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Original Article

Interim Analysis of 48-week Tenofovir Amibufenamide Treatment in Chronic Hepatitis B Patients with Normal Alanine Aminotransferase Levels: The PROMOTE Study

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Abstract

Background and Aims: Chronic hepatitis B virus (HBV)-infected patients may exhibit liver fibrosis and other pathological changes despite normal alanine aminotransferase (ALT). This study aimed to assess the efficacy and safety of tenofovir amibufenamide (TMF) in chronic HBV-infected patients with normal ALT levels. ***Methods:*** The ongoing PROMOTE study (NCT05797714) is the first prospective, multicenter, randomized, open-label, blank-controlled clinical trial involving chronic HBV-infected patients with normal ALT levels. Participants were randomized in a 1:1 ratio to receive either TMF (TMF group) or no treatment (blank control group). The primary efficacy endpoint was the proportion of participants achieving HBV DNA levels <20 IU/mL at 48 weeks. ***Results:*** A total of 197 participants were enrolled, with 95 in the TMF group and 102 in the blank control group. At 48 weeks, a significantly greater proportion of participants in the TMF group achieved HBV DNA levels <20 IU/mL compared with the control group (74.2% vs. 9.0%, $P < 0.001$). The TMF group demonstrated more pronounced reductions in HBV DNA (−2.63 vs. −0.22 $\log_{10}$ IU/mL, $P < 0.001$), HBsAg (−0.07 vs. −0.04 $\log_{10}$ IU/mL, $P = 0.02$), and ALT levels (−14.09% vs. 0%, $P = 0.003$) compared with the blank control. In the TMF group, the proportion of participants with high-normal ALT levels (20–40 IU/L) was reduced. No significant differences were observed between the groups in creatinine, glomerular filtration rate, bone turnover biomarkers, lipid profiles, or phosphorus levels. ***Conclusions:*** TMF treatment demonstrates significant efficacy in chronic HBV-infected patients with normal ALT levels and shows a favorable safety profile regarding bone, renal, and lipid parameters. The PROMOTE study is ongoing, and further results at 96 and 144 weeks are expected to provide additional insights.

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Introduction

For patients with hepatitis B virus (HBV) infection, elevated alanine aminotransferase (ALT) levels indicate liver damage and extensive pathological changes and are also associated with a higher risk of developing hepatocellular carcinoma

(HCC).[1] Previous research has demonstrated that chronic HBV-infected patients may exhibit liver fibrosis and other pathological changes despite having substantial normal ALT levels.[2] This important finding has been corroborated by recent meta-analyses.[3,4] Consequently, antiviral treatment should be considered for patients with normal ALT levels. Notably, there remains a lack of prospective, large-sample, randomized controlled trials (RCTs) with a blank control design to evaluate the short- and long-term benefits of antiviral treatment in patients with chronic HBV (CHB) infection and normal ALT levels. Prior RCTs have shown that various antiviral therapies can effectively suppress HBV replication in HBeAg-positive patients with normal ALT levels.[5] Moreover, ALT levels within the normal range may carry different clinical implications. A large-scale retrospective analysis has revealed that patients with high-normal ALT levels exhibit more pronounced liver histopathological changes compared with those with low-normal ALT levels.[6] Thus, evaluating treatment options for patients with normal ALT levels is of considerable clinical importance.

Tenofovir amibufenamide (TMF) is a novel nucleotide reverse transcriptase inhibitor and a second-generation prodrug of tenofovir, introduced to the mainland Chinese market in 2021. Clinical evidence suggests that TMF demonstrates antiviral efficacy in CHB that is not inferior to that of tenofovir disoproxil fumarate (TDF), while offering better bone and renal safety profiles.[7,8] Additionally, TMF appears to offer potential advantages over TDF in terms of ALT normalization rates after 48 weeks of treatment.[7] Notably, TMF contains an additional methyl group compared to TAF, which enhances both plasma stability and cell membrane permeability.[8] Recent real-world studies comparing TMF to TAF in CHB patients have indicated that TMF achieves comparable or superior effectiveness.[9,10] Although Phase III trials of TMF have included participants with normal ALT levels (<1× upper limit of normal [ULN]),[7,8] no specific subgroup analyses addressing efficacy and safety in this cohort have been conducted. Therefore, further investigation into the efficacy and safety of TMF in chronic HBV-infected patients with normal ALT levels is warranted.

Methods

Study design

The PROMOTE study (ClinicalTrials.gov, Registration No. NCT05797714) is conducted across 12 research centers in China. Commencing in June 2022 and projected to conclude in June 2026, the trial aimed to evaluate the efficacy and safety of TMF in patients with chronic HBV infection who have not previously received treatment and have normal ALT levels. Eligible participants were randomized into two groups: the TMF group, which received TMF treatment, and the blank control group, which received no antiviral therapy. The study design includes a core follow-up period from week 0 to week 48, followed by an extended follow-up period until week 144. Follow-up visits were conducted every 12 weeks during the first 48 weeks and every 24 weeks thereafter, until the final visit at week 144. This interim analysis reports the 48-week results following the core follow-up period, including the primary endpoint, other critical efficacy endpoints, and safety.

This trial adheres to the 2024 Declaration of Helsinki, abides by the 2018 Declaration of Istanbul, and follows Good Clinical Practice guidelines. Ethical approval was obtained from the Ethics Committee of Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine (Approval No. 2021 Clinical Ethics Review [382]), as well as from the institutional review boards of each participating center. Written informed consent was obtained from all participants. The preparation of the manuscript adheres to the CONSORT reporting guidelines. All authors had access to the study data and reviewed and approved the final manuscript.

Patients

The main inclusion criteria were 1) age 18–65 years, 2) documented evidence of chronic HBV infection, 3) documented ALT levels ≤ 1× ULN (i.e., ≤40 IU/L), 4) serum HBV DNA levels >20 IU/mL at screening, and 5) no prior treatment with any nucleoside analogs (NAs) or interferon therapy. The main exclusion criteria were 1) co-infection with hepatitis C virus, hepatitis E virus, hepatitis D virus, or human immunodeficiency virus, 2) comorbid autoimmune liver disease, metabolic-associated fatty liver disease, or drug-induced liver injury, 3) radiological diagnosis of HCC, and 4) cirrhosis.

