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Original Article

96-Week Treatment of Tenofovir Amibufenamide and Tenofovir Disoproxil Fumarate in Chronic Hepatitis B Patients

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Abstract

Background and Aims: Tenofovir amibufenamide (TMF) is a novel phosphoramidated prodrug of tenofovir with noninferior efficacy and better bone and renal safety to tenofovir disoproxil fumarate (TDF) in 48 weeks of treatment. Here, we update 96-week comparison results. ***Methods:*** Patients with chronic hepatitis B were assigned (2:1) to receive either 25 mg TMF or 300 mg TDF with matching placebo for 96 weeks. The virological suppression was defined as HBV DNA levels <20 IU/mL at week 96. Safety was evaluated thoroughly with focusing on bone, renal, and metabolic parameters. ***Results:*** Virological suppression rates at week 96 were similar between TMF and TDF group in both HBeAg-positive and HBeAg-negative populations. Noninferior efficacy was maintained in the pooled population, while it was first achieved in patients with HBV DNA ≥7 or 8 log10 IU/mL at baseline. Non-indexed estimated glomerular filtration rate for renal safety assessment was adopted, while a smaller decline of which was seen in the TMF group than in the TDF group (p=0.01). For bone mineral density, patients receiving TMF displayed significantly lower reduction levels in the densities of spine, hip, and femur neck at week 96 than those receiving TDF. In addition, the lipid parameters were stable after week 48 in all groups while weight change still showed the opposite trend. ***Conclusions:*** TMF maintained similar efficacy at week 96 compared with TDF with continued superior bone and renal safety profiles (NCT03903796).

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Keywords: Hepatitis B; Liver function tests; Viral hepatitis; Liver; Osteoporosis.

Abbreviations: AEs, adverse events; ALT, alanine aminotransferase; BMD, bone mineral density; CHB, chronic hepatitis B; CrCl, creatinine clearance rate; DXA, dual energy X-ray absorptiometry; eGFR, estimated glomerular filtration rate; FIB-4, fibrosis-4; HBV, hepatitis B virus; HDL, high-density lipoprotein; ISCD, International Society for Clinical Densitometry; LDL, low-density lipoprotein; LSM, liver stiffness measure; PTH, parathyroid hormone; TAF, tenofovir alafenamide; TC, total cholesterol; TDF, tenofovir disoproxil fumarate; TG, triglyceride; TMF, tenofovir amibufenamide.

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Introduction

Hepatitis B virus (HBV) infection is a major global public health concern. In 2019, the estimated global prevalence of chronic HBV infection was 4.1%, corresponding to 316 million infected people.[1] Although a marked decline was seen in

the prevalence of HBV across all ages and in children younger than 5 years of age between 1990 and 2019, the number of HBV-related deaths continues to increase.[1] Most patients with chronic HBV infection maintain immune tolerance in their younger ages. However, once immune activation occurs, chronic hepatitis B (CHB) may progress into liver failure, cirrhosis, or hepatocellular carcinoma, which resulted in about 55,500 deaths globally per year.[1,2]

To date, four licensed nucleos(t)ide analogs are recommended for first line therapy in treatment of CHB in mainland China i.e., entecavir, tenofovir disoproxil fumarate (TDF), tenofovir alafenamide (TAF) and tenofovir amibufenamide (TMF; codename: HS-10234).[3,4] They all have been shown to halt or even reverse disease progression.[5,6] However, unless a functional cure is achieved, the anti-HBV treatment is generally life-long, which imposes high requirements for the efficacy and safety of these drugs.[2] Among these, using entecavir may be restricted by the high proportion of lamivudine-experienced patients in mainland China,[7–9] whereas the use of TDF is often complicated due to its bone and renal safety profile in an aging population.[10–12] TAF, as an upgrade of TDF, has shown its noninferior efficacy and improved bone and renal safety in a general population. However, noninferiority of TAF regarding virological suppression was not established in patients with HBV DNA ≥7 or 8 $\log_{10}$ IU/mL for the first 96 weeks of treatments.

TMF, another prodrug of tenofovir, produced by ProTide technology,[13] has structurally one more methyl group than TAF, which may lead to higher stability in peripheral blood and improved intracellular conversion. *In vitro*, TMF has a lower EC_{50} in HepG2.2.15 cells than TAF and TDF.[14] In the reported 48-week results, TMF was statistically noninferior to TDF in antiviral efficacy, while displaying improved bone and renal safety. In June, 2021, TMF was approved in mainland China and was incorporated into the 2021 China National Reimbursement Drug List. In this report, we present the extended 96-week results of that study.

Methods

Study design and participants

This was a multicenter, randomized, double-blind, phase III noninferiority study. Eligible patients were randomly assigned in a 2:1 ratio to be orally treated with 25 mg of TMF (Hansoh Pharmaceuticals Co., Ltd, Jiangsu, China) or 300 mg of TDF (GlaxoSmithKline, Tianjin, China) once daily with a matching placebo of the unassigned study drug for the first 96 weeks. The details of the designs have been published.[15] In brief, we enrolled patients 18–65 years of age with confirmed chronic hepatitis B diagnosed by HBV DNA levels of ≥20,000 IU/mL and alanine aminotransferase (ALT) levels 1–10 times the upper limit of normal (ULN), and those with clear decompensation or hepatocellular carcinoma were excluded. Full eligibility criteria are given in the Supplementary File 2 and the study was registered at ClinicalTrial.gov (NCT03903796).

Procedures and outcomes

Patient visits occurred every 4 weeks from treatment week 4 to 12 and subsequently every 12 weeks. In addition, genotypic resistance tests were performed using serum samples at baseline and at the visit period of the viral breakthrough occurred. Dual energy X-ray absorptiometry (DXA) scans were used to assess the bone mineral density (BMD) every 24 weeks with centralized quality control.[16] Biomarkers of bone turnover were assayed as well.

The efficacy endpoint at week 96 was defined as the proportion of patients with HBV DNA less than 20 IU/mL, which was assessed at the central laboratory with a lower limit of 10 IU/mL. Other prespecified efficacy and safety endpoints at week 96 were concordant with those at week 48. For a more appropriate evaluation, the results of estimated glomerular filtration rate (eGFR-epi) were not indexed to the body surface area (calculated by the serum creatinine based chronic kidney disease epidemiology collaboration equation). In addition, the creatinine clearance rate by the Cockcroft-Gault equation (CrCl-cg) was also provided.[17] The renal tubular function was assessed by the *de novo* hypophosphatemia, serum parathyroid hormone (PTH) elevation and the clinical diagnosis of renal tubular dysfunction, which is based on the *de novo* appearance of at least three of five characteristics: hypophosphatemia, hypouricaemia, serum creatinine elevation, proteinuria, or glucosuria.[18] For bone safety evaluation, osteopenia and osteoporosis were diagnosed according to the International Society for Clinical Densitometry (ISCD) standard in this study.[19]

Statistical analysis

Justification for the sample size has been reported previously. Noninferiority was claimed by the same margin of −12% at week 48. Efficacy was assessed using the full analysis set (FAS) and the per-protocol analysis set. Safety was assessed using a safety analysis set. For the efficacy analysis by FAS, the missing values were imputed using the last observation carried forward (LOCF) method, which follows the week-48 analysis. This setting was based on the evidence that TDF has high potency for viral suppression and a low incidence of viral breakthrough. In this case, adopting a missing-equals-failed approach in data imputation may lead to an efficacy underestimation for the efficacy of TDF, which is the active control of our study. Hence, the LOCF should be a more conservative method for statistical estimation. Besides, sensitivity analysis with missing-equals-failed approach was also performed. The rate difference and its two-sided 95% confidence interval were calculated using a Cochran-Mantel-Haenszel (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-naïve vs. treatment-experienced). SAS version 9.4 (Cary, NC, USA) was used for all analyses.

