Clinical Infectious Diseases

MAJOR ARTICLE

Pradefovir Treatment in Patients With Chronic Hepatitis B: Week 24 Results From a Multicenter, Double-Blind, Randomized, Noninferiority, Phase 2 Trial

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Background. Pradefovir is a liver-targeted prodrug of adefovir, a nucleoside/nucleotide analogue with antiviral activity against hepatitis B virus (HBV) DNA polymerase. This phase 2 study compared the efficacy and safety of oral pradefovir (30, 45, 60, or 75 mg) versus tenofovir disoproxil fumarate (TDF; 300 mg) and aimed to identify the most appropriate dose of pradefovir for the forthcoming phase 3 study.

Methods. Treatment-naive and experienced (not on treatment >6 months) patients with chronic hepatitis B were eligible.

Results. A total of 240 participants were randomized and treated in the study (48 per group). Approximately 80% were hepatitis B e antigen (HBeAg) positive, and 10% had liver cirrhosis. The reductions from baseline in HBV DNA levels achieved at week 24 were 5.40, 5.34, 5.33, and 5.40 $\log_{10}$ IU/mL, with pradefovir doses of 30-, 45-, 60-, and 75-mg, respectively, compared with 5.12 $\log_{10}$ IU/mL with TDF. However, HBeAg loss was attained by more participants who received 45-, 60-, or 75-mg pradefovir than by those receiving TDF (12%, 6%, and 9% vs 3%). The TDF group exhibited a more significant increase in serum creatinine than the pradefovir 30- and 45-mg groups, and serum phosphate levels were comparable among all groups. Most adverse events (AEs) were mild (grade 1). No treatment-related severe AEs were reported. Overall, AEs and laboratory abnormalities were comparable to those in the TDF group.

Conclusions. Pradefovir and TDF exhibited comparable reductions in HBV DNA levels. All treatments were safe and well tolerated.

Keywords: pradefovir, tenofovir disoproxil fumarate, hepatitis B, efficacy, safety,

Hepatitis B virus (HBV) infection is a global health problem, but its prevalence shows considerable geographic variability. According to the World Health Organization, nearly 257 million people are chronically infected with HBV, and 68% of cases occur in Africa and the Western Pacific [1]. Worldwide, approximately 887 000 people die of HBV infection–related diseases annually [2]. The prevalence of HBV surface antigen (HBsAg) is 2% (39 million) in Southeast Asia and 6.0% (1.15 billion) in the Western Pacific. In China, the current prevalence of HBsAg is estimated to be 5%–6%, representing approximately 70 million patients, including 20–30 million with chronic hepatitis B (CHB). Therefore, liver disease caused by CHB represents a major burden in China [3–5].

Received 17 May 2021; editorial decision 28 August 2021; published online 6 September 2021.

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Clinical Infectious Diseases® 2022;74(11):1925–32

https://doi.org/10.1093/cid/ciab763

Antiviral drugs can effectively inhibit HBV replication, reduce liver inflammation and cirrhosis, and delay the occurrence of liver failure, decompensated cirrhosis, liver cancer, and other complications [6–8]. Currently, the antiviral drugs primarily used in clinical practice are nucleoside/nucleotide analogues (NAs) and interferon [2, 9]. Lamivudine was the first approved oral therapy for HBV infection, but its efficacy is limited by the high incidence of drug resistance [10]. Adefovir dipivoxil exhibits activity against wild-type and lamivudine-resistant HBV [11], but dose-limiting renal and bone toxic effects, as well as the risk of resistance, limit its clinical use [12, 13]. Entecavir, tenofovir disoproxil fumarate (TDF), and tenofovir alafenamide fumarate (TAF) are currently recommended as first-line treatments for CHB.

Pradefovir is a liver-targeted prodrug of oral bioequivalent adefovir, which is converted to drug-active 9-(2-phosphonomethoxyethyl)adenine (PMEA; adefovir) via catalysis by liver CYP3A4 enzyme [14, 15]. Adefovir dipivoxil is metabolized in vivo, with a 1:1 kidney to liver ratio of PMEA levels; thus, the drug has a high risk of nephrotoxicity. However, pradefovir mesylate targets the liver and leads to a kidney-to-liver PMEA ratio of 1:20 after activation, which greatly reduces its nephrotoxicity. In HBV-infected transgenic mouse models, this drug significantly inhibits HBV replication. In addition, pradefovir has shown good antiviral activity and safety in phase 1 clinical trials, both in China and elsewhere [16].

The results of the phase 2 clinical trial of pradefovir are presented herein. For this clinical trial, a randomized, double-blind, noninferiority, positive drug parallel contrast research design was applied, with TDF as the control drug for evaluating the efficacy and safety of pradefovir.

METHODS

Study Design

This randomized, double-blind, noninferiority trial was conducted across 23 centers in China and is registered with ClinicalTrials.gov (NCT00230503) and ChinaDrugTrials.org.cn (CTR20180426). The primary objectives were to evaluate the safety and tolerability of multiple oral doses of pradefovir (30, 45, 60, or 75 mg), compared with 300-mg TDF given once daily for 24 weeks and to identify the most appropriate pradefovir dose for the forthcoming phase 3 study. The study protocol was approved by the institutional review board or independent ethics committee at each participating site, and the study was designed and conducted according to the principles of the Declaration of Helsinki and Good Clinical Practice. Written informed consent was obtained from all participants before enrollment.

