced), baseline HBV DNA (≥8 $\log_{10}$ IU/mL vs < 8 $\log_{10}$ IU/mL), baseline ALT (>ULN vs ≤ULN) and treatment compliance (≥95% vs < 95%; Table S5 and Figure S3).

In the pooled population, only four patients experienced HBV DNA increase in more than 1 $\log_{10}$ IU/mL from nadir or became detectable after undetected. Resistance surveillance was conducted according to the protocol. Mutations (rtv173L + rtL180M + rtM204V) were detected in one treatment-experienced patient in TMF group and were all proved to be pre-existing by baseline samples.

3.3 | Other efficacy endpoints

For the HBeAg-positive patients, the rate of HBeAg loss in patients receiving TMF was 17.2% (82/478) at week 48, which was not significantly different from that in patients receiving TDF (15.9% [39/246]). For the patients with positive HBeAg and negative HBeAb, no significant difference was detected between the two treatment groups in the incidence rate of seroconversion (Table 2). At week 48, only one patient achieved HBsAg loss, but the seroconversion was not observed.

After 48 weeks of treatment, the observed median (IQR) decrease in ALT level from baseline was −68.5 (−137, −32) U/L in patients receiving TMF, which was statistically larger than −60.6 (−125.4, −26) U/L in patients receiving TDF. Regarding ALT normalisation according to local laboratory criteria (ULN range from 35 to 72 for men, 35 to 69 for women), there was no significant difference in all populations (Table 2). However, when the ULN recommended by the AASLD was adopted (≤35 U/L for men and ≤25 U/L for women), the proportion of ALT normalisation in the TMF group was statistically higher than that in the TDF group in the pooled populations. The rates of ALT normalisation were 72.1% in the TMF group and 64.6% in the TDF group, with an adjusted difference of 7.4% (95% CI 1.22-13.63; $P = 0.017$). In HBeAg-positive population, a significant difference was also observed. In contrast, the ALT normalisation rate in HBeAg-negative population was just numerically higher in TMF group than that in the TDF group. The rates of ALT normalisation were 74.6% in the TMF group and 67.4%