Randomization

The participants were randomized via an interactive web response system and stratified by screening centers, to the TMF and blank control groups in a 1:1 ratio. This study is an open-label trial without the use of placebos or blinding methods.

Treatment

Participants assigned to the TMF group received 25 mg of TMF orally once daily for 144 continuous weeks. For participants who did not achieve HBV DNA levels <20 IU/mL after 96 weeks of continuous TMF treatment, ETV could be added at the investigator's discretion, and treatment continued until 144 weeks.

Participants assigned to the blank control group did not receive any antiviral drug and were followed up until week 144. During the first 48 weeks, if ALT levels exceeded 2× ULN in HBeAg-positive individuals or 1× ULN in HBeAg-negative individuals, the patient was eligible to switch to TMF treatment, provided that other potential causes of elevated ALT were ruled out, as determined by the investigator. Following an update in the guidelines, from weeks 48 to 144, patients in the control group who experienced ALT levels exceeding 1× ULN were eligible to commence TMF therapy, regardless of HBeAg status.

Study endpoints

The primary efficacy endpoint of this study was the proportion of participants who achieved serum HBV DNA levels <20 IU/mL at week 48. Secondary efficacy endpoints evaluated at week 48 included the following key measures: 1) The extent of HBV DNA reduction from baseline; 2) For HBeAg-positive participants: the proportion who achieved HBeAg loss; 3) For HBeAg-positive participants: the proportion who achieved HBeAg loss and seroconversion; 4) For HBsAg-positive participants: the proportion who achieved HBsAg loss; 5) For HBsAg-positive participants: the proportion who achieved HBsAg loss and seroconversion; 6) The reduction in HBsAg levels from baseline; and 7) The change in liver fibrosis markers from baseline.

Bone and renal safety were assessed through changes from baseline in bone biomarkers (β-CTx and P1NP), serum creatinine levels, glomerular filtration rate, and blood lipid profiles, including triglyceride, low-density lipoprotein, and high-density lipoprotein levels. Adverse events occurring throughout the study were systematically recorded. These events were classified according to the Common Terminology

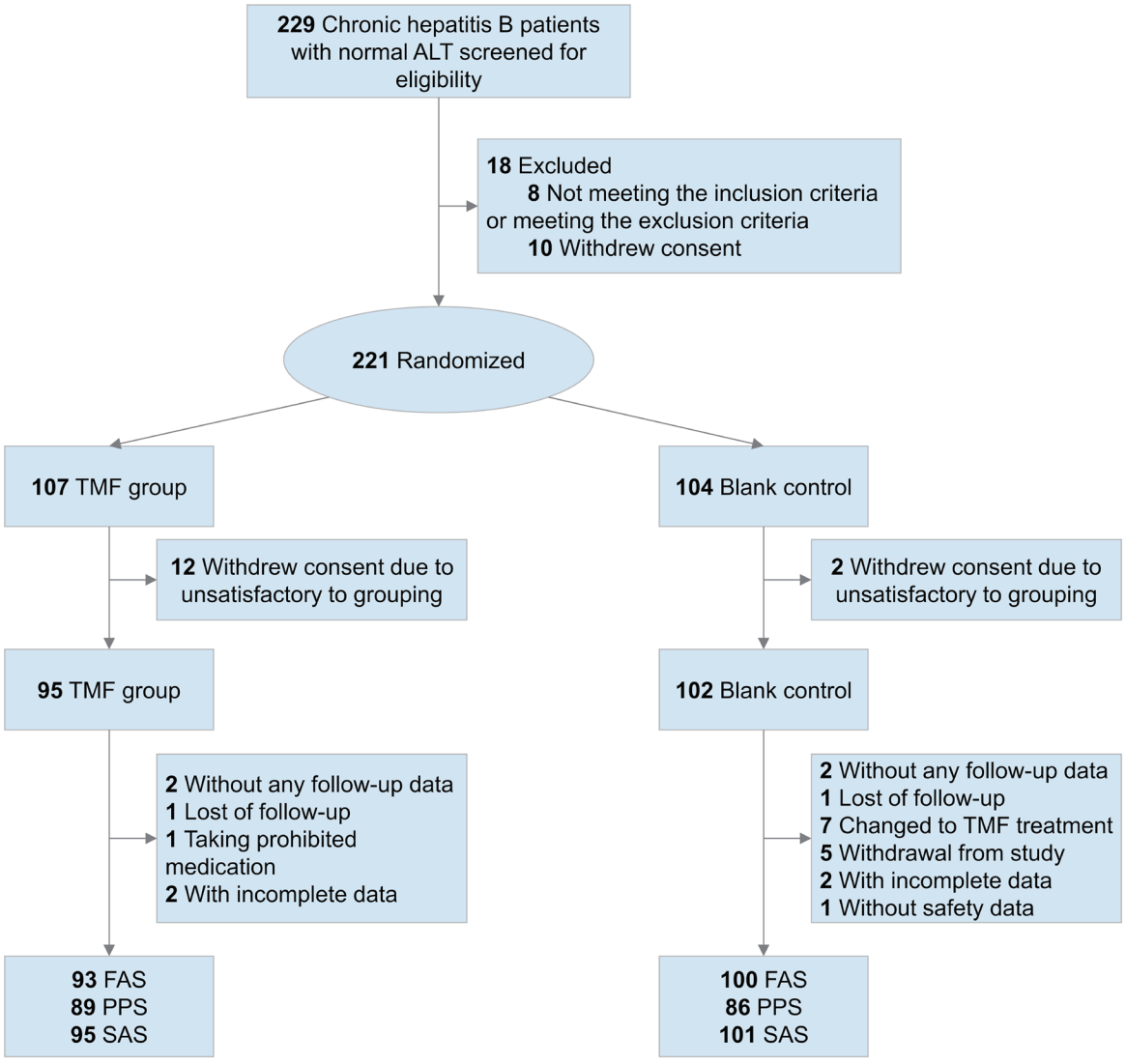

Fig. 1. Participant flowchart. Note: One patient received clarithromycin, which was a prohibited medication due to its strong inhibition of CYP3A4 and P-gp with significant effects on the metabolism and transport of multiple drugs. TMF, tenofovir amibufenamide; FAS, full analysis set; PPS, per protocol set; SAS, safety analysis set.