Participants

Patients (male or female) were eligible if they met the following inclusion criteria: age 18–65 years; CHB with plasma HBV DNA level >20 000 IU/mL if hepatitis B e antigen (HBeAg) positive or >2000 IU/mL if HBeAg negative; and high serum alanine aminotransferase (ALT) level (1.2–10 times the upper limit of normal [ULN]), except in patients with confirmed liver inflammation or hepatic fibrosis of grade 2 or above. Treatment-naive and treatment-experienced patients (previous treatment with interferon or NAs if stopped ≥6 months before screening), as well as those with advanced fibrosis (stage F3) or compensated cirrhosis (stage F4) (≤10%), were included.

The following exclusion criteria were applied: allergy to NAs, liver failure, decompensated cirrhosis, hepatocellular carcinoma, or any severe liver disorder other than HBV infection; total bilirubin >2 times the ULN; chronic kidney disorder or estimated glomerular filtration rate ≤70 mL/min; or positive results for human immunodeficiency virus or hepatitis C virus. Patients with adefovir or TDF resistance also were excluded.

Procedures

Patients were screened 14 days before enrollment, and eligible patients were stratified according to HBeAg status (180 were HBeAg positive; 60, HBeAg negative) and randomly assigned (1:1:1:1:1) to receive pradefovir (30, 45, 60, or 75 mg) or TDF (300 mg) once daily for 24 weeks, after which all patients switched to or continued TDF for an additional 4 weeks of observation. After that, patients either continued the treatment or started alternative therapies. Participants returned to the clinic every 4 weeks for laboratory assessments of serum biochemical and hematological profiles, liver function, renal function (eg, serum creatinine level, glomerular filtration rate, and proteinuria), and HBV DNA levels.

HBV DNA testing was performed using the Roche COBAS AmpliPrep/Taqman assay (Roche Molecular Systems), with lower and upper limits of quantitation of 20 and 10^8 IU/mL, respectively. Hepatitis B serological markers (HBsAg, HBeAg, etc) were assessed every 12 weeks in a central laboratory (Teddy Clinical Research Laboratory). Resistance surveillance and genotypic analysis of HBV polymerases were conducted for all participants at baseline and for participants whose HBV DNA viral load decreased by <1 $\log_{10}$ IU/L from baseline to week 12. Safety and tolerability assessments were conducted throughout the study, and all adverse events (AEs), treatment discontinuations, and patient deaths were recorded.

End Points

The primary efficacy end point was defined as the reduction in serum HBV DNA level from baseline to after 24 weeks of treatment. Secondary end points included the proportions of participants with undetectable HBV DNA (<29 IU/mL), with ALT normalization, and with HBeAg loss or seroconversion at week 24.

Safety was assessed at every study visit by monitoring AEs, vital signs, physical examination findings, clinical laboratory

test results, 12-lead electrocardiography, and upper abdominal ultrasonographic scans from the start of treatment up to 4 weeks after the last dose of study drug. AEs were coded using the Medical Dictionary for Regulatory Activities, version 17.1 (MedDRA MSSO) and graded using National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.03. The attribution of causality for any AE to the study drug was at the discretion of the investigator according to a national adverse drug reaction vigilance procedure. An adverse drug reaction is defined as any AE that is definitely, probably, or possibly caused by the use of the study drug, as assessed by the investigator.

Statistical Analysis

The planned study enrollment was 180 HBeAg-positive and 60 HBeAg-negative patients with CHB, for a total of 240 randomized patients. The proportion with compensated liver cirrhosis was intended to be <20%, and the proportion of treatment-experienced patients, <35%. Serum HBV DNA levels were analyzed after logarithmic transformation. For the primary efficacy analysis, HBV DNA levels <20 IU/mL were considered to be 20 IU/mL, and the change from baseline to week 24 in HBV DNA ($\log_{10}$ IU/mL) was analyzed using the Bonferroni method for multiple comparisons, with significance set at $P < .05/10 = .005$. The proportions of participants with undetectable HBV DNA within patient subsets and the potential effects of HBV DNA e antigen presentation (HBeAg positive or negative) were analyzed using Pearson χ^2 or Fisher exact tests. Safety results were descriptively summarized and compared, also using Pearson χ^2 or Fisher exact tests. Differences were considered statistically significant at $P \leq .05$ (2 sided). All statistical summaries and analyses were performed using the SAS software package, version 9.4 (SAS Institute).

RESULTS

Study Population

From 31 May 2018 through 9 January 2019, a total of 330 patients were screened. Of these, 90 patients were excluded for not meeting the eligibility criteria, leaving 240 participants for randomization (48 per group). All 240 randomized participants received ≥1 dose of the assigned drugs. A total of 13 participants did not complete the study. One patient withdrew from the pradefovir 45-mg group at week 8 owing to a serious AE (SAE) that was determined to be unrelated to the study drug (hepatocellular carcinoma). Three patients did not complete the treatment owing to AEs determined to be likely related to the study drugs: 1 patient in the pradefovir 75-mg group who withdrew at week 20 because of gastritis, 1 in the pradefovir 30-mg group who experienced proteinuria and withdrew at week 16, and 1 in the TDF group who experienced increased creatinine and withdrew at week 14. Another 6 patients withdrew from the study for unknown reasons. Three participants were lost to follow-up. No participants discontinued the study due to lack of efficacy (Figure 1).