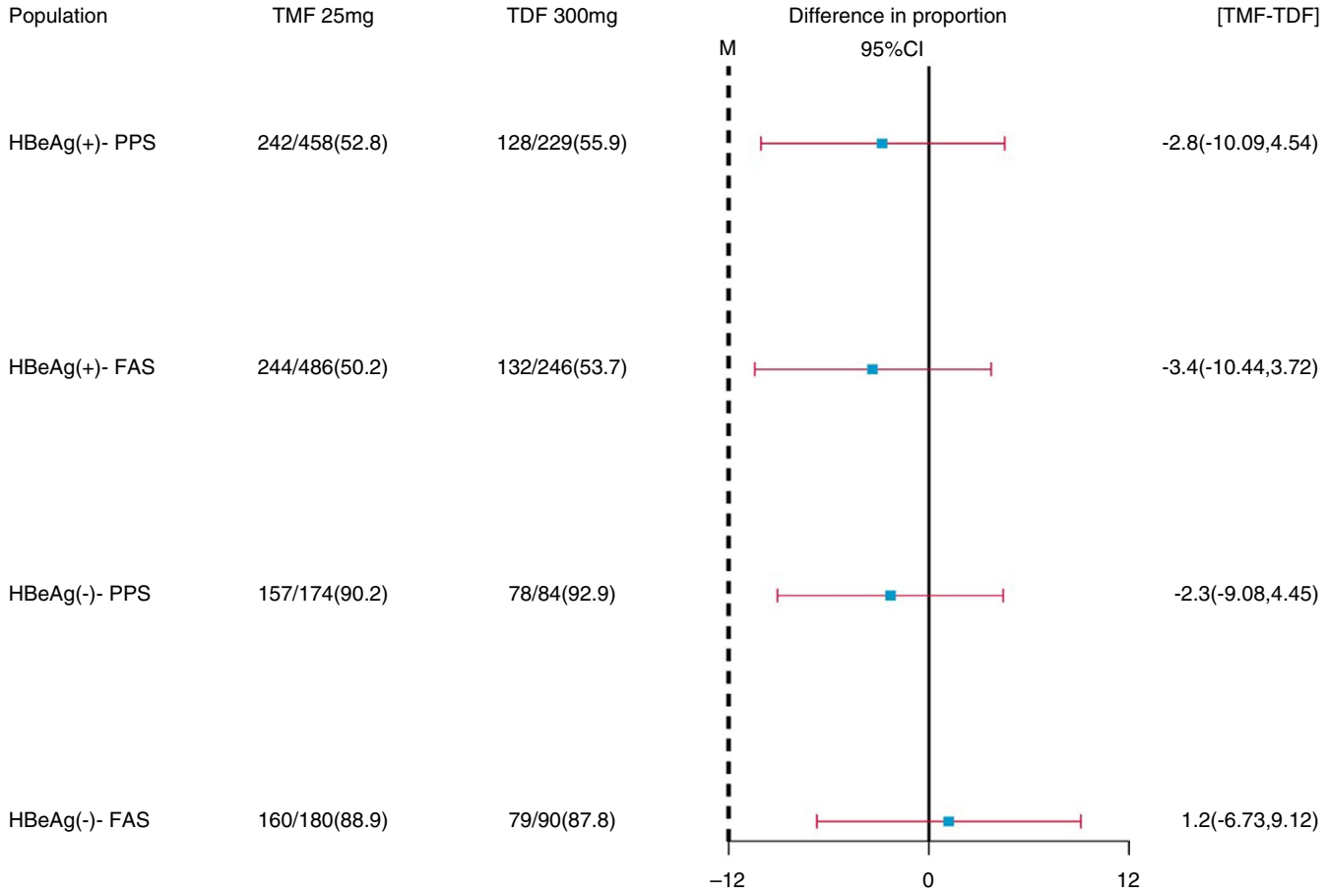

FIGURE 2 The non-inferiority of virological suppression in all analysis set. The non-inferiority of virological suppression, which is defined as HBV DNA <20 IU/mL at week 48, was met in the per-protocol set and full-analysis set of HBeAg-positive or -negative chronic hepatitis B patients receiving tenofovir amibufenamide 25 mg or tenofovir disoproxil fumarate 300 mg. FAS, full analysis set. PPS, per-protocol set

in the TDF group, with an adjusted difference of 7.2% (95% CI −4.79 to 19.12; $P = 0.229$) in the HBeAg-negative population. Regarding AST level, the observed median (IQR) decrease from baseline was −34.6 (−73.6, −15) U/L in patients receiving TMF, which was statistically larger than −32.5 (−68.3, −12) U/L in patients receiving TDF.

The regression of liver fibrosis was assessed by FIB-4 score and LSM in this study (Tables S6 and S7). The values of FIB-4 scores and LSM have all significantly decreased from baseline in both treatment groups. For the HBeAg-positive patients, a significantly greater decline of FIB-4 score was observed in patients receiving TMF than TDF (−0.48 ± 0.026 vs −0.35 ± 0.034, with a least-squares method difference of −0.12; $P = 0.002$). This inter-group difference was not observed in HBeAg-negative populations. The decrease in LSM at week 48 from baseline was not significantly different between the TMF group and the TDF group, either in HBeAg-positive or -negative population.

3.4 | General safety

Generally, both study treatments were well tolerated (Table 3). Most adverse events were mild to moderate in severity. There are 613 (92.0%) patients receiving TMF and 303 (90.2%) patients receiving TDF experienced adverse events during 48 weeks of treatment. For these, only half were deemed as study drug related. The incidence of grade 3 or 4 adverse events (18.2%-16.4%) was slightly higher than expected. Notably, the grade 3 or 4 laboratory abnormalities were not reported separately in our study (Table S13; grade 3 or 4 laboratory abnormalities: TMF 12.5% *vs* TDF 10.1%). The incidence of grade 3 or 4 study-drug-related adverse events was low and distributed equally in each group (TMF 6.0% vs TDF 6.3%). Serious adverse events, discontinuation of treatment due to adverse events, primary liver cancers and deaths, were uncommon in this study (Table 3).

Specifically, adverse events with an incidence ≥5% were upper respiratory tract infection (184 [27.6%] patients receiving TMF vs 75 [22.3%] patients receiving TDF), hyperuricaemia (59 [8.9%] vs 20 [6.0%]), hepatic steatosis (56 [8.4%] vs 19 [5.7%]), nasopharyngitis (51 [7.7%] vs 22 [6.5%]), hypophosphataemia (43 [6.5%] vs 28 [8.3%]) and urinary tract infection (34 [5.1%] vs 18 [5.4%]). The most common grade 3 and 4 adverse events were abnormal investigations of serum ALT and AST. Of these, nine (1.4%) patients receiving TMF and nine (2.7%) patients receiving TDF experienced an ALT flare that all of these occurred within the first 1-3 months of the