Criteria for Adverse Events version 5.0, and their relationship to the study medication was evaluated.

Statistical analysis

The sample size, determined to be 200 participants, was based on estimates from previous studies[7,11]; details are available in the Supplementary File 1. The full analysis set (FAS) included all participants who were randomly assigned and received at least one dose of TMF for those in the TMF group, excluding cases who violated major inclusion criteria or had no post-randomization observational data. The per protocol set (PPS) comprised participants from the FAS who adhered to the study protocol, took the assigned study medication, did not use prohibited medications during the trial, and completed the case report form as specified. The safety analysis set (SAS) included all randomized participants with recorded safety data and at least one dose of the study medication for those in the TMF group. The details of handling missing values and statistical analysis are provided in the Supplementary File 1.

Statistical significance was defined as a two-sided *P*-value ≤ 0.05. Statistical analyses were performed using SPSS version 19.0 or higher and SAS version 9.4 or higher.

Results

Characteristics of the participants

As illustrated in Figure 1, a total of 229 patients were screened for this study, with 211 subsequently randomized. Fourteen participants withdrew their consent due to dissatisfaction with their group assignment. Consequently, 197 participants were enrolled, with 95 assigned to the TMF group and 102 to the blank control group. The FAS comprised 193 participants, the PPS included 175 participants, and the SAS encompassed 196 participants.

In the FAS, the two groups were generally well-balanced, although the prevalence of hypertension was notably higher in the TMF group (10.8% vs. 2.0%) (Table 1). Baseline char-

Table 1. Baseline characteristics of enrolled participants in FAS

Characteristics	Total (n = 193)	TMF group (n = 93)	Blank control group (n = 100)
Age (years), median (Q1, Q3)	41.0 (33.0, 49.0)	45.0 (34.0, 53.0)	40.0 (33.0, 46.3)
Age >30 years, n (%)	168 (87.0)	80 (86.0)	88 (88.0)
Gender, male, n (%)	87 (45.1)	45 (48.4)	42 (42.0)
Positive HBeAg, n (%) [n = 188]	48 (25.5)	23 (25.0)	25 (26.0)
HBsAg (log_{10} IU/mL)			
Overall patients, median (Q1, Q3) [n = 188]	3.29 (2.51, 4.08)	3.36 (2.57, 4.21)	3.27 (2.36, 4.05)
HBeAg positive patients, median (IQR) [n = 48]	4.73 (4.43, 4.87)	4.76 (4.70, 4.86)	4.56 (4.17, 4.88)
HBeAg negative patients, median (IQR) [n = 140]	2.94 (2.26, 3.54)	2.89 (2.36, 3.66)	3.01 (2.22, 3.48)
Serum HBV DNA (log_{10} IU/mL)			
Overall patients, median (Q1, Q3)	3.68 (2.97, 5.77)	3.69 (2.94, 6.13)	3.64 (3.01, 5.68)
HBeAg positive patients, median (IQR) [n = 48]	8.35 (8.07, 8.59)	8.41 (8.28, 8.59)	8.34 (7.87, 8.51)
HBeAg negative patients, median (IQR) [n = 140]	3.34 (2.78, 3.96)	3.36 (2.78, 4.08)	3.32 (2.78, 3.85)
Serum HBV DNA >8 log_{10} IU/mL, n (%)	38 (19.7)	21 (22.6)	17 (17.0)
ALT (IU/L)			
Overall patients, median (Q1, Q3) [n = 192]	22.00 (17.00, 31.00)	23.00 (18.75, 32.02)	20.50 (16.00, 30.25)
HBeAg positive, median (IQR) [n = 48]	25.00 (16.00, 33.25)	26.00 (18.50, 33.00)	23.00 (15.00, 34.00)
HBeAg negative, median (IQR) [n = 139]	21.00 (17.00, 30.50)	22.00 (18.75, 31.02)	20.00 (16.00, 26.50)
GFR (mL/m), median (Q1, Q3) [n = 191]	99.80 (87.55, 118.40)	98.20 (85.20, 116.00)	102.25 (89.17, 118.62)
GFR <90 mL/m, n (%) [n = 191]	58 (30.4)	32 (34.4)	26 (26.5)
Creatinine (μmol/L), median (Q1, Q3) [n = 192]	65.10 (56.00, 79.00)	66.00 (57.00, 78.00)	64.00 (54.50, 81.00)
Liver stiffness measurement (kPa), median (Q1, Q3) [n = 192]	5.30 (4.50, 6.50)	5.30 (4.57, 6.62)	5.30 (4.50, 6.40)
FIB4, median (Q1, Q3) [n = 191]	0.98 (0.69, 1.21)	0.99 (0.71, 1.31)	0.94 (0.68, 1.17)
β-CTx (ng/mL), median (Q1, Q3) [n = 69]	0.41 (0.28, 0.59)	0.42 (0.29, 0.73)	0.39 (0.26, 0.53)
P1NP (ng/mL), median (Q1, Q3) [n = 24]	46.42 (39.36, 65.76)	46.42 (38.37, 68.25)	46.43 (42.02, 63.58)
History of hepatitis B treatment, n (%)	0	0	0
Hypertension, n (%)	12 (6.2)	10 (10.8)	2 (2.0)
Diabetes, n (%)	4 (2.1)	4 (4.3)	0
Cardiovascular and cerebrovascular diseases, n (%)	0	0	0
Dyslipidemia, n (%)	5 (2.6)	3 (3.2)	2 (2.0)
Fatty liver, n (%)	7 (3.6)	3 (3.2)	4 (4.0)

FAS, full analysis set; TMF, tenofovir amibufenamide; Q, quartile; HBsAg, Hepatitis B surface antigen; HBeAg, hepatitis B e-antigen; IQR, interquartile range; HBV DNA, hepatitis B virus DNA; ALT, alanine aminotransferase; GFR, glomerular filtration rate; β-CTx, β-isomerized C-terminal telopeptides; P1NP, procollagen type I intact N-terminal propeptide.