The demographic and other baseline characteristics of the randomized participants were similar across the 5 groups (Table 1). The participants included 183 men and 57 women and had a mean age of 34 years (range, 18–65 years). The proportion of HBeAg-positive participants was 72% (172 of 240). Fifty-four participants had received nucleoside/nucleotide analogues treatments previously, including adefovir dipivoxil (5%), entecavir (14%), telbivudine (10%), lamivudine (3%), and TDF (4%), and 13 (2%) had a history of interferon use. At the time of screening, the mean liver stiffness measurement was 10.68 kPa, and 21 (9%) participants had liver cirrhosis. The mean baseline ALT concentration was 156.5 IU/L (range, 18–476 IU/L). However, the pradefovir 30-mg group had a significantly higher mean serum HBV DNA level (7.54 $\log_{10}$ IU/mL) and a higher proportion of patients with HBV DNA levels ≥8 $\log_{10}$ IU/mL than the other 4 groups.

Virologic and Biochemical Responses

At week 24, all 5 treatment groups exhibited significant reductions in serum HBV DNA levels from baseline (Figure 2A). The reductions from baseline were 5.40, 5.34, 5.33, and 5.40 $\log_{10}$ IU/mL for the pradefovir 30-, 45-, 60-, and 75-mg groups, respectively, compared with 5.12 $\log_{10}$ IU/mL for the TDF group (Table 2). The proportions of participants with HBV DNA levels <29 IU/mL were 27%, 54%, 48%, and 58%, respectively, for the 4 pradefovir groups, compared with 42% for TDF (Figure 2B). A dose–response relationship was demonstrated among the pradefovir treatment groups. HBeAg loss rates did not differ significantly among the pradefovir 30-, 45-, 60-, and 75-mg groups (3%, 12%, 6%, 9%, respectively) and the TDF group (3%; Table 2). The HBeAg seroconversion rates were 0%, 10%, 0%, and 4%. respectively, for the 4 pradefovir groups, compared with 3% for the TDF group. No statistically significant differences were identified between the groups. No HBsAg loss or HBsAg seroconversion was observed throughout the study.

At week 24, the mean reductions in serum ALT were 141.8, 119.1, 107.5, and 83.2 IU/L for the pradefovir 30-, 45-, 60-, and 75-mg groups, respectively, compared with 145.5 IU/L for the TDF group. The proportions of participants who achieved ALT normalization were 83%, 68%, 65%, and 51%, respectively, for the 4 pradefovir groups, compared with 69% for the TDF group (Figure 2C); no statistically significant differences between treatment groups were observed.

Safety

The rates of AEs in the pradefovir 30-, 45-, 60-, and 75-mg groups and the TDF group were 96%, 90%, 90%, 96%, and 98%, respectively. Most AEs were classified as grade 1 or 2 (Table 3). Fourteen SAEs were reported, and none were judged to be related to the study drugs. The rates of SAEs that occurred in the