TABLE 3 Adverse events

	TMF 25 mg (n = 666)	TDF 300 mg (n = 336)
Adverse events	613 (92.0)	303 (90.2)
Adverse events related to study drug	308 (46.2)	177 (52.7)
Grade 3 adverse events	121 (18.2)	55 (16.4)
Grade 3-4 adverse events related to study drug	40 (6.0)	21 (6.3)
Incidence ≥5% adverse events in any treatment group		
Investigations		
Alanine aminotransferase increased	135 (20.3)	64 (19.0)
Aspartate aminotransferase increased	95 (14.3)	50 (14.9)
Blood parathyroid hormone increased	67 (10.1)	37 (11.0)
Blood creatine phosphokinase increased	47 (7.1)	27 (8.0)
Weight decreased	33 (5.0)	39 (11.6)
Bone density decreased	24 (3.6)	31 (9.2)
Total bile acids increased	35 (5.3)	19 (5.7)
Blood bilirubin increased	37 (5.6)	14 (4.2)
Gamma-glutamyl transferase increased	30 (4.5)	18 (5.4)
Infections and infestations		
Upper respiratory tract infection	184 (27.6)	75 (22.3)
Nasopharyngitis	51 (7.7)	22 (6.5)
Urinary tract infection	34 (5.1)	18 (5.4)
Gastrointestinal disorders		
Diarrhoea	36 (5.4)	9 (2.7)
Metabolism and nutrition disorders		
Hyperuricaemia	59 (8.9)	20 (6.0)
Hypophosphataemia	43 (6.5)	28 (8.3)
Hepatobiliary disorders		
Hepatic steatosis	56 (8.4)	19 (5.7)
Renal and urinary disorders		
Proteinuria	31 (4.7)	18 (5.4)
Respiratory, thoracic and mediastinal disorders		
Cough	37 (5.6)	10 (3.0)
Serious adverse events	30 (4.5)	13 (3.9)
Serious adverse events related to study drug	0	1 (0.3)
Discontinuation of treatment due to adverse events	2 (0.3)	4 (1.2)
Death	0	0

Data are n (%).

study and resolved without sequelae. Thirty (4.5%) patients receiving TMF and 13 (3.9%) patients receiving TDF experienced serious adverse events. Serious adverse events that occurred to more than one patient include upper respiratory tract infection, skin injury, hypertension, thyroid cancer and abnormal liver function. Of these, only one case of serious adverse event was deemed to be related to TDF treatment and none was TMF related. Two patients receiving TMF have permanently discontinued the study drug due to pancreatitis and arthralgia (Tables S11–S13).

3.5 | Safety of special interests: bone and renal abnormalities

The safety issues of bone were compared in the pooled population. For bone mineral density, a significantly smaller mean percent decrease was observed in the TMF group, both in the hip and spine bone measurements, when compared with the TDF group at week 48. Regarding hip measurements, we observed a −0.5% ± 0.162 change from baseline in the TMF group and a −2.09% ± 0.213 change in the TDF group. The least-squares mean difference between these two groups reached 1.59% (95% CI: 1.12-2.06; Figure 3A). Regarding spine measurements, we observed a 0.09% ± 0.181 change from baseline for patients receiving TMF and a −1.90% ± 0.239 change for patients receiving TDF, with a least-squares mean difference of 1.99% (95% CI: 1.46-2.52; Figure 3B). A smaller impact of TMF than that of TDF in bone turnover biomarkers was also observed. For bone absorption, patients on TMF treatment had a 7.72% decrease from baseline in β-CTX, while there was a 21.47% increase in patients on TDF treatment. For bone formation, the serum level of P1NP had a 2.27% decrease from baseline in patients receiving TMF and a 22.65% increase in patients receiving TDF (Tables S8 and S9). At week 48, eight patients receiving TMF and two patients receiving TDF experienced bone fracture events. All these patients had a history of injury preceding the fracture. None of the fractures were considered fragility fractures or adverse effects related to TMF.

In the post hoc analysis, osteal deterioration event was defined as more than 5% decrease in bone mineral density from baseline at any one spot of the femur neck, total hip or lumbar (L1-L4), which is presented in Table 4. A significantly lower proportion of osteal deterioration was observed in patients receiving TMF than TDF during 48 weeks of treatment (27.33% vs 41.67%, $P < 0.05$).