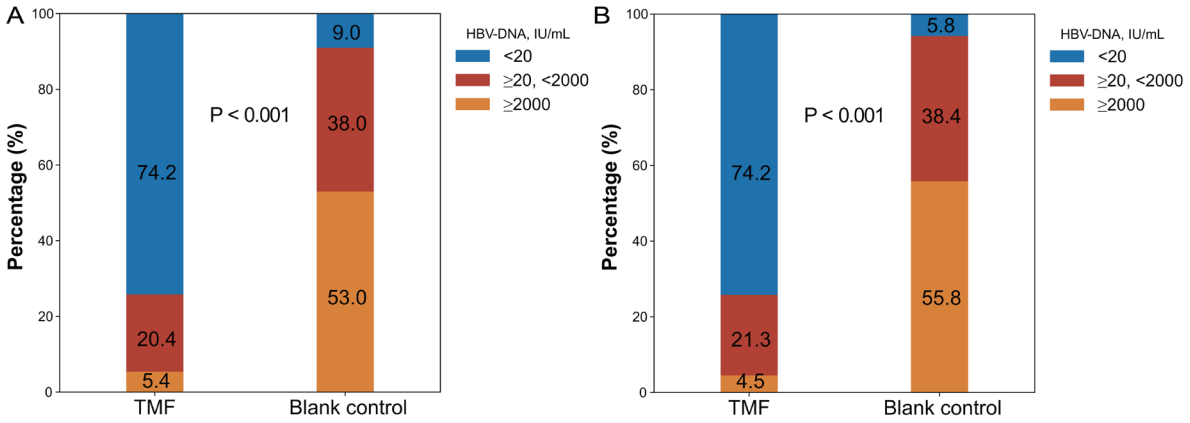

Fig. 2. Proportion of patients in the tenofovir amibufenamide group and the blank control group who achieved the primary endpoint at week 48 in the (A) full analysis set and (B) per protocol set. TMF, tenofovir amibufenamide; HBV-DNA, hepatitis B virus DNA.

acteristics in the PPS were also comparable (Supplementary Table 1).

Primary efficacy endpoint

In the FAS, the proportion of participants achieving HBV DNA levels <20 IU/mL at week 48 was significantly higher in the TMF group compared with the blank control group (74.2% vs. 9.0%; $P < 0.001$) (Fig. 2A and Table 2). The between-group difference (TMF minus blank control) was 65.2% (95% CI: 54.7–75.7). Among those in the TMF group who did not achieve complete virological response, most experienced substantial reductions in HBV DNA, reaching levels between 20 and 2,000 IU/mL (19 participants, 20.4%), with only five individuals exceeding 2,000 IU/mL. A notably higher proportion of participants in the blank control group had HBV DNA levels >2,000 IU/mL (53 participants, 53%). These results were consistent in the PPS (Fig. 2B, Supplementary Fig. 1, and Supplementary Table 2).

Secondary efficacy endpoints

In the FAS analysis, HBV DNA levels decreased by −2.63 $\log_{10}$ IU/mL in the TMF group compared to −0.22 $\log_{10}$ IU/mL in the blank control group ($P < 0.001$) (Table 2 and Fig. 3A) at week 48. One patient in the TMF group achieved seroconversion with HBsAg loss; no such serological changes occurred in the blank control group. HBsAg reduction was also more pronounced in the TMF group (−0.07 vs. −0.04 $\log_{10}$ IU/mL, $P = 0.02$) (Fig. 3B). Although changes in ALT levels from baseline showed no statistically significant difference between groups ($P = 0.055$), the percentage change was significantly greater in the TMF group than in the blank control group (−14.09% vs. 0%, $P = 0.003$) (Fig. 3C). No ALT flares occurred in the TMF group, whereas seven participants in the blank control group experienced ALT flares (7.0%) ($P = 0.014$) (Table 2). These seven cases were deemed by investigators to meet the criteria for disease progression and were subsequently treated with TMF. TMF treatment also reduced the proportion of participants with high-normal ALT (0.5–1× ULN). As shown in Supplementary Figure 1, this proportion declined from 60.9% at baseline to 46.2% at week 48. In the blank control group, the proportions of participants with high-normal or low-normal ALT were both 50% at baseline to week 48. Observations in the PPS, as detailed in Supplementary Table 2, corroborated these findings. We also assessed liver stiffness measurements and FIB-4 scores as markers of liver fibrosis. The results are presented in Table 2 and Supplementary Table 2. Although TMF treatment showed trends toward numerical improvement in liver stiffness, these differences did not reach statistical significance ($P = 0.931$ and $P = 0.81$, respectively, Supplementary Table 2).

Subgroup analysis of efficacy

As demonstrated in Supplementary Table 3, in most subgroups, including males, females, participants over 30 years of age, HBeAg-negative individuals, and those with baseline HBV DNA <8 $\log_{10}$ IU/mL, TMF treatment was associated with a significantly higher proportion of patients achieving HBV DNA < 20 IU/mL at week 48 compared to the blank control group. Supplementary Table 4 illustrates that among participants with baseline HBV DNA ≥ 8 $\log_{10}$ IU/mL, although most in the TMF group did not achieve complete virological response, the majority (85.7%) maintained HBV DNA levels between 20 and 2,000 IU/mL. In contrast, all participants in the blank control group had HBV DNA levels >2,000 IU/mL ($P < 0.001$). Similar results were observed in the HBeAg-positive subgroup.