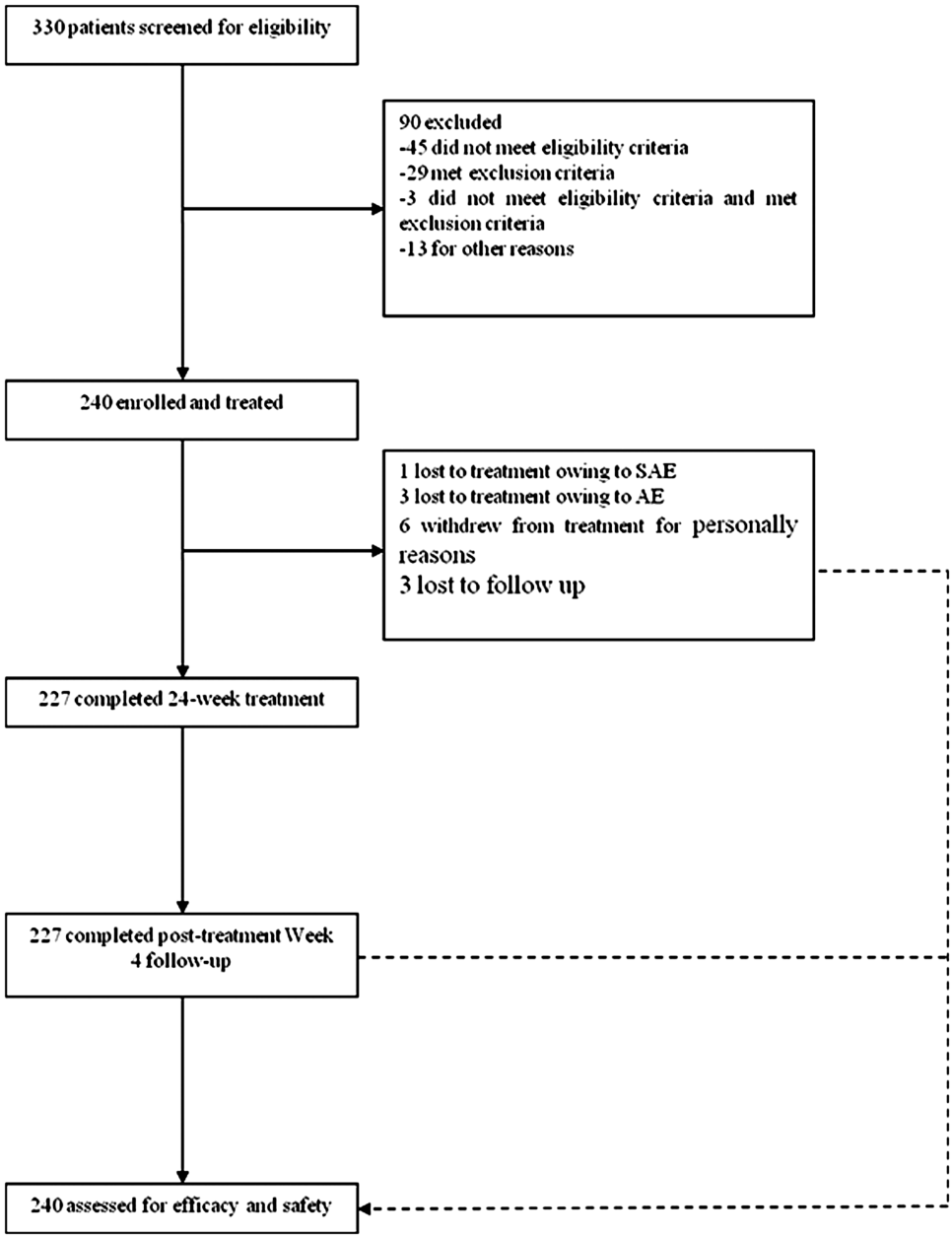

Figure 1. Study flow chart. Abbreviations: AE, adverse event; SAE, serious AE.

pradefovir 30-, 45-, 60-, and 75-mg groups and the TDF 300-mg group were 6%, 6%, 8%, 4%, and 4%, respectively. Three patients were discontinued from the study owing to drug-related grade 3 AEs. The AEs resolved thereafter. No grade 4 AEs or deaths were reported. Overall, the treatments were well tolerated, and the safety profiles were similar among the groups, with no relationships between dose and AEs observed.

The most frequently reported AEs were upper respiratory infection (15%), nausea (10%), abnormal liver function (8%), and malaise (6%) in the TDF group and upper respiratory infection (13%), abdominal distention (5%), and abnormal liver function (11%) in the pradefovir groups (Table 3). The most common laboratory abnormalities (>5%) in the pradefovir groups were decreased cholinesterase (56%), increased creatine kinase MB (30%), hypophosphatemia (10%), increased ALT (10%), increased bilirubin (8%), increased γ-glutamyl transferase (7%), neutropenia (7%), increased aspartate aminotransferase (5%), and increased transaminase (5%).

ALT levels increased to >10 times the ULN were observed in 13 participants, including 3 in the TDF group and 10 in pradefovir groups (3 each treated with 30, 60, or 75 mg and 1 treated with 45 mg). No relationship between dose and ALT increased was observed. In all cases, the ALT level improved to <2 times the ULN. By week 12, normalization was achieved

Table 1. Demographics and Baseline Characteristics of Study Participants by Treatment Group

Characteristic	Participants, No. (%)[a]					
	TDF Group (300 mg) (n = 48)	Pradefovir Groups				
		30 mg (n = 48)	45 mg (n = 48)	60 mg (n = 48)	75 mg (n = 48)	*P* Value
Sex						
Male	38 (79)	38 (79)	34 (71)	37 (77)	36 (75)	.86
Female	10 (21%)	10 (21)	14 (29)	11 (22)	12 (25)	
Age, mean (SD) y	35 (11)	35 (10)	35 (9)	37 (9)	36 (10)	.53
HBV DNA level, mean (SD), $\log_{10}$ IU/mL	7.00 (1.50)	7.54 (1.35)	7.08 (1.49)	6.97 (1.48)	6.81 (1.63)	.17
ALT concentration, mean (SD), IU/L	185 (179)	177 (186)	162 (136)	152 (119)	139 (128)	.35
HBeAg						
Negative	13 (27)	13 (27)	12 (25)	15 (31)	14 (29)	.65
Positive	35 (73)	35 (73)	36 (75)	33 (69)	34 (71)	
Previous NA use	11 (23)	10 (21)	12 (25)	10 (21)	11 (23)	.99
Previous interferon use	2 (4)	2 (4)	2 (4)	2 (4)	5 (10)	.63
Compensated liver cirrhosis						
No	5 (10)	3 (6)	4 (8)	5 (10)	4 (8)	.98
Yes	43 (90)	45 (94)	44 (92)	43 (90)	44 (92)	
LSM, mean (SD, kPa	9.9 (4.3)	11.3 (7.6)	10.7 (9.7)	10.9 (6.3)	10.6 (6.6)	.83

Abbreviations: ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; LSM, liver stiffness measurement; NA, nucleoside/nucleotide analogue; SD, standard deviation; TDF, tenofovir disoproxil fumarate.
[a]Data represent no. (%) of study participants unless otherwise specified.

in 11 participants (85%), despite continuation of treatment. During the study period, 8 participants experienced an ALT flare (defined as serum ALT >2 times the baseline and >10 times the ULN, with or without associated symptoms). Most of these events occurred early in the treatment period (within the first 1–2 months). They resolved without sequelae and were judged to be associated with the underlying CHB. Among these 8 participants, 2 were in the TDF group and 6 in the pradefovir groups (2 treated with 30 mg, 1 with 45 mg, 2 with 60 mg, and 1 with 75 mg; Table 4).