For the assessment of renal safety, the mean change in serum creatinine from baseline in each group was compared in the primary analysis. The increase in 0.60 ± 8.988 μmol/L in the TMF group was significantly smaller than the 1.51 ± 7.975 μmol/L in the TDF group, with a least-squares mean difference of −1.12 μmol/L (95% CI: −2.219, −0.027, $P = 0.045$; Figure 4). Meanwhile, a total of 4.7% (31 of 666) of the patients receiving TMF and 5.4% (18 of 336) of the patients receiving TDF had at least one graded event of proteinuria during the study. No patient in either group experienced adverse events of proximal tubulopathy (including Fanconi syndrome) or renal adverse events resulting in the study drugs discontinuation. Two patients in the TMF group experienced serious adverse events of renal and urinary disorders, which were obstructive nephropathy and renal hydrocele caused by lithiasis.

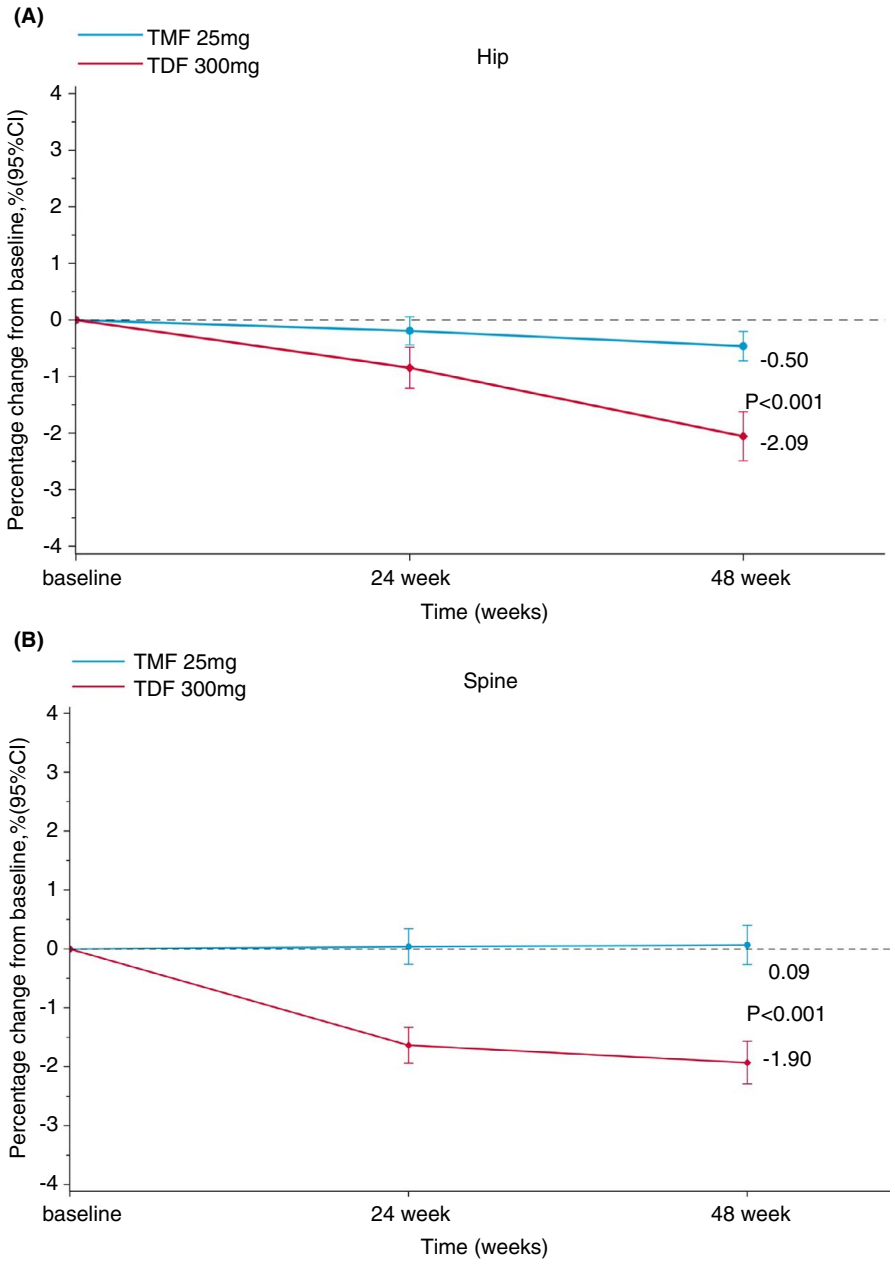

FIGURE 3 Changes in bone mineral density. A, Mean percentage change in hip bone mineral density at weeks 24 and 48 of treatment. Bars are 95% CI. B, Mean percentage change in spine bone mineral density at weeks 24 and 48 of treatment. Bars are 95% CI

In the post hoc analysis, renal deterioration was compared by the percentage of eGFR decrease from baseline, which was confirmed by more than 10% once or 8% twice in a row (Table 4). More patients with renal deterioration event were observed in TDF group than in TMF group during 48 weeks of treatment (38.10% *vs* 29.43%, $P < 0.05$).

