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

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Summary

Background Tenofovir alafenamide is a novel prodrug formulated to deliver the active metabolite to target cells more efficiently than tenofovir disoproxil fumarate at a lower dose, thereby reducing systemic exposure. In patients with HIV, tenofovir alafenamide was as efficacious as tenofovir disoproxil fumarate, with reduced bone and renal toxic effects. We compared the efficacy and safety of the two drugs in patients with HBeAg-positive chronic hepatitis B virus (HBV) infection in a non-inferiority study.

Methods We did this ongoing double-blind, non-inferiority study in 161 outpatient centres in 19 countries. Patients with chronic HBV infection who were positive for the hepatitis B e antigen (HBeAg) were randomly assigned (2:1) to receive either 25 mg tenofovir alafenamide or 300 mg tenofovir disoproxil fumarate with matching placebo. Randomisation was done by a computer-generated allocation sequence (block size six) stratified by plasma HBV DNA concentration and previous treatment experience. The primary efficacy endpoint was the proportion of patients with HBV DNA less than 29 IU/mL at week 48 in all patients who were randomly assigned and received at least one dose of study drug using a missing-equals-failed approach. The pre-specified non-inferiority margin was 10%. Key prespecified safety endpoints were bone and renal parameters at week 48. This study is registered with ClinicalTrials.gov, number NCT01940471.

Findings Of the 1473 patients screened from Sept 11, 2013, to Dec 20, 2014, 875 eligible patients were randomly assigned and 873 received treatment (581 with tenofovir alafenamide and 292 with tenofovir disoproxil fumarate). 371 (64%) patients receiving tenofovir alafenamide had HBV DNA less than 29 IU/mL at week 48, which was non-inferior to the 195 (67%) of patients receiving tenofovir disoproxil fumarate who had HBV DNA less than 29 IU/mL (adjusted difference –3·6% [95% CI –9·8 to 2·6]; p=0·25). Patients given tenofovir alafenamide had a significantly smaller decrease in bone mineral density at hip (mean change –0·10% [95% CI –0·29 to 0·09] *vs* –1·72% [–2·02 to –1·41]; adjusted difference 1·62 [1·27 to 1·96]; p<0·0001) and at spine (mean change –0·42% [–0·66 to –0·17] *vs* –2·29% [–2·67 to –1·92]; adjusted difference 1·88 [1·44 to 2·31]; p<0·0001) as well as smaller mean increases in serum creatinine at week 48 (0·01 mg/dL [0·00–0·02] *vs* 0·03 mg/dL [0·02–0·04]; p=0·02). The most common adverse events overall were upper respiratory tract infection (51 [9%] of 581 patients receiving tenofovir alafenamide *vs* 22 [8%] of 292 patients receiving tenofovir disoproxil fumarate), nasopharyngitis (56 [10%] *vs* 16 [5%]), and headache (42 [7%] *vs* 22 [8%]). 22 (4%) patients receiving tenofovir alafenamide and 12 (4%) patients receiving tenofovir disoproxil fumarate experienced serious adverse events, none of which was deemed by the investigator to be related to study treatment. 187 (32%) of 581 patients in the tenofovir alafenamide group and 96 (33%) of 292 patients in the tenofovir disoproxil fumarate group had grade 3 or 4 laboratory abnormalities, the most common of which were elevations in ALT (62 [11%] of 577 patients receiving tenofovir alafenamide and 36 [13%] of 288 patients receiving tenofovir disoproxil fumarate) and AST (20 [3%] of 577 patients receiving tenofovir alafenamide and 19 [7%] of 288 patients receiving tenofovir disoproxil fumarate).

Interpretation In patients with HBeAg-positive HBV infection, tenofovir alafenamide was non-inferior to tenofovir disoproxil fumarate, and had improved bone and renal effects. Longer term follow-up is needed to better understand the clinical impact of these changes.