Drug-related, mild serum creatinine increases were recorded in 3 patients. One in the TDF group discontinued treatment at

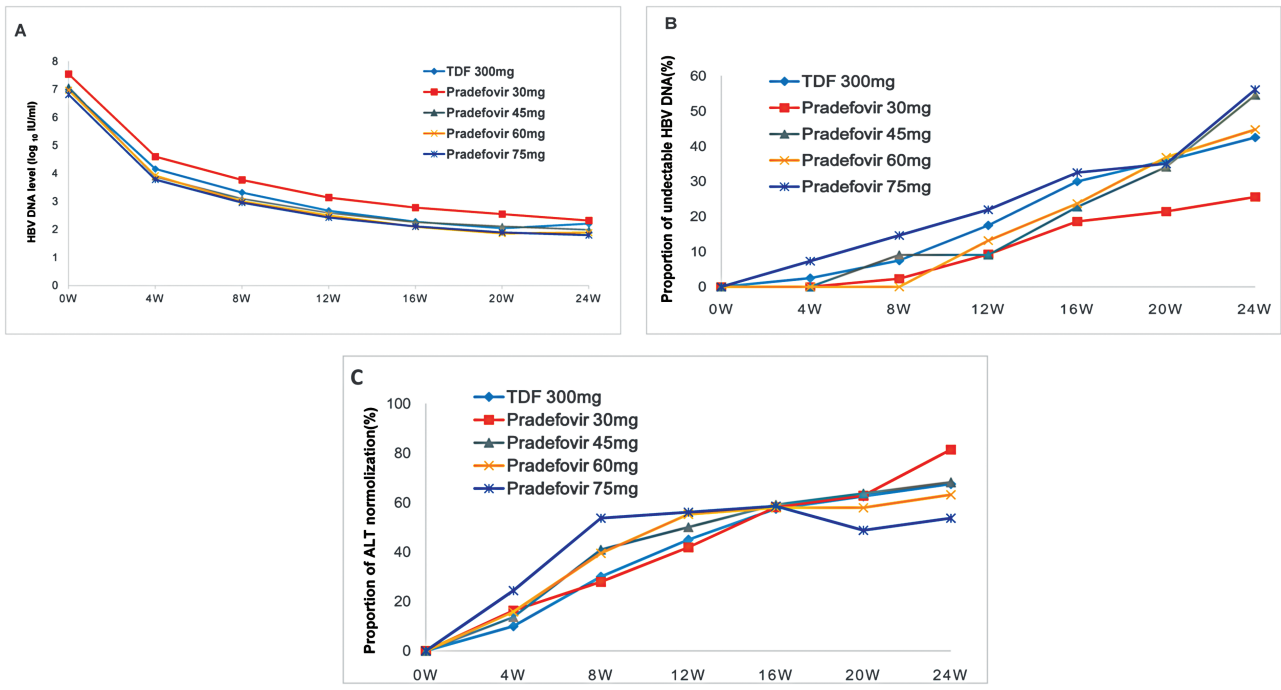

Figure 2. Hepatitis B virus (HBV) DNA levels in study participants during 24-week treatment by group and visit. *A,* HBV DNA levels. *B,* Proportion of participants with undetectable HBV DNA (<29 IU/mL). *C,* Proportion who achieved alanine aminotransferase (ALT) normalization. Abbreviation: TDF, tenofovir disoproxil fumarate.

Table 2. Virologic Response in Study Participants at Week 24 by Treatment Group

Response	TDF (300 mg)	Pradefovir				*P* Value
		30 mg	45 mg	60 mg	75 mg	
Change from baseline in HBV DNA level, mean, $\log_{10}$ IU/mL	5.12	5.40	5.34	5.33	5.40	.85
Proportion of participants, no./total (%)						
With HBV DNA <29 IU/mL	17/40 (43)	11/43 (26)	24/44 (55)	14/38 (37)	23/41 (56)	.02
With HBeAg loss	1/33 (3)	1/35 (3)	4/33 (12)	2/31 (6)	3/33 (9)	.53
With HBeAg seroconversion	1/30 (3)	0/30 (0%)	3/30 (10)	0/26 (0%)	1/24 (4)	.23

Abbreviations: HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; TDF, tenofovir disoproxil fumarate.

week 14. The other 2 cases (1 in the TDF group and 1 in the pradefovir 75-mg group) resolved without intervention, despite continuation of the treatment. Furthermore, higher mean increases in the serum creatinine level from baseline to 24 weeks were observed in the TDF group than in the pradefovir groups (Figure 3A). Similarly, intermittent serum phosphate fluctuations were observed among the 5 treatment groups (Figure 3B), but no clinical meaningful trends were identified.

Table 3. Adverse Events and Laboratory Abnormalities in Study Participants by Treatment Group