3.6 | Safety of special interests: metabolic abnormalities

Though the incidence of each specific lipid disorder-related adverse event was less than 5%, a significantly increased incidence of all lipid-related adverse events was observed in the TMF group, compared with the TDF group (11.4% and 3.0% respectively); such events included hypertriglyceridaemia (4.4% and 1.5% respectively), hyperlipidaemia (3.5% and 0.3% respectively) and increased low-density lipoprotein (2.4% and 0 respectively). Among these events, only three patients in the TMF group were deemed as having grade 3 elevations (Table S13), including one patient had grade 4 elevation at baseline and two others cases who had abnormal triglycerides at baseline and experienced transient grade 3 elevation but recovered to baseline level at the next visit.

Based on a higher incidence of lipid disorders, cardiovascular-related events were thoroughly reviewed. Cardiovascular disease was uncommon during 48 weeks of treatment. No myocardial infarction or chronic heart failure was reported and only two cases of ischaemic vascular disease were observed, including one case of cerebral posterior circulation ischaemia and one case of intracranial venous sinus thrombosis. Both of them were not highly dyslipidaemia-related ischaemia. On the other hand, among patients

Bone or renal function deterioration	TMF, n = 666	TDF, n = 336	*P* value
More than 5% decrease in BMD from baseline at any one spot of femur neck, total hip or lumbar (L1-L4) at week 24 or week 48	182 (27.33)	140 (41.67)	<0.0001
Develop eGFR decreased more than 10% once or 8% twice in a row from baseline	196 (29.43)	128 (38.10)	0.0056

Data are n (%).

Abbreviations: BMD, Bone Mineral Density; eGFR, estimated glomerular filtration rate.

TABLE 4 Bone or renal function deterioration-safety set

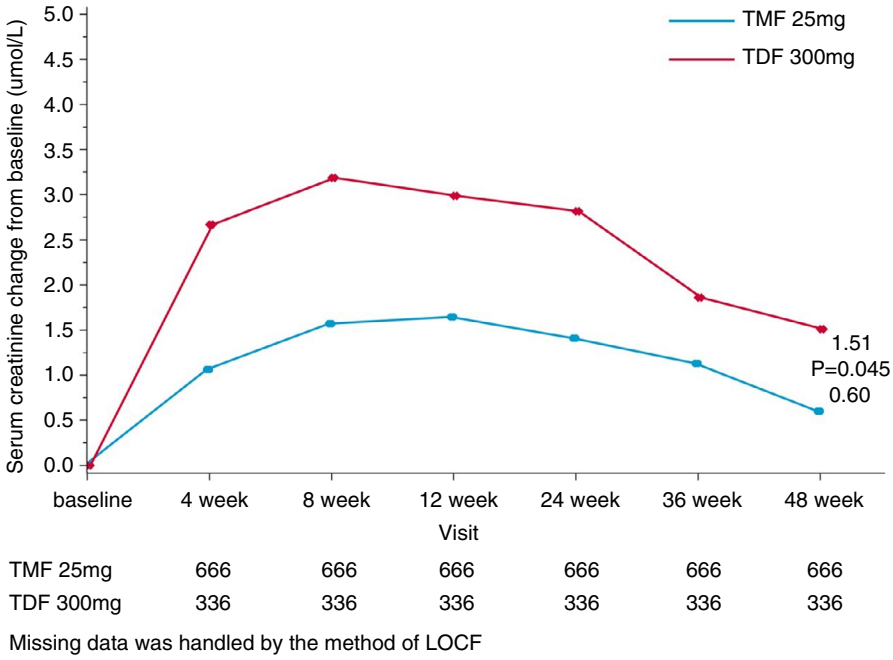

FIGURE 4 Change in serum creatinine, by treatment group. Mean change from baseline in serum creatinine (μmol/L) by study visit.

with dyslipidaemia or cardiovascular diseases at baseline, there are 27 adverse events might be underlying cardiovascular diseases. The distribution of these adverse events was balanced in each group, including 10 cases of transiently elevated creatine kinase, three cases of transiently elevated creatine kinase MB, four cases of chest pain and others. None of the severity of these events was considered as more than grade 3 (Table S15).