Results

Patient population

Among the 1,002 patients who received at least one dose of treatment, 666 were randomized in the TMF group and 336 in the TDF group. Of these, 605 (90.8%) and 297 (88.4%) completed the week 96 assessments, in the TMF and TDF groups. From week 48 to week 96, 62 patients discontinued early on (37 vs. 25 in the TMF and TDF group, respectively, as specified in Fig. 1). The median treatment duration was 96 weeks for both groups and over 99% of the patients had adherence ≥90% (no group difference observed, p=0.608). The baseline characteristics of the two treatment groups were well balanced and have been described in the week 48 report (also provided in Supplementary Table 1). The majority of patients were male (72.2%), with a median age of 35 years and interquartile range (IQR) of 29 to 44. The median level of HBV DNA titers was 7.31 (IQR: 5.86 to 8.23) $\log_{10}$ IU/mL with 38.0% being 8 $\log_{10}$ IU/mL and above. The percentage of patients with previously diagnosed cirrhosis was 19.5% for the HBeAg-positive population and 17.8% for the HBeAg-negative population. As for HBV treatment history, 6.6% of the pooled population had received interferon-based treatment before, and 28.5% of the patients had been treated with oral nucleos(t)ide analogs. The median non-indexed

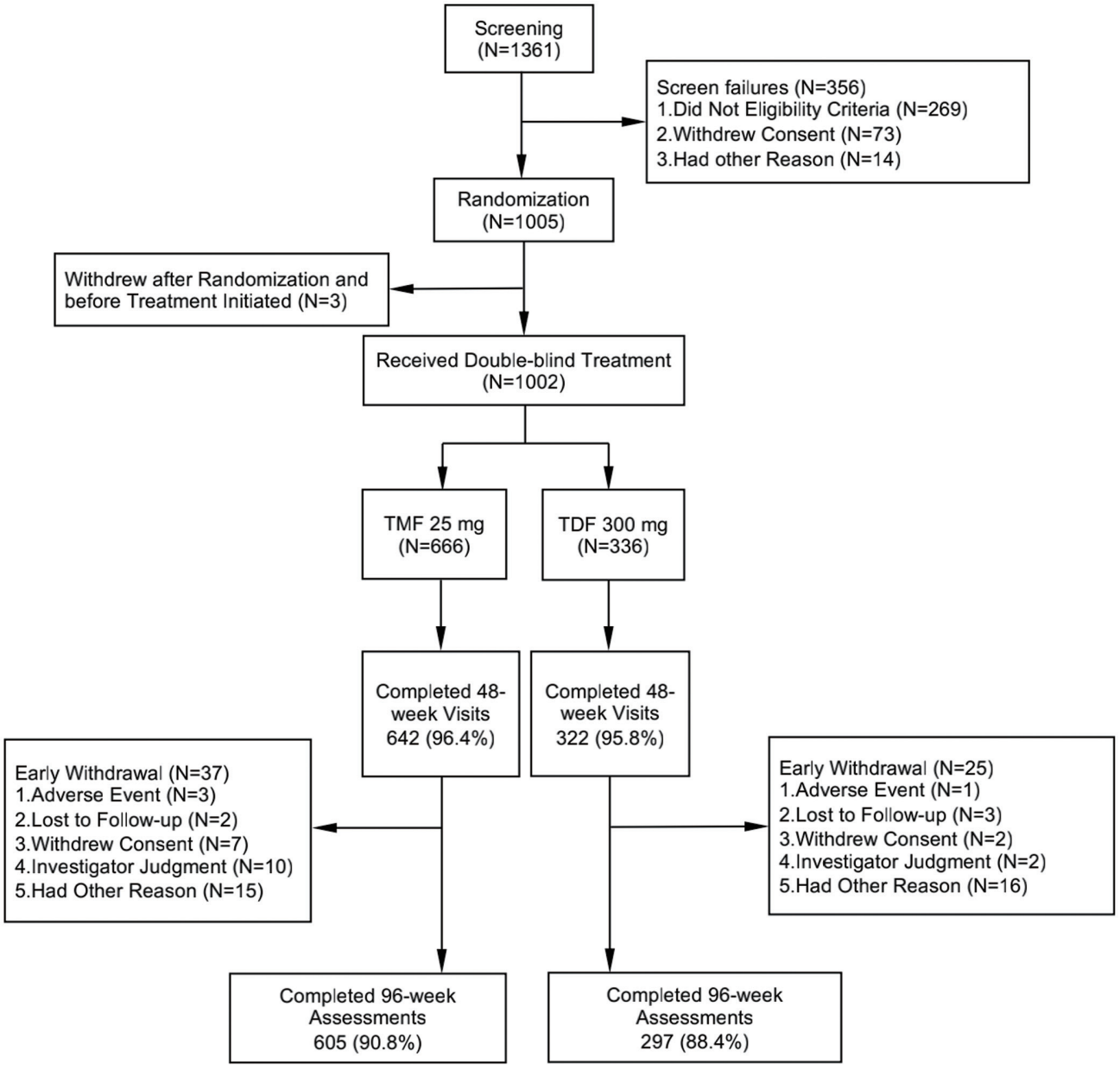

Fig. 1. Patient disposition. Flowchart of the screening, randomization, and study drug exposure. Other reasons included scheduled pregnancy, self-withdrawal, and job transfer. TMF, tenofovir amibufenamide; TDF, tenofovir disoproxil fumarate.

eGFR-epi and CrCl-cg values for patients at baseline were 111.70 mL/min and 114.85 mL/min, respectively. According to the ISCD standard, 12.0% of the subjects presented with osteopenia at baseline, and very few (0.9%) patients had osteoporosis. In the week 48 results, none of the patients had a persistent normal ALT and could be immune tolerant. The incidences of comorbidities, including diabetes, dyslipidemia, hypertension, cardiovascular diseases, upper respiratory tract infection, nasopharyngitis, hepatic steatosis, hyperuricemia, and hypertriglyceridemia were all even between two treatment groups.