AEs and Abnormalities	Participants, No. (%)				
		Pradefovir			
	TDF 300 mg (n = 48)	30 mg (n = 48)	45 mg (n = 48)	60 mg (n = 48)	75 mg (n = 48)
Any AE	47 (98)	46 (96)	43 (90)	43 (90)	46 (96)
Death	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
SAE	2 (4)	3 (6)	3 (6)	4 (8)	2 (4)
AE leading to drug discontinuation	1 (2)	1 (2)	0 (0)	0 (0)	1 (2)
Grade 3 AE	4 (8)	4 (8)	5 (10)	4 (8)	7 (15)
AEs occurring in ≥5%					
Upper respiratory tract infection	7 (15)	8 (17)	8 (17)	2 (4)	7 (15)
Urinary tract infection	0 (0)	3 (6)	5 (10)	1 (2)	1 (2)
Nausea	5 (10)	1 (2)	0 (0)	3 (6)	1 (2)
Diarrhea	1 (2)	0 (0)	0 (0)	0 (0)	3 (6)
Abdominal distention	0 (0)	1 (2)	1 (2)	2 (4)	5 (10)
Abnormal liver function	4 (8)	3 (6)	4 (8)	5 (10)	9 (19)
Malaise	3 (6)	0 (0)	1 (2)	1 (2)	0 (0)
Laboratory abnormalities occurring in >5%					
Increased CK-MB	26 (54)	12 (25)	14 (29)	14 (29)	17 (35)
Increased CK	10 (21)	8 (17)	5 (10)	5 (10)	2 (4)
Hypophosphatemia	2 (4)	5 (10)	2 (4)	6 (13)	5 (10)
Increased ALT	3 (6)	4 (8)	0 (0)	5 (10)	6 (13)
Increased bilirubin	5 (10)	1 (2)	0 (0)	4 (8)	5 (10)
Decreased neutrophil count	2 (4)	4 (8)	1 (2)	3 (6)	4 (8)
Increased GGT	1 (2)	2 (4)	3 (6)	2 (4)	9 (19)
Increased transaminase	3 (6)	3 (6)	4 (8)	3 (6)	1 (2)
Increased AST	1 (2)	2 (4)	0 (0)	3 (6)	4 (8)
Leukopenia	1 (2)	3 (6)	1 (2)	2 (4)	3 (6)
Prolonged APTT	1 (2)	2 (4)	0 (0)	4 (8)	0 (0)
Proteinuria	4 (8)	3 (6)	4 (8)	2 (4)	1 (2)
Hematuria	1 (2)	3 (6)	4 (8)	1 (2)	2 (4)
Urine erythrocytes	4 (8)	2 (4)	1 (2)	1 (2)	0 (0)
Amylase increases	2 (4)	3 (6)	0 (0)	1 (2)	2 (4)
Elevated blood uric acid	4 (8)	2 (4)	2 (4)	2 (4)	1 (2)
Total bile acids increase	0 (0)	1 (2)	4 (8)	1 (2)	1 (2)
Increased creatinine	1 (2)	1 (2)	1 (2)	1 (2)	2 (4)

Abbreviations: AE, adverse event; ALT, alanine aminotransferase; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; CK-MB, creatine kinase MB; GGT, γ-glutamyl transpeptidase; SAE, serious AE; TDF, tenofovir disoproxil fumarate.

Table 4. Alanine Aminotransferase (ALT) Concentrations in Study Participants Who Experienced ALT Flare

Treatment Group	Participant number	ALT, IU/L						
		Baseline	wk 4	wk 8	wk 12	wk 16	wk 20	wk 24
TDF (300 mg)	9	245	679	69	23	18	22	18
	102	119	243	555	99	41	33	23
Pradefovir								
30 mg	10	276	842	50	30	31	27	26
	166	120	920	170	79	33	ND	24
45 mg	83	131	494	25	22	28	23	19
60 mg	153	236	609	50	37	52	37	40
	223	221	621	27	26	19	40	46
75 mg	105	255	565	42	68	83	75	29

Abbreviations: ALT, alanine aminotransferase; ND, not done; TDF, tenofovir disoproxil fumarate.

DISCUSSION

In this phase 2 clinical trial, pradefovir showed good efficacy and an acceptable safety profile compared with TDF. These findings indicate that pradefovir is a promising novel NA for the treatment of CHB.

Regarding HBV DNA viral suppression, our results indicate that pradefovir doses of 30–75 mg proved noninferior to TDF at 300 mg. The response rates did not differ significantly among the treatment groups. The rates of HBeAg loss and serum serological conversion appeared to be better in the pradefovir 45-mg group than in the TDF group (12% vs 3% and 10% vs 3%, respectively), but these differences were not statistically significant. The proportions of participants who achieved ALT normalization were similar among the groups, with very close percentages noted for the pradefovir 45-mg (68%) and the TDF 300-mg (69%) groups.

All treatments were safe and well tolerated, and most AEs were mild. Discontinuation of treatment drugs owing to AEs was very rare (<1% overall), and there were no significant differences in the rates of AEs, SAEs, or laboratory abnormalities among the 5 groups. The rate of drug-related AEs in the pradefovir 45-mg group was almost identical to that in the TDF 300-mg group (both 71%).

ALT flares in CHB are common and may be caused by cell-mediated immune system against HBV-infected hepatocytes [17, 18]. It has been reported during entecavir, TDF, and TAF therapy [19, 20]. In this study, the ALT flares accompanied reductions of ≥3 $\log_{10}$ UL/mL in serum HBV DNA level, and serum ALT levels achieved normalization despite continuation of treatment. Therefore, the serum ALT flares may indicate recovery of the host immune response against HBV.

Long-term treatment with tenofovir has been linked with kidney injury, including acute renal failure, proximal tubulopathy, and, in rare instances, Fanconi syndrome [12, 21–25]. In addition, TDF treatment can cause modest declines in glomerular filtration rates, which may result from subclinical tubular injury [26, 27]. In the current study, the change trend for serum creatinine was analyzed. Participants given pradefovir exhibited smaller increases in serum creatinine levels than those given TDF, particularly patients who received lower pradefovir doses (30 or 45 mg). Notably, serum creatinine levels showed minimal increases and remained within the normal ULN range. In addition, no cases of serious renal injury were observed.