In the post hoc analysis on metabolic abnormalities, the median (IQR) level of total cholesterol was not significantly different from baseline to week 48 in patients receiving TMF (Table 5). However, slight increases were observed in serum LDL-C and total triglyceride, that is a median change of 0.11 (−0.21, 0.51) mmol/L and 0.05 (−0.2,0.37) mmol/L respectively. In contrast, all analysed lipid parameters were statistically decreased in patients receiving TDF, with a median change of −0.69 (−1.13, −0.26) mmol/L, −0.3 (−0.61, 0.03) mmol/L and −0.09 (−0.32, 0.11) mmol/L in total cholesterol, LDL-C and total triglyceride respectively. For HDL-C, a significant decrease was observed in both groups, but it was much more intense in patients receiving TDF, which was −0.12 (−0.28, 0.05) mmol/L in the TMF group and −0.26 (−0.44, −0.12) mmol/L in the TDF group. Meanwhile, a significant increase in total cholesterol to HDL-C ratio from baseline to week 48 was observed in both groups, which was 0.28 (−0.11, 0.67) in the TMF group and 0.14 (−0.17, 0.51) in the TDF group. When two study treatments were compared, there were no differences in all lipid parameters at baseline and significantly opposing effects were yielded after 48 weeks of treatment other than HDL-C. Additionally, weight and BMI were also investigated, and both of these parameters increased in patients receiving TMF at week 48 while decreased in patients receiving TDF.

4 | DISCUSSION

In the first 48 weeks of treatment in this randomised control trial, TMF was non-inferior to TDF with respect to all primary and secondary efficacy endpoints in patients with compensated chronic HBV infection.

The non-inferiority of virological response between TMF and TDF was established among the HBeAg-positive or -negative population in all study sets. Meanwhile, the subgroup analysis did not reveal any significant differences between two treatments in subgroups with different baseline characteristics. However, when

TABLE 5 Metabolic abnormalities in each group at baseline and week 48-full analysis set

Parameter	Treatment	Baseline	Week 48	Change from baseline	*P* value		
					Intra-group	Inter-group	
						Baseline	Week 48
TC (mmol/L)	TMF	4.55 (3.96, 5.19)	4.54 (4.01, 5.2)	0.01 (−0.43, 0.47)	0.4567	0.8176	<0.0001
	TDF	4.59 (4, 5.15)	3.91 (3.43, 4.41)	−0.69 (−1.13, −0.26)	<0.0001		
LDL-C (mmol/L)	TMF	2.61 (2.08, 3.18)	2.73 (2.24, 3.26)	0.11 (−0.21, 0.51)	<0.0001	0.6871	<0.0001
	TDF	2.59 (2.13, 3.1)	2.3 (1.95, 2.78)	−0.3 (−0.61, 0.03)	<0.0001		
HDL-C (mmol/L)	TMF	1.39 (1.16, 1.64)	1.27 (1.09, 1.5)	−0.12 (−0.28, 0.05)	<0.0001	0.2127	<0.0001
	TDF	1.38 (1.2, 1.68)	1.11 (0.97, 1.33)	−0.26 (−0.44, −0.12)	<0.0001		
TC/HDL-C ratio	TMF	3.26 (2.73, 3.93)	3.59 (2.98, 4.25)	0.28 (−0.11, 0.67)	<0.0001	0.1768	0.0083
	TDF	3.2 (2.72, 3.88)	3.35 (2.94, 4)	0.14 (−0.17, 0.51)	<0.0001		
Triglyceride (mmol/L)	TMF	1.03 (0.81, 1.35)	1.1 (0.77, 1.53)	0.05 (−0.2, 0.37)	<0.0001	0.5667	<0.0001
	TDF	0.99 (0.79, 1.31)	0.9 (0.66, 1.2)	−0.09 (−0.32, 0.11)	0.0003		
Weight (kg)	TMF	65 (58, 73.5)	66 (58, 74.2)	0.8 (−0.9, 2.5)	<0.0001	0.9204	<0.0001
	TDF	65.03 (57, 74)	63 (56, 70.8)	−1 (−3, 0.5)	<0.0001		
BMI	TMF	23.24 (21.21, 25.48)	23.73 (21.22, 25.71)	0.28 (−0.32, 0.87)	<0.0001	0.8218	<0.0001
	TDF	23.11 (21.11, 25.53)	22.49 (20.88, 24.62)	−0.38 (−1.07, 0.2)	<0.0001		

Data are median (IQR).