Virological response

The primary efficacy endpoint at week 96 according to FAS is shown in Table 1. (see the per-protocol set results in Supplementary Table 2). In the HBeAg-positive population, 70.8% of patients in the TMF treatment group achieved HBV DNA <20 IU/mL, compared with 72.0% in the TDF treatment group (difference and 95% CI: −1.1%, −7.8% to 5.6%, p=0.746). In the HBeAg-negative population, the proportions of HBV DNA <20 IU/mL were 93.9% and 93.3% in the TMF and TDF groups respectively (difference: 0.4%; 95% CI: −5.8% to 6.7%; p=0.889). Both lower bounds of the 95% CI of the difference were above the predefined noninferiority margin. Similarly, there were no statistical differences in the proportion of patients achieving HBV DNA levels <29 IU/mL or <69 IU/mL between the two treatment groups, despite their HBeAg status. The mean decline of HBV DNA titers from

Table 1. Primary and secondary efficacy endpoints at week 96 in the full-analysis set

	HBeAg-positive				HBeAg-negative			
	TMF 25 mg (N=486)	**TDF 300 mg (N=246)**	**Difference (95% CI)**	***p*-value**	**TMF 25 mg (N=180)**	**TDF 300 mg (N=90)**	**Difference (95% CI)**	***p*-value**
HBV DNA <20 IU/mL	344/486 (70.8)	177/246 (72.0)	−1.1 (−7.78, 5.55)	0.746	169/180 (93.9)	84/90 (93.3)	0.4 (−5.77, 6.66)	0.889
HBV DNA <29 IU/mL	363/486 (74.7)	194/246 (78.9)	−4.1 (−10.31, 2.07)	0.204	170/180 (94.4)	84/90 (93.3)	1.0 (−5.16, 7.14)	0.748
HBV DNA <69 IU/mL	406/486 (83.5)	211/246 (85.8)	−2.2 (−7.63, 3.21)	0.435	173/180 (96.1)	85/90 (94.4)	1.5 (−4.00, 7.06)	0.569
HBeAg loss[a]	129/478 (27.0)	59/246 (24.0)	3.2 (−3.35, 9.68)	0.349	–	–	–	–
HBeAg seroconversion[b]	63/417 (15.1)	26/206 (12.6)	2.5 (−3.16, 8.19)	0.399	–	–	–	–
HBsAg loss[c]	1/486 (0.2)	1/246 (0.4)	NC	NC	2/180 (1.1)	0/90 (0.0)	NC	NC
Mean ALT change from baseline (SD)	−108.7 (113.8)	−95.7 (116.4)	−7.4 (−13.50, −1.34)	0.017	−96.9 (108.1)	−83.2 (106.4)	−3.1 (−8.32, 2.08)	0.238
ALT normalization rate[d]	356/479 (74.3)	154/242 (63.6)	10.7 (3.45, 17.90)	0.003	129/173 (74.6)	59/86 (68.6)	6.0 (−5.99, 17.92)	0.315
Mean[e] FIB-4 score change from baseline (SE)	−0.51 (0.03)	−0.40 (0.04)	−0.1 (−0.18, −0.02)	0.013	−0.96 (0.10)	−0.84 (0.11)	−0.12 (−0.29, 0.04)	0.144
Mean[e] LSM value change from baseline (SE)	−3.71 (0.14)	−4.05 (0.19)	0.34 (−0.09, 0.77)	0.118	−3.76 (0.46)	−4.15 (0.50)	0.38 (−0.39, 1.16)	0.328

Data are n/N or n/n (%) unless otherwise stated. ANCOVA model was used to compare change from baseline at week 96 between treatments for continuous outcomes, including HBsAg, ALT, FIB-4 score and LSM value. [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 HBsAg, [d]The upper limit of normal is 35 U/L for male and 25 U/L for female, [e]Least squares mean. HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; ALT, alanine aminotransferase; AASLD, American Association for the Study of Liver Diseases; FAS, full analysis set; CI, confidence interval; NC, not calculated.

baseline to week 96 was similar in the TMF and TDF groups (Supplementary Fig. 1; HBeAg-positive patients: −6.38 $\log_{10}$ IU/mL vs. −6.42 $\log_{10}$ IU/mL, p=0.795; HBeAg-negative patients: −6.08 $\log_{10}$ IU/mL vs. −5.78 $\log_{10}$ IU/mL, p=0.279). In the HBeAg-positive population, the proportions of patients with undetectable HBV DNA at week 96 were 21.0% and 21.1% in the TMF and TDF groups, respectively (difference; −0.1%; 95% CI: −6.3% to 6.0%; p=0.970), while the proportions of patients in the HBeAg-negative population were 48.3% and 41.1% (difference: 7.4%; 95% CI: −5.2% to 19.9%; p=0.253).

Among the HBeAg-positive patients, 164 had observed treatment failure (i.e., finished the week 96 visit and had an HBV DNA >20 IU/mL or virological breakthrough at any visit) that 111 received TMF (22.8%) and 53 received TDF (21.5%). Among those, there was a similar proportion of patients with viral loads ≥20 IU/ml and <69 IU/ml in the TMF group (52.3%) and the TDF group (56.6%). For the remaining patients with HBV DNA >69 IU/mL, 49 receiving TMF and 19 receiving TDF had plateaus of viral loads (decrease <1 $\log_{10}$ IU/mL from week 48 to week 96). In the HBeAg-negative population, only eight patients receiving TMF and no patients receiving TDF had observed treatment failure at week 96 visit.

Twenty-two patients (2.2%) had virological breakthrough in each group during 96 weeks of treatment. Hereinto, eight patients in the TMF group developed virological breakthrough after week 48, compared with five patients in the TDF group. All 22 patients underwent gene sequencing for resistance test. Only one patient in the TMF group was found to have mutations on rtv173L, rtL180M, and rtM204V which were consistently detected at baseline and week 48.

As for subgroup analysis defined by baseline characteristics, the primary efficacy endpoint showed no significant interactions in HBeAg-positive or negative populations by FAS and per-protocol set (Supplementary Figs. 2 and 3 and Supplementary Table 3). Hereinto, the noninferiority of virological suppression was also seen in subsets of patients with high viral load at baseline. For patients with HBV DNA ≥7 $\log_{10}$ IU/mL, the CMH adjusted differences between TMF and TDF treatment groups were 0.8% (95% CI: −7.70% to 9.24%, p=0.859) or 11.7% (95% CI: −10.50% to 33.83%, p=0.239) in HBeAg-positive or -negative populations, respectively. As the lower bound of the 95% CIs did not exceed the margin of −12%, the noninferiority was established, which is a similar case in subgroups of patients with HBV DNA ≥8 $\log_{10}$ IU/mL.

Serological response and HBsAg dynamics

Among patients who were HBeAg-positive at baseline, 27.0% achieved HBeAg loss at week 96 in the TMF arm, compared with 24.0% in the TDF controls (p=0.349 for group difference). Among patients who were seropositive for HBeAg and negative for anti-HBe at baseline, 15.1% achieved HBeAg seroconversion in the TMF group and 12.6% in the TDF controls respectively (p=0.399 for group difference). At week 96, totally four patients achieved HBsAg loss (three in the TMF group and one in the TDF group), but none achieved

HBsAg seroconversion. The *post-hoc* analysis of serum HBsAg dynamics was provided (Supplementary Table 4). In the HBeAg-positive population, the mean declines at week 96 in the TMF group and TDF group were −0.28±0.926 (SD) and −0.30±0.892 $\log_{10}$ IU/mL, respectively (p<0.001 in both groups). The changes were not significant from week 48 to week 96, and the intergroup difference was not significant at each time point. A numerically higher proportion of patients with a ≥1 $\log_{10}$ IU/mL decline were seen in the TMF group that TDF group (18.4% vs. 13.3%, p=0.104).