The current guidance from the American Association for the Study of Liver Diseases recommends entecavir, TDF, and TAF as first-line treatments for CHB [28]. Dohyeong Lee suggested that serum phosphate measurement is a sensitive method for predicting kidney injury in patients taking NAs; however, the

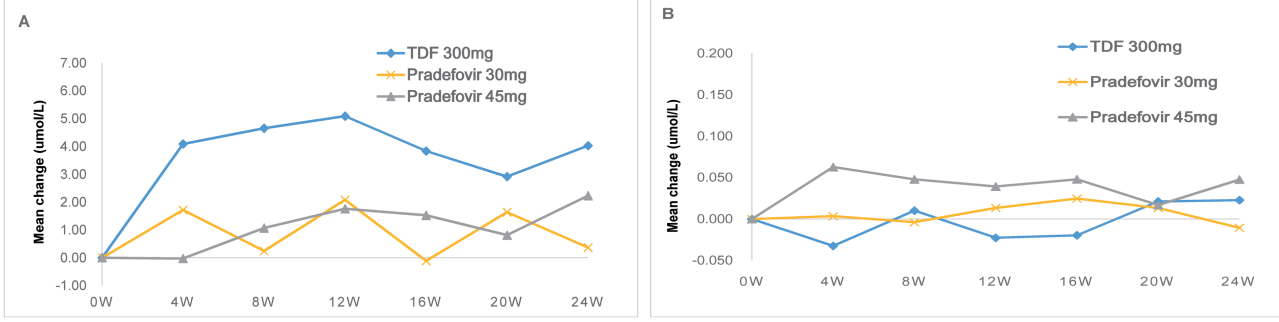

Figure 3. Changes in serum creatinine and phosphate levels in study participants during 24-week treatment by group and visit. *A,* Changes in serum creatinine levels in the tenofovir disoproxil fumarate (TDF) and pradefovir (30- and 45-mg) groups. *B,* Changes in phosphate levels in the TDF and pradefovir (30- and 45-mg) groups.

use of hypophosphatemia as a predictor of kidney injury and the characteristics of patients who experience this condition when taking TDF have yet to be clearly established [29]. In the current study, serum phosphate levels were closely monitored, and similar intermittent serum phosphate fluctuations were observed among the 5 treatment groups (Figure 3B). However, no clinically meaningful trends were identified.

Although the rates of virological response did not differ significantly among the groups, the proportion of patients with an HBV DNA level <29 IU/mL at week 24 was numerically higher in the pradefovir 45-mg group than in the pradefovir 30-mg and TDF groups. The pradefovir 45-mg group also showed numerical advantages in the rates of HBeAg loss and serum serological conversion compared with the pradefovir 30-mg and TDF groups. Although the rates of AEs were similar among all groups, the pradefovir 45-mg group showed the best profile overall. Most importantly, the pradefovir 30-mg and 45-mg groups showed safer trends in serum creatinine changes, suggesting a better renal safety profile. Considering the safety and efficacy profiles of the treatment groups and the risk–benefit balance, once-daily use of pradefovir at 45 mg may be recommended for forthcoming phase 3 studies to further investigate the efficacy and safety of pradefovir for the long-term treatment of patients with CHB.

The possible limitation of this study is that the treatment period was only 24 weeks. However, viral resistance to long-term adefovir therapy is rare, with reported cumulative rates of resistance of 0%, 3%, 11%, 18%, and 28%, respectively, at 1, 2, 3, 4, and 5 years of treatment [30]. No drug-resistant HBV mutant was observed in this study. However, the 24-week duration might not be long enough to conclusively show that pradefovir will lead to a lower incidence of bone and renal events. A randomized, double-blind, multicenter, phase 3 trial is now recruiting participants to compare long-term efficacy and safety of pradefovir versus TDF in patients with CHB in China (NCT04543565). The study will enroll 900 participants to be randomly assigned (2:1) to receive pradefovir (45 mg) or TDF (300 mg) once daily for 144 weeks.

Notes

Financial support. This work was supported by Xi'an Xintong Pharmaceutical Research Co., Ltd. and national major project funding of the 13th Five-Year Plan of China (code 2016ZX09101008-001).

Potential conflicts of interest. The authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