Median of TC, LDL, HDL, TC/HDL-C and TG use Wilcoxon signed-rank test for intra-group comparison, Wilcoxon rank-sum test for inter-group comparison. Mean of Weight and BMI use paired t-test for intra-group comparison, analysis of covariance (ANCOVA) for inter-group comparison.

Abbreviations: HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; TC, total cholesterol.

compared with other studies with TDF or tenofovir alafenamide treatment, a lower virological response rate of the HBeAg-positive population in this study was also observed. A stricter criterion of virological response in our study than those in other studies may mainly explain this situation. Adopting a standard of HBV DNA <69 IU/mL, more than 73% of HBeAg-positive patients in this study, receiving TMF or TDF, achieved a virological response at week 48, which was highly comparable with the virological response rate of TDF treatment in global (76.0%) or Chinese registrational trials (76.7%).[30,33] Adopting a standard of HBV DNA <29 IU/mL, the proportion of virological response in HBeAg-positive patients at week 48 was numerically 5%-6% lower than that reported in the separate cohort enrolled in mainland China of the tenofovir alafenamide registration trial.[34] From a statistical standpoint, a high degree of crossover in 95% CI (51.77, 69.64) of virological response rate should be noted. Meanwhile, the mean change in HBV DNA levels from baseline was similar for each treatment.[34] Similar situations exist when compared with tenofovir alafenamide study of non-China patients.[18] Although numerically larger differences could be observed across these two trials, a longer median duration of study drug exposure, which is 57 weeks (IQR 48-72) in the GS-US-320-0110 study, should also be noted. In the HBeAg-negative population, virological response rates were highly comparable among three prodrugs of tenofovir.[17,30,33]

The non-inferiority of efficacy was also observed by the numerically higher proportions of HBeAg loss and seroconversion in patients receiving TMF over TDF. In the TMF group, 17.2% of the HBeAg-positive patients had HBeAg loss, and around half of them (9.4%) had achieved HBeAg seroconversion. After 48 weeks of treatment of TMF, very few patients experienced a virological breakthrough during the 48 weeks of treatment. No new genotype substitution and new drug resistance site were found. This proves the high drug resistance barrier of TMF treatment. Longer term drug resistance monitoring is ongoing.

It seemed to become a common characteristic of tenofovir prodrugs using ProTide technique that TMF also showed a significantly higher ALT normalisation rate in pooled and HBeAg-positive population when compared with TDF. Although this benefit did not reach statistical significance in HBeAg-negative population, it should be a consequence in future follow-up as a significantly further decrease in ALT levels from baseline was observed in patients receiving TMF than those receiving TDF. As another point of reference, tenofovir alafenamide had previously proved this advantage in HBeAg-negative population with a lower baseline ALT level (67.0 U/L, IQR 44-102 vs 84.8 U/L, IQR 58.3-148).

Regression of fibrosis was assessed by surrogate markers in this study. In HBeAg-positive population, a significantly larger decline of FIB-4 score was observed in TMF group than TDF group. It may be partially explained by the higher ALT normalisation rate by TMF treatment and a much larger decline in ALT levels than AST levels by both treatments. In contrast, the lack of a significantly further decrease in LSM values in the TMF group over the TDF group may still

advocate a further investigation or longer follow-up on the benefits of fibrosis regression.

Besides the non-inferiority in anti-viral efficacy, two study treatments presented different characteristics on safety profiles. Generally, both 25 mg TMF and 300 mg TDF appeared to be safe and well tolerated and the incidence of adverse events, serious adverse events and laboratory abnormalities were similar in each group. However, TMF presented a better safety profiles for bone mineral and renal function while TDF was found to have a unique lipid lowering effects.

The side effect of long-term TDF treatment on hip and spine bone mineral density has been confirmed in patients with HIV infection and chronic HBV infection.[16,35-37] In this study, the benefit of less bone mineral density decrease was observed in the TMF group over the TDF group, with adjusted percentage differences of 1.12%-2.06% or 1.46%-2.52% in hip or spine respectively. Lower incidence of hypophosphataemia and osteoporosis and less increase in bone turnover markers in the TMF group also confirmed this advantage. Moreover, there are 14.34% more patients in TDF group than TMF group experienced osteal deterioration event, which is more reflective to indicate a clinically significant damage (Table 4). It should be notice that we have enrolled a young population in this study. Bone mineral density decreases continuously after it reaches the peak around 30-40 years old, and the rate of loss increases with age.[38,39] 48 weeks of TDF treatment in our study lead to about 2% decrease in hip or spine bone mineral density in a median 35-year-old population. In comparison, a larger detrimental impact of TDF was witnessed with older ages in tenofovir alafenamide registrational trial in HBeAg-negative patients.[17] Further study on older populations is needed to measure the increased incidence of fragility fracture for this effect.