Safety

At week 48, there were no significant differences between the TMF and blank control groups in changes from baseline in creatinine (1.00 vs. 1.50 μmol/L), glomerular filtration rate (−1.60 vs. −1.65 mL/m), and serum phosphorus (−0.01 vs. 0.02 mmol/L), with all *P*-values >0.05 (Fig. 4 and Supplementary Table 5). The changes in β-CTX (assessed in 66 evaluable participants) after 48 weeks of treatment were −0.01 in the TMF group and −0.05 in the blank control group ($P = 0.441$). For P1NP (measured in 21 evaluable participants), changes from baseline were −11.70 and −6.85 in the TMF and blank control groups, respectively ($P = 0.622$). In terms of blood lipid levels, changes in total cholesterol (−0.07 vs. −0.02 mmol/L) and other parameters, including triglyceride, low-density lipoprotein, and high-density lipoprotein levels, showed no significant differences between groups at week 48 (all $P > 0.05$, Supplementary Table 5).

As detailed in Supplementary Table 6, TMF showed a favorable safety profile. Most treatment-emergent adverse events were mild, with only 2.1% in the TMF group and 1.0% in the blank control group graded ≥3. Only one participant discontinued TMF due to adverse events. No deaths were reported during the study.

Table 2. Summary of efficacy at week 48 in FAS

Efficacy indicators	TMF group (n = 93)	Blank control group (n = 100)	*P*
Serum HBV DNA <20 IU/mL			<0.001
Number of patients, n (%) [95%CI]	69 (74.2) [64.1–82.7]	9 (9.0) [4.2–16.4]	
Proportion difference between TMF and blank control, % [95%CI]	65.2 [54.7–75.7]	-	
Serum HBV DNA level			<0.001
<20 IU/mL	69 (74.2)	9(9.0)	
20–2,000 IU/mL	19 (20.4)	38 (38.0)	
>2,000 IU/mL	5 (5.4)	53 (53.0)	
Serum HBV DNA			
Log_{10} IU/mL, median (Q1, Q3)	1.00 (1.00, 1.41)	3.41 (2.29, 5.01)	<0.001
Change from baseline (log_{10} IU/mL), median (Q1, Q3)	−2.63 (−4.71, −1.88)	−0.22 (−0.71, 0.02)	<0.001
Percentage change from baseline (%), median (Q1, Q3)	−72.45 (−77.29, −64.73)	−4.64 (−21.45, 0.45)	<0.001
HBeAg loss, n (%)	0	1 (4.0)	>0.999
HBeAg loss and HBeAg seroconversion, n (%)	0	1 (4.0)	>0.999
HBsAg loss, n (%)	1 (1.1)	0	0.482
HBsAg loss and HBsAg seroconversion, n (%)	1 (1.1)	0	>0.999
HBsAg			
Log_{10} IU/mL, median (Q1, Q3)	3.34 (2.47, 4.13)	3.23 (2.32, 4.02)	0.672
Change from baseline (log_{10} IU/mL), median (Q1, Q3) [n = 188]	−0.07 (−0.16, −0.01)	−0.04 (−0.09, 0.01)	0.02
Percentage change from baseline (%), median (Q1, Q3) [n = 188]	−2.39 (−4.23, −0.57)	−1.33 (−2.98, 0.58)	0.022
Liver stiffness measurement			
kPa, median (Q1, Q3) [n = 185]	5.30 (4.80, 6.60)	5.40 (4.43, 6.20)	0.372
Change from baseline (kPa), median (Q1, Q3) [n = 184]	0.00 (−0.67, 1.00)	0.15 (−0.90, 1.18)	0.827
Percentage change from baseline (%), median (Q1, Q3) [n = 184]	0.00 (−11.23, 22.38)	3.21 (−15.94, 22.52)	0.912
FIB4			
Median (Q1, Q3)	0.99 (0.72, 1.37)	0.90 (0.64, 1.13)	0.126
Change from baseline, median (Q1, Q3) [n = 191]	0.00 (−0.11, 0.12)	0.00 (−0.11, 0.10)	0.848
Percentage change from baseline (%), median (Q1, Q3) [n = 191]	−0.13 (−11.09, 10.97)	0.36 (−10.09, 16.18)	0.745
ALT			
Median (Q1, Q3)	20.00 (16.00, 27.00)	20.50 (17.00, 26.25)	0.417
Change from baseline, median (Q1, Q3) [n = 192]	−3.00 (−8.25, 1.00)	0.00 (−7.00, 3.00)	0.055
Percentage change from baseline (%), median (Q1, Q3) [n = 192]	−14.09 (−33.33, 6.02)	0.00 (−21.27, 21.43)	0.003
ALT flare*	0	7 (7.0)	0.014

*ALT flare: During the study, subjects experienced ALT > 2 × ULN (40 IU/L for HBeAg-positive patients or ALT > ULN (40 IU/L) for HBeAg-negative patients), and other causes were excluded by the investigators. FAS, full analysis set; TMF, tenofovir amibufenamide; CI, confidence interval; HBV DNA, hepatitis B virus DNA; Q, quartile; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e-antigen.