In the HBeAg-negative population, the mean declines at week 96 in the TMF group and TDF group were −0.43±0.749 (SD) and −0.34±0.372 $\log_{10}$ IU/mL, respectively. The declines were significant from week 48 to week 96, while the intergroup difference was not significant at each time point. The proportion of patients with a ≥1 $\log_{10}$ IU/mL decline were similar in the TMF group and TDF group (9.9% vs. 8.8%, p=0.995).

ALT response and fibrosis regression

For the pooled population, the TMF group achieved a nearly 10% greater ALT normalization rate (74.4% vs. 64.9%, p=0.002) and a significantly larger mean reduction in ALT levels from baseline to week 96 (−105.5 U/L vs. −92.4 U/L, p=0.009). In the HBeAg-positive population, patients in the TMF group had a significantly higher ALT normalization rate at week 96 compared with patients in the TDF group (74.3% vs. 63.6%, p=0.003; Table 1), and greater mean reduction in ALT levels from baseline (−108.7 U/L vs. −95.7 U/L, p=0.017; Table 1). Furthermore, patients in the TMF group had higher rates of ALT normalization since week 24, and the superiority was maintained up to week 96 (p=0.003) (Supplementary Fig. 4). In the HBeAg-negative population, the ALT normalization rate and the ALT decline from baseline were only numerically higher in the TMF group than that in the TDF group.

Values of liver stiffness measure (LSM) and fibrosis-4 (FIB-4) scores decreased significantly from baseline to week 96 in both treatment groups (Supplementary Tables 5 and 6). The decrease in LSM at week 96 from baseline was not significantly different between these two groups, either in HBeAg-positive (p=0.118) or negative population (p=0.328). For the FIB-4 score of HBeAg-positive patients, a significantly greater decline was seen in patients receiving TMF than that in TDF (−0.51±0.03 vs. −0.40±0.04, p=0.013). This significant intergroup difference was not observed in the HBeAg-negative populations.

General safety

After 96 weeks of treatment, both treatment groups were well tolerated regarding their general safety (Table 2). Most adverse events were mild to moderate in severity. Only one case of study drug-related serious adverse event (SAE) was seen in the TDF group. The incidence of grade ≥3 study drug-related adverse events (AEs) was low and distributed equally in two groups (6.3% in the TMF group and 6.8% in the TDF group). Three patients in the TMF group paused the treatment and only one was due to study drug-related AEs. Five patients in each group had permanent study drug discontinuation and only two of the five instances in the TDF group were associated with study drug-related AEs. Specifically, adverse events with an incidence of ≥5% were upper respiratory tract infection (216 [32.4%] patients receiving TMF vs. 96 [28.6%] patients receiving TDF), hepatic steatosis (92 [13.8%] vs. 30 [8.9%]), hyperuricemia (89 [13.4%] vs. 29 [8.6%]), hypophosphatemia (85 [12.8%] vs. 40 [11.9%]), nasopharyngitis (64 [9.6%] vs. 32 [9.5%]) and hypertriglyceridemia (51 [7.7%] vs. 12 [3.6%]). The most frequent grade ≥3 adverse events were abnormal investigations of serum ALT and aspartate aminotransferase (AST) levels, which mainly occurred within 48 weeks.

Safety of special interest: renal and bone abnormalities

Renal safety results at week 96 are shown in Table 3. At week 96, a significantly smaller decrease in the median of CrCl-cg was seen in patients receiving TMF compared with patients receiving TDF (−3.01 mL/min vs. −6.65 mL/min, respectively, p<0.001). A similar difference was seen for non-indexed eGFR-epi (−1.68 mL/min vs. −3.12 mL/min for TMF vs. TDF, p=0.010). Moreover, there were significantly fewer patients with a ≥10% sustained decline of non-indexed eGFR-epi from baseline in the TMF group compared with the TDF group (6.0% vs. 11.0%, TMF vs. TDF, p=0.005). The incidences of *de novo* hypophosphatemia were similar between the two groups, but patients in the TMF group had a lower incidence of elevated serum PTH at week 96 (10.0% vs. 16.9%, p=0.005). Only one patient in the TDF group had developed clinically diagnosis of renal tubular dysfunction, and the deterioration of proteinuria was uncommon and even in each group. None of the patients in the TMF and TDF group experienced adverse events of proximal tubulopathy or adverse renal events, resulting in discontinuation of the study drugs. Two patients in the TMF group reported serious adverse events of renal and urinary disorders, which were obstructive nephropathy and renal hydrocele.

The bone safety profile was assessed in pooled population. As for the BMD at week 96, the mean percentage change from baseline for all three measurement spots remained significantly different, with a greater decrease in the TDF group (Fig. 2 and Table 4). From week 48 to week 96, significant declines were seen at hip and femur neck in patients receiving TDF, of which the least square mean declines were −0.39±0.199% and −0.95±0.253%, respectively (all p<0.05). For patients receiving TMF, only the BMD at femur neck significantly declined from week 48, of which the least square mean decline was −0.43±0.193% (p<0.001). In contrast, there were significantly fewer patients in the TMF group experiencing a >5% decrease in BMD from baseline at femur neck, total hip or lumbar spine (L1-L4). There was no statistically significant difference in the incidences of osteopenia and osteoporosis diagnosed by ISCD standard between two treatment groups. From week 48 to week 96, only nine patients experienced a bone fracture, of whom seven received TMF and two others received TDF. However, none were fragility fractures.

The adverse impact on bone turnover biomarkers at week 96 was also assessed (Supplementary Tables 7 and 8). As for bone absorption, patients in the TMF group had a mean percentage change of +8.26% in serum c-type collagen sequence levels, but patients in the TDF group had a 39.14% increase (p<0.001 for group difference) from baseline to week 96. In terms of bone formation, the serum level of procollagen type 1 N-terminal propeptide decreased by 13% in the TMF group and increased by 3.9% in the TDF group (p<0.001 for group difference).