For progressive renal dysfunction, a higher risk was observed in the TDF treatment. In other words, it indicated better renal safety in the TMF treatment. In the presented study, patients in the TMF group had a significantly smaller increase in serum creatinine compared with those receiving TDF. Besides, 8.67% more patients with renal deterioration event were observed in the TDF group than in the TMF group. Previously, renal tubular injury such as proximal tubular disease and Fanconi syndrome caused by long-term use of TDF was already reported by other studies.[14,15,40,41] A 10-year clinical study showed that 5.1% of CHB patients had renal insufficiency during TDF treatment.[42] Therefore, TMF may be a better choice for the long-term treatment of CHB patients, especially for those at risk of renal impairment.

Besides the benefits in the prevention of osteal and renal toxicity of TDF, tenofovir alafenamide treatment was reported with a higher incidence of dyslipidaemia and weight gain in CHB and HIV patients.[17,18,43] Similarly, a significantly higher incidence of dyslipidaemia and more weight gain was observed with the TMF treatment than TDF treatment. However, we did not observe more direct evidence rather than laboratory abnormalities. The incidence of grade 3 or 4 dyslipidaemia in TMF group was uncommon and all cases were resolvable. As major adverse outcomes of dyslipidaemia, the incidence of cardiovascular disease or relevant abnormalities was low and distributed equally in two treatment groups, even in patients who already had dyslipidaemia or cardiovascular disease at baseline. In fact, we observed a lowering effect rather than less increase by TDF treatment in all lipid parameters, even in serum HDL-C. Another study may explain this effect that TDF decreased serum cholesterol levels by upregulating hepatic CD36 via PPAR-α activation.[44] It was previously reported that the incidence of metabolic syndrome, dyslipidaemia or abdominal obesity increases in CHB patients after virological response achieved.[45,46] Hence, it seems that the lowering dosage benefit by ProTide technology in TMF or tenofovir alafenamide deprived the lipid lowering effect of TDF at the same time.

Comparing with LDL-C levels, the total cholesterol to HDL-C ratio is a stronger predictor of cardiovascular disease.[47] In our study, both TMF and TDF treatment presented a significant increase in this ratio. Based on this, we could not conclude that the more decrease in lipid parameters of TDF will turn into benefits of cardiovascular disease. Additionally, weight and BMI were increased in patients receiving TMF but reduced in patients receiving TDF. However, the extent of change was quite small and most patients were still within a normal BMI after 48 weeks of treatment. Hence, the *pros and cons* of this effect was unable to be adjudicated.

Good trial quality was warranted by a low drop-out rate (4.1%), and good compliance was observed in this study. However, there are still several limitations. First, a comparator arm utilising tenofovir alafenamide was not included as it had not come into the market in mainland China at the study initiation. However, TDF is still one of first-line options and we also observed a lipid-lowering effect of TDF in this study. Furthermore, though increase in total cholesterol to HDL-C ratio was observed, the increased risk of cardiovascular in 10 years was not obtained as abdomen circumference was not collected in this study.[48] Meanwhile, it should be pointed out that the enrolled subjects were relatively young but we are facing an aging population of CHB nowadays. Based on these limitations, this study has been extended into a 10-year real-world cohort with control groups of entecavir or tenofovir alafenamide and more complete investigations included.

In conclusion, TMF offers a better treatment choice with non-inferior efficacy, a higher rate of ALT normalisation and a better osteal and renal safety profile than TDF for CHB patients. Although losing the lipid lowering effect, TMF was not confirmed to have an increased risk of cardiovascular disease than TDF. Hence, we believed TMF 25 mg QD can be recommended for the treatment of adult patients with HBeAg-positive or HBeAg-negative CHB.

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AUTHORSHIP

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Author contributions: J.H., J.N. and, C.S. and ZH.L. involved indesigned the study conception and design. The TMF Study Group carried out acquisition ofperformed the research and collected the data. C.L. carried out statistical analysis. ZH.L. and X.L. involved in interpretation of the data and drafting the manuscriptpaper. All authors involved in critical revision of the manuscript for important intellectual contentAQ7. All the authors approved the final draftversion of the manuscriptAQ8.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from Hansoh Pharmacy Co. Restrictions apply to the availability of these data, which were used under license for this study. Data are available from Jinlin Hou with the permission of Hansoh Pharmacy Co.

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SUPPORTING INFORMATION

Additional supporting information will be found online in the Supporting Information section.

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