References

1. World Health Organization. Global Hepatitis Report. Geneva, **2017**.
2. The guidelines of prevention and treatment for chronic hepatitis B (2019 version). Zhonghua Gan Zang Bing Za Zhi **2019**; 27:938–61.
3. Ott JJ, Stevens GA, Groeger J, Wiersma ST. Global epidemiology of hepatitis B virus infection: new estimates of age-specific HBsAg seroprevalence and endemicity. Vaccine **2012**; 30:2212–9.
4. Xiao J, Wang F, Wong NK, et al. Global liver disease burdens and research trends: analysis from a Chinese perspective. J Hepatol **2019**; 71:212–21.
5. Wang H, Men P, Xiao Y, et al. Hepatitis B infection in the general population of China: a systematic review and meta-analysis. BMC Infect Dis **2019**; 19:811.
6. Xu X, Zhang C, Shi C, et al. Antiviral therapy effectively improves liver hemodynamics as evidenced by serum biomarker and contrast-enhanced ultrasound examinations in patients with hepatitis B cirrhosis. PeerJ **2018**; 6:e5484.
7. Hong-liang O, Li M. Hepatocyte growth promoting factor combined with antiviral drug in the treatment of chronic hepatitis B and its effect on liver function, viral replication and fibrosis index. Biomed Res **2017**; 28:4.
8. Zhou Y, Hu C, Yuan G, et al. Antiviral and antifibrotic therapies reduce occurrence of hepatocellular carcinoma in patients with chronic hepatitis B and liver fibrosis: a 144-week prospective cohort study [in Chinese]. Nan Fang Yi Ke Da Xue Bao **2019**; 39:633–40.
9. Roade L, Riveiro-Barciela M, Esteban R, Buti M. Long-term efficacy and safety of nucleos(t)ides analogues in patients with chronic hepatitis B. Ther Adv Infect Dis **2021**; 8:2049936120985954.
10. Lai CL, Dienstag J, Schiff E, et al. Prevalence and clinical correlates of YMDD variants during lamivudine therapy for patients with chronic hepatitis B. Clin Infect Dis **2003**; 36:687–96.
11. Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, et al; Adefovir Dipivoxil 438 Study Group. Adefovir dipivoxil for the treatment of hepatitis B e antigen-negative chronic hepatitis B. N Engl J Med **2003**; 348:800–7.
12. Gara N, Zhao X, Collins MT, et al. Renal tubular dysfunction during long-term adefovir or tenofovir therapy in chronic hepatitis B. Aliment Pharmacol Ther **2012**; 35:1317–25.
13. Fung SK, Chae HB, Fontana RJ, et al. Virologic response and resistance to adefovir in patients with chronic hepatitis B. J Hepatol **2006**; 44:283–90.
14. Lin CC, Fang C, Benetton S, Xu GF, Yeh LT. Metabolic activation of pradefovir by CYP3A4 and its potential as an inhibitor or inducer. Antimicrob Agents Chemother **2006**; 50:2926–31.
15. Tillmann HL. Pradefovir, a liver-targeted prodrug of adefovir against HBV infection. Curr Opin Investig Drugs **2007**; 8:682–90.
16. Ding Y, Zhang H, Li X, et al. Safety, pharmacokinetics and pharmacogenetics of a single ascending dose of pradefovir, a novel liver-targeting, anti-hepatitis B virus drug, in healthy Chinese subjects. Hepatol Int **2017**; 11:390–400.
17. Nair S, Perrillo RP. Serum alanine aminotransferase flares during interferon treatment of chronic hepatitis B: is sustained clearance of HBV DNA dependent on levels of pretreatment viremia? Hepatology **2001**; 34:1021–6.
18. Perrillo RP. Acute flares in chronic hepatitis B: the natural and unnatural history of an immunologically mediated liver disease. Gastroenterology **2001**; 120:1009–22.
19. Shindo M, Chayama K, Mochida S, et al. Antiviral activity, dose-response relationship, and safety of entecavir following 24-week oral dosing in nucleoside-naive Japanese adult patients with chronic hepatitis B: a randomized, double-blind, phase II clinical trial. Hepatol Int **2009**; 3:445–52.
20. Chan HL, Fung S, Seto WK, et al; GS-US-320-0110 Investigators. Tenofovir alafenamide versus tenofovir disoproxil fumarate for the treatment of HBeAg-positive chronic hepatitis B virus infection: a randomised, double-blind, phase 3, non-inferiority trial. Lancet Gastroenterol Hepatol **2016**; 1:185–95.
21. Maggi P, Montinaro V, Leone A, et al. Bone and kidney toxicity induced by nucleotide analogues in patients affected by HBV-related chronic hepatitis: a longitudinal study. J Antimicrob Chemother **2015**; 70:1150–4.
22. Cooper RD, Wiebe N, Smith N, Keiser P, Naicker S, Tonelli M. Systematic review and meta-analysis: renal safety of tenofovir disoproxil fumarate in HIV-infected patients. Clin Infect Dis **2010**; 51:496–505.
23. Hall AM, Hendry BM, Nitsch D, Connolly JO. Tenofovir-associated kidney toxicity in HIV-infected patients: a review of the evidence. Am J Kidney Dis **2011**; 57:773–80.
24. Viganò M, Brocchieri A, Spinetti A, et al. Tenofovir-induced Fanconi syndrome in chronic hepatitis B monoinfected patients that reverted after tenofovir withdrawal. J Clin Virol **2014**; 61:600–3.
25. Gracey DM, Snelling P, McKenzie P, Strasser SI. Tenofovir-associated Fanconi syndrome in patients with chronic hepatitis B monoinfection. Antivir Ther **2013**; 18:945–8.
26. Hall AM, Edwards SG, Lapsley M, et al. Subclinical tubular injury in HIV-infected individuals on antiretroviral therapy: a cross-sectional analysis. Am J Kidney Dis **2009**; 54:1034–42.
27. Mallet V, Schwarzinger M, Vallet-Pichard A, et al. Effect of nucleoside and nucleotide analogues on renal function in patients with chronic hepatitis B virus monoinfection. Clin Gastroenterol Hepatol **2015**; 13:1181–8 e1.
28. Terrault NA, Bzowej NH, Chang KM, Hwang JP, Jonas MM, Murad MH; American Association for the Study of Liver Diseases. AASLD guidelines for treatment of chronic hepatitis B. Hepatology **2016**; 63:261–83.
29. Lee D, Yun BC, Seo KI, et al. Risk factors associated with hypophosphatemia in chronic hepatitis B patients treated with tenofovir disoproxil fumarate. Medicine (Baltimore) **2019**; 98:e18351.
30. Ryu SH, Chung YH. Resistance to adefovir in patients with chronic hepatitis B. Korean J Hepatol **2006**; 12:484–92.