Safety of special interest: metabolic abnormalities

At week 96, a significantly larger decline in the levels of all serum lipid parameters from baseline was seen in the TDF group than that in the TMF group (Fig. 3), while a significantly lower increase in the total cholesterol/high-density lipoprotein (TC/HDL) ratio was seen correspondingly. However, the median changes from week 48 to week 96 were

Table 2. Safety of TMF and TDF at week 96

	n (%)	
	TMF 25 mg (N=666)	**TDF 300 mg (N=336)**
Patients with any adverse event	640 (96.1)	311 (92.6)
Study drug-related AE	371 (55.7)	211 (62.8)
Patients with SAE	68 (10.2)	33 (9.8)
Study related SAE	0 (0.0)	1 (0.3)
Patients with grade 3 and above AE	168 (25.2)	80 (23.8)
Patients with study drug-related AE ≥ grade 3	42 (6.3)	23 (6.8)
AE leading to study drug interruption	3 (0.5)	0 (0.0)
AE leading to study drug permanent discontinuation	5 (0.8)	5 (1.5)
Patients with any AE in ≥5% patients	575 (86.3)	281 (83.6)
Laboratory abnormality	385 (57.8)	213 (63.4)
Alanine aminotransferase increased	176 (26.4)	89 (26.5)
Aspartate aminotransferase increased	125 (18.8)	74 (22.0)
Blood parathyroid hormone increased	93 (14.0)	48 (14.3)
Blood creatine phosphokinase increased	64 (9.6)	34 (10.1)
Bone density decreased	43 (6.5)	43 (12.8)
Weight decreased	43 (6.5)	43 (12.8)
Blood bilirubin increase	52 (7.8)	19 (5.7)
Gamma−glutamyl transferase increased	48 (7.2)	22 (6.5)
Total bile acids increased	40 (6.0)	21 (6.3)
Weight increased	40 (6.0)	9 (2.7)
Platelet counts decreased	30 (4.5)	17 (5.1)
Infection and infestations	298 (44.7)	141 (42.0)
Upper respiratory tract infection	216 (32.4)	96 (28.6)
Nasopharyngitis	64 (9.6)	32 (9.5)
Urinary tract infection	48 (7.2)	27 (8.0)
Pharyngitis	30 (4.5)	18 (5.4)
Metabolism and nutrition disorders	196 (29.4)	72 (21.4)
Hypophosphatasemia	85 (12.8)	40 (11.9)
Hyperuricemia	89 (13.4)	29 (8.6)
Hypertriglyceridemia	51 (7.7)	12 (3.6)
Hyperlipidemia	34 (5.1)	2 (0.6)
Hepatobiliary disorders	121 (18.2)	43 (12.8)
Hepatic steatosis	92 (13.8)	30 (8.9)
Gallbladder polyps	34 (5.1)	17 (5.1)
Gastrointestinal disorders	96 (14.4)	42 (12.5)
Diarrhea	46 (6.9)	14 (4.2)
Toothache	34 (5.1)	15 (4.5)
Abdominal pain	22 (3.3)	17 (5.1)
Respiratory, thoracic, and mediastinal disorders	48 (7.2)	15 (4.5)
Cough	48 (7.2)	15 (4.5)
Renal and urinary disorders	36 (5.4)	23 (6.8)
Proteinuria	36 (5.4)	23 (6.8)
Blood and lymph disorders	15 (2.3)	24 (7.1)
Anemia	15 (2.3)	24 (7.1)

AEs, adverse events; TDF, tenofovir disoproxil fumarate; TMF, tenofovir amibufenamide.

Table 3. Renal safety outcomes at week 96

	TMF 25 mg (N=666)	TDF 300 mg (N=336)
CrCl (mL/min)		
Baseline		
Median [q1, q3]	115.51 (100.03,135.62)	114.33 (97.44,132.54)
Change at week 96		
Median [q1, q3]	−3.01 (−13.24,7.27)	−6.65 (−15.70,2.81)
Least squares mean (SE)*	−2.75 (0.76)	−6.71 (0.99)
Nonindexed eGFR (mL/min)		
Baseline		
Median [q1, q3])	111.73 (101.91,123.51)	111.47 (100.53,121.25)
Change at week 96		
Median [q1, q3]	−1.68 (−8.00,3.27)	−3.12 (−9.83,1.72)
Least squares mean (SE)*	−2.35 (0.45)	−4.07 (0.59)
Sustained decline of nonindexed eGFR at week 96* (≥10%)	40 (6.0%)	37 (11.0%)
De novo hypophosphatemia (<2.5 mg/dL)	46 (7.6%)	22 (7.1%)
De novo PTH elevation*	54 (10.0%)	48 (16.9%)
Clinical diagnosis of renal tubular dysfunction†	0	1 (0.3%)
Deterioration of proteinuria	22 (3.3%)	10 (3.0%)

CrCl, creatinine clearance rate, calculated by Cockcroft−gault equation; eGFR, estimated glomerular filtration rate, calculated by CKD-EPI equation; PTH, parathyroid hormone. *Difference is statistically significant; †The clinical diagnosis of renal tubular dysfunction was specified in method. TDF, tenofovir disoproxil fumarate; TMF, tenofovir amibufenamide.