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Introduction

Nearly 250 million people worldwide are thought to be chronically infected with hepatitis B virus (HBV).[1] Chronic hepatitis B infection causes progressive fibrosis of the liver, which can lead to cirrhosis, liver decompensation, and hepatocellular carcinoma.[2–4] More than 780 000 people die each year as a result of complications of chronic hepatitis B.[5] Clinical guidelines

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Research in context

Evidence before this study

We searched PubMed in April 2013 using the search terms "HBV", "hepatitis B virus", "chronic HBV", "tenofovir disoproxil fumarate", "bone toxicity", and "nephrotoxicity", for clinical trials published from inception to April 30, 2013, restricted to English language publications. Tenofovir disoproxil fumarate effectively suppresses viral replication in patients chronically infected with hepatitis B virus (HBV) with a very low rate of viral resistance, but its long-term use is associated with bone and renal toxic effects in some patients. Tenofovir alafenamide was developed to lower systemic tenofovir exposure without reducing intrahepatic concentrations of the active metabolite. In phase 2 and 3 trials in patients with HIV, tenofovir alafenamide has shown similar antiviral efficacy to tenofovir disoproxil fumarate, with significantly reduced effect on renal function and bone mineral density. In the only trial in patients with HBV to date—a phase 1b dose-ranging study in 51 treatment-naive patients without cirrhosis—tenofovir alafenamide was safe and well-tolerated, and produced declines of HBV DNA similar to tenofovir disoproxil fumarate.

Added value of this study

This phase 3 double-blind non-inferiority trial compared tenofovir alafenamide with tenofovir disoproxil fumarate in patients with HBeAg-positive chronic HBV infection. The results showed that tenofovir alafenamide was non-inferior to tenofovir disoproxil fumarate in antiviral efficacy, and had significantly less effects on the secondary outcomes of bone mineral density and renal function after 48 weeks of treatment. The rates of HBsAg loss and seroconversion at week 48 were low in both treatment groups.

Implications of all the available evidence

Our findings suggest that tenofovir alafenamide appears to be a good alternative to tenofovir disoproxil fumarate in patients with chronic HBV infection. Whether the short-term benefits we observed in this 48-week trial will translate into improvements in bone and renal health in patients receiving long-term treatment remains to be seen.

recommend treatment for all patients with immune active chronic hepatitis B, with the goal of reducing the risk of liver-related complications.[6–8] Although few patients achieve seroclearance of the hepatitis B surface antigen (HBsAg), which is indicative of functional cure, suppression of viral replication through antiviral treatment has been demonstrated to slow—and in some cases reverse—disease progression.[9–11] The initial phase of immune reactivity against HBV is characterised by the presence of hepatitis B e antigen (HBeAg) in the serum and widely fluctuating concentrations of HBV DNA and liver enzymes. About 10–20% of patients will lose HBeAg and have the option of discontinuing antiviral therapy, but most patients require long-term suppressive therapy.[12]

Tenofovir is a nucleotide analogue that, following intracellular metabolism to its active form, tenofovir diphosphate, inhibits reverse transcription of HBV and HIV-1.[13] Tenofovir disoproxil fumarate, an orally bioavailable prodrug of tenofovir, was approved in 2008 to treat patients with HBV infection. Although tenofovir disoproxil fumarate has demonstrated potent antiviral activity in patients with chronic HBV infection with no resistance throughout 8 years of use, its long-term use is tied to renal toxic effects in some patients and is associated with reductions in mineral bone density and increases in markers of bone turnover.[14–16] Tenofovir alafenamide, a novel prodrug of tenofovir, was developed to have greater stability in plasma than tenofovir disoproxil fumarate, thereby enabling more efficient delivery of the active metabolite to target cells at a substantially lower dose.[17,18] When tenofovir alafenamide is given at a dose of 25 mg to patients with HBV or HIV infection, circulating concentrations of tenofovir were about 90% lower than concentrations with the standard 300 mg dose of tenofovir disoproxil fumarate.[19,20] The reduced systemic exposure of tenofovir offers the potential for an improved safety profile compared with tenofovir disoproxil fumarate, a benefit that has been demonstrated in a recent clinical trial in patients with HIV infection.[21]

The aims of this phase 3 trial were to compare the efficacy and safety of 48 weeks of tenofovir alafenamide with that of tenofovir disoproxil fumarate in treatment-naive and treatment-experienced patients with immune-active HBeAg-positive chronic hepatitis B.

Methods

Study design and participants

We did this randomised, double-blind, non-inferiority study in 161 outpatient centres in Australia, Bulgaria, Canada, France, Hong Kong, India, Italy, Japan, New Zealand, Poland, Romania, Russia, Singapore, South Korea, Spain, Taiwan, Turkey, UK, and USA.