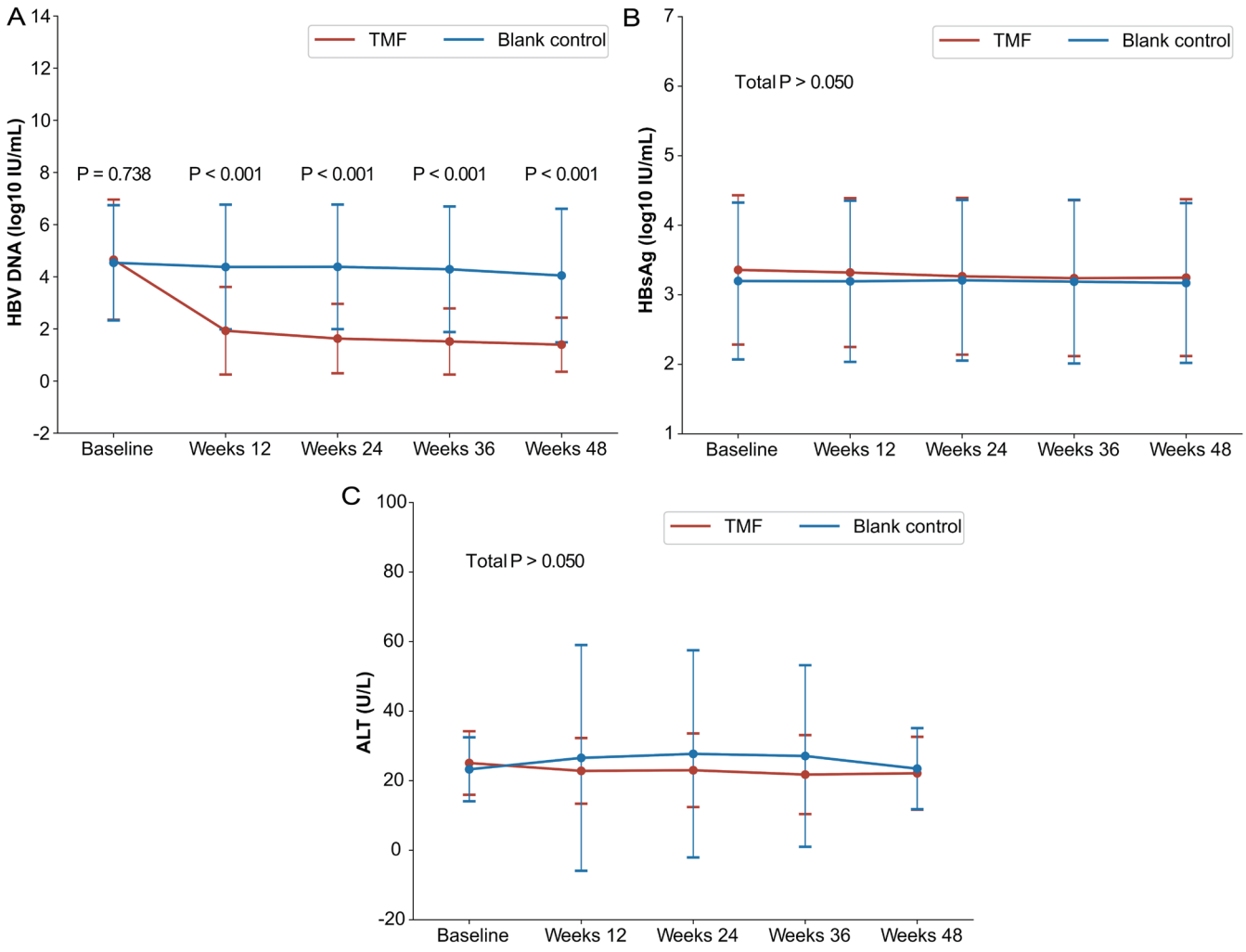

Fig. 3. Changes in hepatitis B virus DNA (A), hepatitis B surface antigen (B), and alanine aminotransferase (C) levels over time. TMF, tenofovir amibufenamide; HBV-DNA, hepatitis B virus DNA; HBsAg, hepatitis B surface antigen; ALT, alanine aminotransferase.

Discussion

Previous studies of NAs have predominantly focused on antiviral treatment in patients with elevated ALT (e.g., a mild increase of <2× the ULN). However, the benefits of antiviral treatment in patients with normal ALT have largely been overlooked. For instance, the pivotal Phase III registrational study of TMF included participants with normal ALT but did not perform a subgroup analysis for these patients.[7,8] This consideration is essential because ALT levels do not adequately reflect the degree of liver inflammation or the integration of the HBV genome into hepatocyte DNA.[2–4] Moreover, some patients with normal ALT levels may still benefit from NA treatment. The patients in this study were not considered part of the population requiring treatment according to conventional perspectives. In recent years, with the urgent need to increase the treatment rate of individuals infected with HBV, many researchers have advocated for extending treatment to larger populations, or even to all infected individuals. This shift is particularly pronounced in China, which bears the heaviest global burden of CHB. Treating all infected individuals seems to align with an optimal strategy that offers the best cost-benefit ratio.[12] Early effective treatment may be delayed in such patients, leading to liver inflammation and fibrosis despite normal ALT levels,[13] and increasing the risk of disease progression to HCC.[14] Even within the normal range, higher ALT levels still indicate a higher risk of progression to cirrhosis and HCC.[15] Therefore, exploring appropriate antiviral treatment for patients with normal ALT remains an urgent issue. The available evidence is mostly derived from small sample-size, single-center, or retrospective studies. A retrospective study of 117 Chinese patients showed that chronic HBV-infected patients with normal ALT and detectable HBV DNA, but not meeting the treatment guideline criteria, could achieve favorable complete virological responses after 24 weeks of TAF treatment.[16] In the RCT, Chan *et al*.[5] examined the effects of TDF combined with emtricitabine versus TDF alone in chronic HBV-infected patients with high viral load and normal ALT. The combination therapy achieved better viral suppression than TDF alone. Xing *et al*.[17] performed a retrospective analysis of 79 treatment-naïve chronic HBV-infected patients treated with TAF for 24 weeks. They found that TAF reduced viral load in patients with normal ALT, with effects similar to those observed in patients with elevated ALT. Furthermore, the risk of significant clinical events in untreated chronic HBV-infected patients with normal ALT is higher than in patients