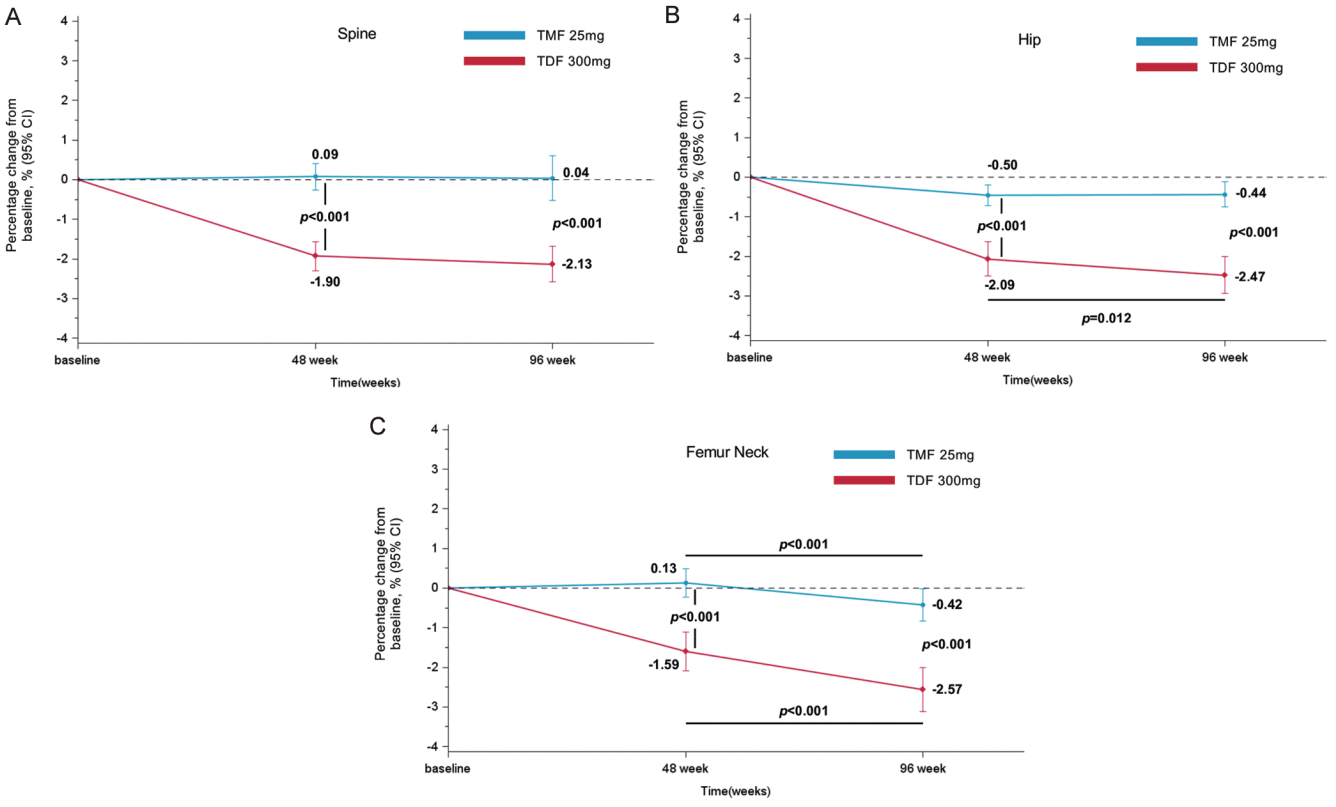

Fig. 2. Percentage change of bone mineral density. Mean percentage changes of BMD in the spine (A), hip (B), and femur neck (C) at week 48 and 96 treatment. Bars are 95% CI. TMF, tenofovir amibufenamide; TDF, tenofovir disoproxil fumarate; BMD, bone mineral density.

Table 4. Bone safety outcomes at week 96

	TMF 25 mg (N=666)	TDF 300 mg (N=336)	*p*-value* for intergroup comparison
Mean percentage change in BMD from baseline (SD)			
Hip[a]	−0.44 (4.104)	−2.47 (4.206)	<0.001
Femur neck[a]	−0.42 (5.258)	−2.57 (5.075)	<0.001
Spine (L1-L4)[b]	0.04 (7.291)	−2.13 (4.109)	<0.001
Mean percentage change in BMD from week 48 (SD)			
Hip	0.05 (3.393)	−0.38 (3.208)	0.032
(Intragroup *p*-value)	0.66	0.01	
Femur neck	−0.49 (4.418)	−0.95 (3.892)	0.074
(Intragroup *p*-value)	<0.001	<0.001	
Spine(L1-L4)	−0.01 (6.388)	−0.19 (3.182)	0.69
(Intra−group *p*-value)	0.14	0.41	
Incidences of >5% decline in BMD from baseline			
Hip[a]	62 (9.6%)	69 (21.3%)	<0.001
Femur neck[a]	95 (14.7%)	90 (27.8%)	<0.001
Spine (L1-L4)[b]	65 (10.0%)	56 (17.1%)	0.001
De novo osteopenia[†]	22/573 (3.8%)	18/294 (6.1%)	0.124
De novo osteoporosis[‡]	2/47 (4.3%)	5/32 (15.6%)	0.115

[a]Available cases was 646 in the TMF group and 324 in TDF group; [b]Available cases was 652 in the TMF group and 328 in TDF group. **P*-value was calculated by least square mean; [†]Diagnosed as ISCD standard and proportions were reported in patients without osteoporosis at baseline; [‡]Diagnosed as ISCD standard and proportions were reported in male patients aged >50 or menopausal female patient without osteoporosis at baseline. BMD, bone mineral density; TDF, tenofovir disoproxil fumarate; TMF, tenofovir amibufenamide.

mild for all parameters in each group (Table 5). In the TDF group, none of the serum lipid parameters, except for the TC/HDL ratio, changed significantly from week 48 onward. For the TMF group, a mild median (IQR) change of −0.02 (−0.14, 0.07) mmol/L in HDL, and 0.05 (−0.21, 0.38) in TC/HDL ratio were seen with statistical significance. Besides, the intergroup differences were significant for the change of HDL levels only. Cardiovascular diseases (CVDs) related AEs were reviewed, although they were rare events (Supplementary Table 9). No apparent difference in the incidence rate was seen. Only a single case of cardiomyopathy, cerebral venous sinus thrombosis, cardiomyopathy, or myocardial ischemia was seen in the TMF group.

Consistent with week 48, patients in the TMF treatment group continued to gain weight at week 96 (a median increase of 1.0 [IQR −1.00, 3.10] kg from baseline, $p<0.001$; a median increase of 0.30 [IQR −1.00, 2.00] kg from week 48, $p<0.001$). In contrast, the TDF group did not maintain a significant weight loss at week 96 compared with week 48 ($p=0.378$), while the group difference was still significant ($p<0.001$).

Discussion

We have previously reported that TMF had noninferior efficacy and improved safety profiles compared with TDF through the first 48-week treatment of this large, randomized, double-blind, phase III trial in mainland China. However, overall virological suppression rate was relatively low in each group and the short follow-up duration may not have been sufficient for safety assessments. Here, we presented the results of 96 weeks of treatment with TMF in a double-blind, active control setting.

At week 96, the virological response rate of each group continued to show noninferiority between the TMF and TDF therapy, either in the HBeAg-positive or negative populations. Prespecified subgroup analyses revealed no significant difference in virological suppression. In patients with baseline HBV DNA >7 $\log_{10}$ IU/mL or >8 $\log_{10}$ IU/mL, TMF also established noninferior efficacy over TDF. With a larger pooled sample size in its phase III trials, TAF did not establish the noninferior efficacy in this subgroup of patients.[20] For HBeAg-positive population at week 48, 50.2% of patients in the TMF group and 53.7% of patients in the TDF group achieved HBV DNA <20 IU/mL, which are both lower than expected. With prolonged treatment, about 20% more patients in each group achieved virological response at week 96. For those who did not achieve HBV DNA <20 IU/mL at week 96, over 52% have HBV DNA titers lower than 69 IU/mL. Hence, more patients could be expected to achieve virological response with further treatment.

The proportion of HbeAg seroconversion and HbeAg loss were similar in the two treatment groups at week 96. From week 48 to week 96, the proportion of HbeAg seroconversion increased from 9.4% to 15.1% in the TMF group and 8.3% to 12.6% in the TDF group. In terms of HbsAg, comparable declines were seen in the two treatment groups up to week 96. Further analysis showed that the mean reduction of qHBsAg were numerically larger in HBeAg-negative patients than that in HBeAg-positive ones at week 96, which is not under the previous understanding of HbsAg dynamics.[21,22] The lower serum ALT levels at baseline in the HBeAg-positive patients compared with previous studies may be one of the possible answers.[22] A further decline from week 48 to week 96 in HBeAg-negative population was another uncommon situation. Though only four patients achieved HbsAg loss during 96 weeks of treatment, over 34% of the HBeAg-positive patients and 20% of the HBeAg-negative patients achieved a

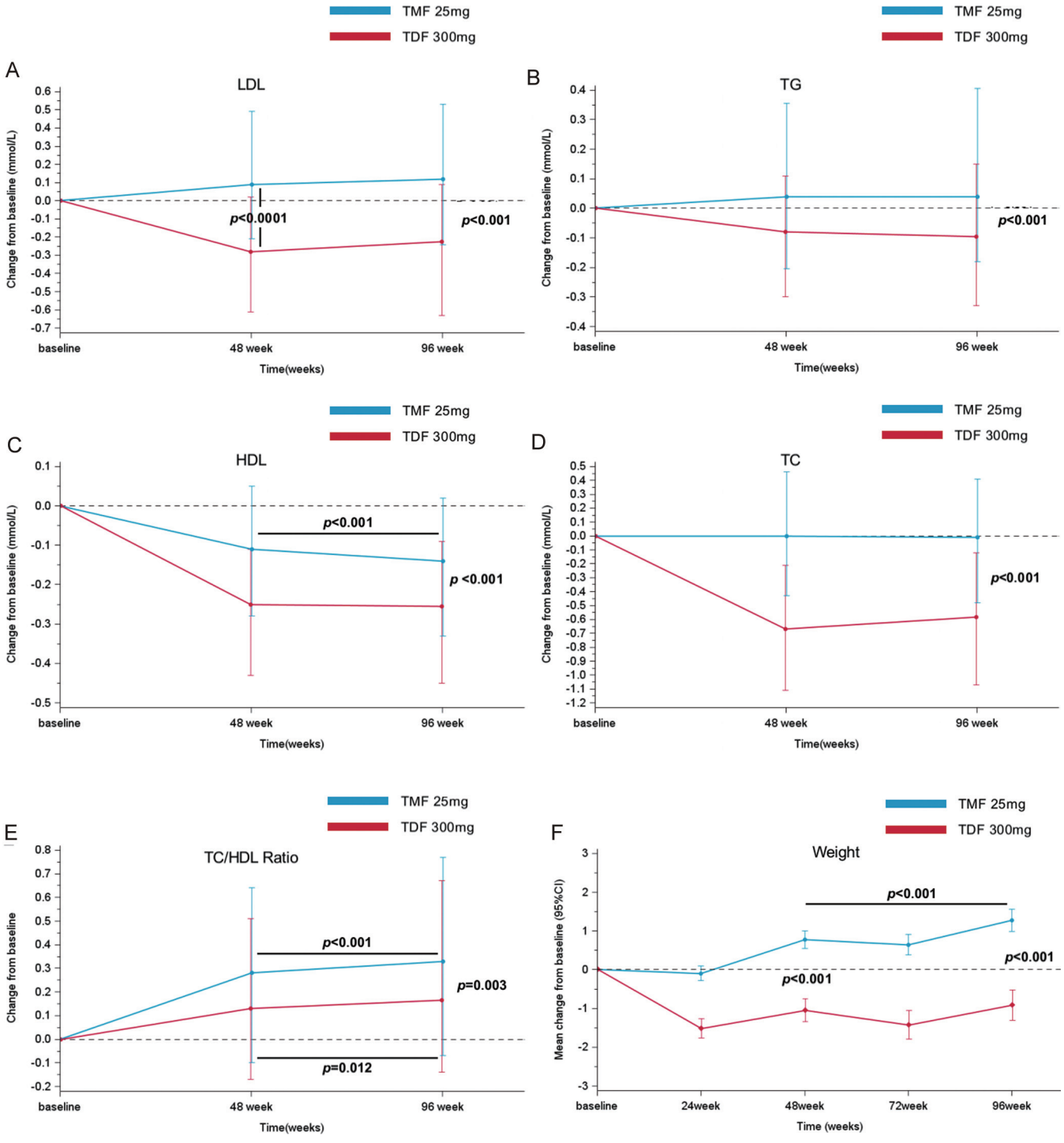

Fig. 3. Change of metabolic parameters. Median changes of serum LDL (A), TG (B), HDL (C), TC (D) and TC/HDL ratio (E) from baseline at week 48 and 96 treatment. Mean changes of body weight (F) by visit. All the inter- and intragroup differences of the changes in lipid parameters and body weight by visit were not statistically significant unless marked by *in the figure. Bars are 95% CI. TMF, tenofovir amibufenamide; TDF, tenofovir disoproxil fumarate; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglyceride.

≥0.5 $\log_{10}$ IU/mL decline of serum HbsAg and almost half of them experienced a ≥1 $\log_{10}$ IU/mL decline, which was predictive for HbsAg loss.[22]

At week 48, more patients in the TMF group achieved ALT normalization than those in the TDF group. This superior effect of TMF over TDF continued through 96 weeks of treatment, confirming that TMF had a durable advantage over TDF in terms of ALT normalization. In this week 96 analysis, the rate of ALT normalization was significantly higher in the pooled patients receiving TMF than in those receiving

Table 5. Change of metabolic parameters in each group from week 48 to week 96

	Intergroup comparison				*p*-value
	TMF 25 mg (N=666)		TDF 300 mg (N=336)		
	Median (q1, q3)	*p*-value	Median (q1, q3)	*p*-value	
TC (mmol/L)	−0.04 (−0.36, 0.31)	0.052	0 (−0.27, 0.29)	0.415	0.056
LDL (mmol/L)	0.02 (−0.27, 0.30)	0.095	0 (−0.21, 0.24)	0.343	0.592
HDL (mmol/L)	−0.02 (−0.14, 0.07)	<0.001	0 (−0.10, 0.08)	0.303	0.031
TC/HDL	0.05 (−0.21, 0.38)	<0.001	0.02 (−0.19, 0.34)	0.012	0.395
TG (mmol/L)	0 (−0.25, 0.32)	0.282	0 (−0.18, 0.19)	0.961	0.586
Weight (kg)	0.30 (−1.00, 2.00)	<0.001	0.00 (−1.00, 1.50)	0.378	<0.001

The *p*-value was calculated by two-sided Wilcoxon rank sum for intergroup comparison and Wilcoxon signed-rank for intragroup comparison except for weight change calculated by ANCOVA. HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride.

TDF. The findings in the HBeAg-positive patients (74.3% vs. 63.6%) followed those in the pooled patients. For HBeAg-negative patients, the ALT normalization rate showed no statistical difference between TMF and TDF at week 96. HbeAg-negative patients receiving TAF treatment had a significantly higher ALT normalization rate than those receiving TDF in its phase III trials. The discrepancy between the TMF study and the TAF study may be related to the less strict criteria of ALT normalization and higher baseline ALT levels in our study. As for other efficacy endpoints, significant differences were not observed between treatment arms other than the reduction in the FIB-4 score among HBeAg-positive patients. As expected, the incidences of serological responses increased with prolonged treatment duration in all groups.

In general safety, both 25 mg TMF and 300 mg TDF were well tolerated at week 96. Similar incidences of AE and SAE were seen in the two groups. From week 48 to week 96, the proportions of patients who had at least one AE, AE ≥ grade 3, or SAE increased evenly in each group by around 5–7%.[15] Hereinto, study-drug related cases only accounted for a small proportion and there were no new study drug-related SAEs after week 48. For AEs reported by investigators, patients in the TMF group were seen to have slightly higher incidences of weight increased, hyperuricemia, hypertriglyceridemia, hyperlipidemia, hepatic steatosis and diarrhea and slightly lower incidences of weight decrease, proteinuria, abdominal pain, and anemia than those in the TDF group.