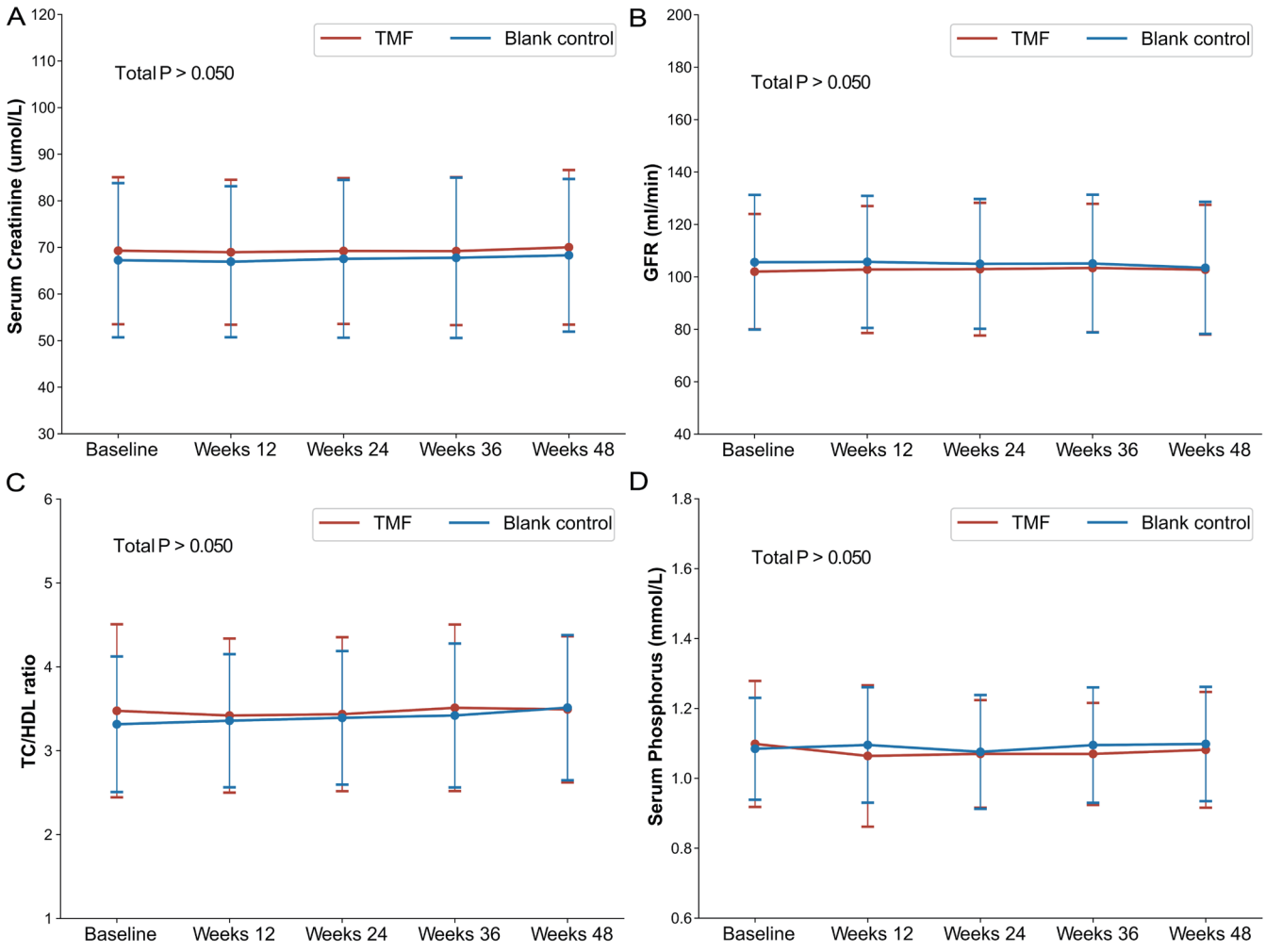

Fig. 4. Changes in creatinine (A), glomerular filtration rate (B), total cholesterol/high-density lipoprotein ratio (C), and serum phosphorus (D) within 48 weeks. TMF, tenofovir amibufenamide; GFR, glomerular filtration rate; TC/HDL, total cholesterol /high-density lipoprotein.

with abnormal ALT who receive treatment.[18] Thus, there remains an urgent need for large-scale RCTs to explore both the short-term and long-term benefits of antiviral treatment for chronic HBV-infected patients with normal ALT levels. As the first prospective, multicenter, large-scale RCT using a blank control design, the PROMOTE study investigates the efficacy of NAs in chronic HBV-infected patients with normal ALT levels and provides high-quality evidence for this patient population. The PROMOTE study is ongoing, with long-term follow-up results at 96 weeks and 144 weeks to be reported in future publications. This study not only demonstrates the efficacy and safety of TMF in chronic HBV-infected patients with normal ALT levels but also provides valuable data on the therapeutic benefits of NAs for this population and supports guideline recommendations for antiviral therapy in individuals with normal ALT levels.

In the present study, ALT levels in the TMF group decreased as early as 12 weeks after starting treatment and remained low throughout the 48-week study period. TMF also decreased the proportion of patients with ALT levels >0.5× the ULN, highlighting the improvement in ALT levels achieved following treatment. Furthermore, patients with high-normal ALT levels could achieve low-normal ALT levels after treatment. Therefore, the decrease in ALT suggests a reduction in the risk of disease progression, offering further benefits for these patients. It is also noteworthy that in the blank control group, seven cases of ALT flare met the disease progression criteria, leading to the switch of these participants to TMF. These ALT flares further support the risk of disease activity and progression without timely treatment, highlighting the necessity of antiviral treatment in chronic HBV-infected patients with normal ALT. The difference between FIB-4 and liver stiffness after 48 weeks of TMF treatment was not significant, partly because this study excluded patients with cirrhosis, and the degrees of liver stiffness and fibrosis were relatively low. Non-invasive markers such as liver stiffness measurement and FIB-4 are less sensitive in patients with milder cirrhosis, making it difficult to show significant changes in a short period. The effect of antiviral treatment on the progression of liver fibrosis needs to be further evaluated with longer follow-up periods.