The major safety concerns of the long-term use of TDF are renal toxicity and reductions of BMD. Our previous results at week 48 showed that patients who received TMF had better bone and renal safety. In the present report, these benefits persisted. As for renal function, there were significantly fewer declines of eGFR-epi and CrCl-cg in patients receiving TMF than that in patients receiving TDF at week 96. Specifically, patients receiving TMF had a median decline of 3.01 mL/min in CrCl-cg, compared with 6.65 mL/min in patients receiving TDF. The amount of intergroup difference seems smaller than that was reported by TAF trials.[20] However, the Cockcroft-Gault equation was developed to estimate the clearance rate of creatinine, which usually over-estimates the GFR.[17] The eGFR calculated by the CKD-EPI equation was recommended among individuals with normal or only mildly reduced GFR.[23,24] On the other side, the change of body weight exhibited a opposite trend between the two treatment arms, indicating that the traditional eGFR indexed to body surface area may not be appropriate.[25] With the non-indexed unit, the decline of eGFR seemed milder in both treatment arms (−1.68 mL/min for TMF, −3.12 mL/min for TDF). However, the intergroup difference in eGFR decline still remained significant, and 5% more patients presented with a ≥10% sustained decline of eGFR-epi in the TDF group (p=0.005). Besides, patterns of kidney injury due to TDF also include proximal tubular dysfunction.[26–28] In our study, only one patient in the TDF group presented with a clinical diagnosis of renal tubular dysfunction. Hypophosphatasemia in TDF-treated patients is usually considered a sign of renal tubular damage, however, the incidence of *de novo* hypophosphatemia was even in each group. Notably, a significantly higher incidence of serum PTH level elevation was seen in the TDF group over TMF group. Given that hypophosphatemia is actually the result of PTH elevation secondary to calcium loss by the renal tubular damage,[29–32] the PTH elevation may be a more straightforward sign of underlying tubular dysfunction. As described above, TMF may cause lower levels of eGFR decline and less tubular damage than TDF.

Like renal findings, a smaller percentage decline of BMD from baseline to week 96 was seen in patients receiving TMF than that in patients receiving TDF. This result continued to support the improved bone safety of TMF over TDF, either in hip, femur neck, or lumbar spine. Though not common in each group, when compared with TMF group, numerically higher incidences of *de novo* osteopenia or osteoporosis were still seen in the TDF group than TMF group. Besides, there were more patients in the TDF group experienced a >5% decline of BMD than patients in the TDF group (7.1–13.1% at different spots), which was more reflective of a higher risk of fragility fractures. Of note, in patients receiving TDF, the amount of BMD decline from week 48 to 96 was much lower than from baseline to week 48, and the intergroup difference was significant only regarding the hip. According to the 144-week comparison of TAF and TDF, the BMD loss of TDF treatment did not further enlarge since week 48 at the spine and week 96 at the hip.[33–36] Hence, it is reasonable to speculate that the bone loss mainly occurred in the first or second year of TDF treatment.

Although having renal or osteal toxicity in some patients, TDF treatment was found to have a lipid or weight lowering effect, which was firstly described in HIV infected patients.[33–35] In the week 48 results of our study, levels of fasting TC, LDL, HDL, and weight were all significantly reduced by TDF therapy. Hence, TMF therapy seems to have a plausible higher incidence of metabolism and nutrition disorder and hepatic steatosis than TDF therapy. However, none of the fasting lipid parameters significantly changed from week 48 to week 96 in the TDF group. Similarly, the change of lipid parameters in patients receiving TMF was mild from week 48 to week 96 that significantly change was only seen in HDL by a median 0.02 mmol/L decrease. Besides, like the findings

at week 48, we did not observe significant differences in the incidences of cardiovascular diseases between two treatment arms at week 96. Hence, we consider that long-term TMF treatment will have a neutral effect on serum lipids. Indeed, other studies observed a stable profile of lipid parameters for 5-year treatment of TAF or in previously entecavir treated patients after switching to TAF.[36,37]

A median weight gain of 0.3 kg was seen in the TMF group from week 48 to week 96, which is much lower than that from baseline to week 48. Weight gain is a common condition in CHB patients after virological suppression.[38,39] As reported by the same study mentioned above, weight decrease was seen after switching to TAF for 48 weeks in patients treated by entecavir, compared with weight increase in patients previously treated by TDF. This result further confirmed the weight lowering effect of TDF, rather than weight increasing by TAF or TMF treatment. Because of these points, weight gained in patients receiving TMF should not be a major safety concern either.

There are several limitations in this trial. First, the enrolled patients in this trial were relatively young, but we are facing an older CHB population in real-world with increasing comorbidities like bone and renal disorders.[40] Therefore, the significantly higher incidence of bone and renal toxicity of TDF over TMF may still be underestimated in our study. Also, the hyperlipidemia usually correlates with ALT elevation. Hence, the second limitation lies because we did not carry out multivariate analysis toward ALT normalization, especially considering that a higher incidence of hyperlipidemia was seen in the TMF group. It is reasonable to carry out deeper investigations toward ALT normalization soon. Last but not the least, considering the requirement of long-term suppression of HBV, the efficacy and safety results of 96 weeks may still be insufficient. Hence, this study has been extended into a 10-year real-world follow-up study. After 96 weeks of treatment, TMF maintained similar effectiveness in viral suppression to TDF with significantly less bone and renal toxicity.

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Conflict of interest

ZL has served as a speaker for Bristol Myers Squibb (BMS), Gilead Sciences, and Hansoh Pharma. QJ has served as a speaker for Abbott, Amoytop Biotech, Chiatai Tianqing, Gilead, and has received research funding from Kawin Technology, Chiatai Tianqing, Hansoh Pharma, Johnson and Johnson (J&J), BMS, HEC Pharmaceutical, TaiGen Biotechnology, Gilead Science. JN has served as a speaker for and has received research funding from HEC Pharmaceutical, Xintong Pharmacy, Hansoh Pharma, Qilu Pharmaceutical, Kelun Industry, Roche, J&J, GlaxoSmithKline (GSK), Assembly, Gilead, Brii Biosciences, Huahui Health, Zhimeng Biopharma, Hengrui Medicine, Aligos, Hepu Pharmaceutical, Ascletis Pharma, Sanhome, Ginkgo pharma, Changzhou Yinsheng pharma. JH has received consulting fee from AbbVie, Arbutus, BMS, Gilead Sciences, J&J, Roche and received grants from BMS and J&J. QW, CP, WJ, CL, and CS are employees of Hansoh Pharmaceuticals Co., Ltd. JN has has been an associate editor of *Journal of Clinical and Translational Hepatology* since 2013, and JH has has been an executive associate editor of *Journal of Clinical and Translational Hepatology* since 2021. The other authors have no conflict of interests related to this publication.

Author contributions

Guarantors of the article (JH, JN), study conception and design (JH, JN, CS, ZL), acquisition of data, (TMF Study Group), statistical analysis (CL), interpretation of the data and drafting of the manuscript (ZL, PX), and critical revision of the manuscript for important intellectual content (all authors).

Ethical statement

Written informed consent was obtained from all participated patients before enrollment, and the study was approved by the institutional review board or independent ethics committees at each site and was conducted following the principles of the Declaration of Helsinki and Good Clinical Practice guidance.

Data sharing 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 Pharmaceuticals Co., Ltd.

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