The present study raised no significant concerns regarding bone, renal, and lipid parameters, as supported by previous studies.[7–10] NAs have been considered safe and generally well-tolerated; however, some patients experience cumulative toxicity with prolonged use of oral antiviral agents, particularly manifesting as skeletal and renal injuries.[19,20] These results preliminarily suggest that TMF may

have favorable safety characteristics regarding bone and renal toxicity among NAs. The use of a no-treatment (blank control) comparator in this study allows for a more accurate assessment of TMF's bone and renal toxicity. The 48-week safety results indicated no significant differences between TMF and the blank control regarding bone and renal parameters, providing further reassurance about TMF's safety profile. On the other hand, the blood lipid safety of second-generation tenofovir has always been an important concern for clinicians. Previous studies of TAF reported elevated blood lipids, which could influence cardiovascular risk.[21] The Phase III RCT of TMF[7,8] showed that TMF had a tendency to increase blood lipids compared to TDF, but the impact of TMF on blood lipids remained stable after 48 weeks of treatment. This might be related to TDF's effect of reducing blood lipids, and the tendency of TMF to increase blood lipids did not increase the risk of cardiovascular events. The present study compared changes in blood lipids with the blank control group, indicating that TMF did not increase the risk of blood lipid alterations. Moreover, regarding blood lipid safety, the impact of TMF on total cholesterol levels is smaller than that of TAF.[10] The favorable safety profile of TMF provides a solid foundation for its long-term use, and outcomes at 96 and 144 weeks are anticipated to provide more definitive insights.

In the future, antiviral treatment may be expanded to both HBeAg-positive and HBeAg-negative patients with normal ALT, eventually reaching a "treat-all" strategy.[22,23] The necessity of antiviral treatment for HBeAg-negative patients with normal ALT is higher, and they usually achieve better outcomes when they receive antiviral treatment,[24] aligning with the present study. Therefore, starting antiviral treatment as early as possible for HBeAg-negative patients with normal ALT should be considered. HBeAg-positive patients with normal ALT may include those in the immune tolerance period. These patients still have a high risk of cccDNA integration and clonal hepatocyte expansion, meaning that patients in the immune tolerance period also have the risk of developing HCC.[25] Therefore, it is also necessary to treat HBeAg-positive patients with normal ALT. In this study, the proportion of HBeAg-positive individuals was small, and the antiviral efficacy at week 48 was not ideal. However, in HBeAg-positive patients with high viral load, TMF treatment significantly reduced HBV DNA levels. This reduction is associated with a decreased risk of disease progression and a lower incidence of hospitalization due to acute hepatitis flares compared to those who remain untreated. Furthermore, we observed that in a small proportion of patients (9.0%) in the control group who did not receive antiviral treatment, HBV DNA levels were lower than 20 IU/mL. This may be explained by the natural course of the infection, during which chronic carriers present fluctuations in HBV DNA levels, and many patients may even experience spontaneous HBsAg loss accompanied by undetectable HBV DNA. Previous data showed that 10.2% of HBsAg carriers presented HBV DNA levels below the detection limit without treatment during follow-up, a proportion similar to that observed in the blank control group in this study.[26]

Two subcategories of patients showed no significant decreases in HBV DNA levels: those younger than 30 years and those who were HBeAg-positive. It should be noted that TMF showed an improving, albeit nonsignificant, trend compared to the control group, which may be due to the small sample size in the ≤30 years subgroup. Nonetheless, previous RCTs evaluating TMF at 48 and 96 weeks did not show evidence of reduced efficacy in younger populations.[7,8] On the other hand, the observed lack of difference in HBV DNA levels in the HBeAg-positive subgroup was expected, as antiviral therapy presents challenges in HBeAg-positive populations. This does not necessarily suggest that TMF antiviral therapy is not useful in HBeAg-positive subgroups. Notably, long-term follow-up in previous RCTs found that the HBeAg-positive subgroup achieved HBV DNA <20 IU/mL in 50.2% of patients after one year, increasing to 70.8% after two years, showing significant progressive improvement.[7,8] Thus, continued treatment would likely bring additional benefits. Furthermore, TMF significantly reduced viral load in the HBeAg-positive subgroup compared with the blank control, particularly evident in the differences between HBV DNA levels of 20–2,000 IU/mL and >2,000 IU/mL. The baseline viral loads were relatively high, so it is difficult for these to be decreased below 20 IU/mL within 48 weeks. The evaluation in the HBeAg-positive population should be further addressed with a longer follow-up duration.

This study has limitations that should be acknowledged. The study did not employ a placebo-controlled, double-blind design, and the open-label design cannot avoid potential bias from subjective factors. Additionally, the analysis of treatment compliance was not performed in this study. The limited number of HBeAg-positive patients is also a limitation. Further investigation of antiviral efficacy in this subgroup is warranted in future studies.

Conclusions

TMF treatment shows significant efficacy in chronic HBV-infected patients with normal ALT levels, along with favorable safety profiles for bone, renal function, and blood lipids. The PROMOTE study is ongoing, and further reports at 96 and 144 weeks are anticipated to provide additional insights.

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Conflict of interest

ZH, FN and YH are employees of Jiangsu Hansoh Pharmaceutical Group Co., Ltd. The authors have no conflict of interests related to this publication.

Author contributions

Study concept and design (QX, LQ, FN, YH, ZH), acquisition of data (QX, LQ, HG, YShen, LT, PH, FQ, XW, YQ, SZ, JLv, YShi, JLi, YJ, FN, YH, ZH), analysis and interpretation of data (QX, LQ, FN, YH, ZH), drafting of the manuscript (HG, YShen, LT), critical revision of the manuscript for important intellectual content (QX, LQ, HG, YShen, LT, PH, FQ, XW, YQ, SZ, JLv, YShi, JLi, YJ, FN, YH, ZH), administrative, technical, or material support (QX, LQ, HG, YShen, LT, FN, YH, ZH), and study supervision (QX, LQ, HG, YShen, LT, PH, FQ, XW, YQ, SZ, JLv, YShi, JLi, YJ, FN, YH, ZH). All authors have made significant contributions to this study and have approved the final manuscript.

Ethical statement

This trial adheres to the 2024 Declaration of Helsinki, abides by the 2018 Declaration of Istanbul, and follows Good Clinical Practice guidelines. Ethical approval was obtained from the Ethics Committee of Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine (Approval No. 2021 Clinical Ethics Review [382]), as well as from the institutional review boards of each participating center. Written informed consent was obtained from all participants. The preparation of the manuscript adheres to the CONSORT reporting guidelines. This study has been registered with ClinicalTrials.gov (Registration No. NCT05797714).

Data sharing statement

The data that support the findings of this study are available from the corresponding author (QX) upon reasonable request.

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