We enrolled patients who were aged at least 18 years (with no upper age limit) with HBeAg-positive chronic hepatitis B infection (with HBV DNA concentrations of at least 20 000 IU/mL), alanine aminotransferase (ALT) concentrations of higher than 60 U/L in men or higher than 38 U/L in women and at most ten times the upper limit of normal (ULN), and estimated creatinine clearance of at least 50 mL/min (by the Cockcroft-Gault method). We excluded patients with platelet counts of 50 000 cells per µL or less, haemoglobin of less than 10 g/dL, albumin of less than 3 g/dL, and total bilirubin of more than 2·5 times the ULN. Patients with evidence of decompensation (ie, clinical ascites, encephalopathy, or variceal haemorrhage) and those with hepatocellular carcinoma were also excluded. Patients who had not previously received treatment for HBV

infection and those who had been previously treated or were undergoing treatment for HBV infection were eligible (full eligibility criteria are in the appendix p 4).

Before enrolment and before any study procedures begun, written informed consent was obtained from all patients. The study was approved by the institutional review board or independent ethics committees at all participating sites and was done in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice.

Randomisation and masking

Patients were randomly assigned (2:1) within 45 days of screening to receive tenofovir alafenamide or tenofovir disoproxil fumarate. All patients received placebo tablets matching the alternative treatment (ie, patients assigned to receive tenofovir alafenamide also received a matching tenofovir disoproxil fumarate placebo tablet, and vice versa). Study investigators determined eligibility, obtained a participant number, and received automated treatment assignment via an interactive voice and web response system. The computer-generated allocation sequence (with a block size of six) was created by a third party (Bracket, San Francisco, CA, USA). Each patient received a unique patient number during randomisation. Patients, investigators, and all study personnel, including those assessing outcomes, were masked to treatment assignment throughout the 48 weeks of the double-blind phase. Randomisation was stratified by screening HBV DNA concentrations (≥8 $\log_{10}$ IU/mL *vs* <8 $\log_{10}$ IU/mL) and previous oral antiviral treatment (treatment naive *vs* treatment experienced).

Procedures

Patients received tenofovir alafenamide 25 mg orally once daily or tenofovir disoproxil fumarate 300 mg orally once daily. Study visits occurred every 4 weeks starting at treatment week 4 until treatment week 48. Laboratory assessments included haematological analysis, serum chemistry tests, fasting lipid parameters, and measures of renal function (serum creatinine, estimated glomerular filtration rate, proteinuria by dipstick), as well as quantitative markers of proteinuria (protein-to-creatinine ratio, albumin-to-creatinine ratio, retinol binding protein-to-creatinine ratio, β_2-microglobulin-to-creatinine ratio; Covance Laboratories, Indianapolis, IN, USA). Percentage change in bone mineral density was assessed in all patients by dual energy x-ray absorptiometry scans of the lumbar spine and hip at screening, and at weeks 24 and 48 of treatment (and every 24 weeks thereafter). Biomarkers of bone turnover were also assessed, including C-type collagen sequence, which is associated with bone resorption, and bone-specific alkaline phosphatase, osteocalcin, and procollagen type 1 N-terminal propeptide, which are all associated with bone formation. An optional pharmacokinetics substudy open to all enrolled patients willing to provide informed consent was done at the week 4, 8, and 12 visit.

Outcomes

The primary efficacy endpoint was the proportion of patients with HBV DNA less than 29 IU/mL at week 48 of treatment as determined by PCR (COBAS TaqMan HBV Test for use with the High Pure System; Roche Diagnostics, Indianapolis, IN, USA), which was centrally assessed. The lower limit of quantitation of the assay is 29 IU/mL, and the lower limit of detection is 10 IU/mL. A key prespecified secondary efficacy endpoint was the proportion of patients with HBeAg loss and with HBeAg seroconversion to anti-HBe at week 48. Key prespecified secondary safety endpoints at week 48 included percent change in hip bone mineral density, percent change in spine bone mineral density, and change from baseline in serum creatinine. Other prespecified efficacy endpoints were the proportion of patients with plasma HBV DNA less than 29 IU/mL (target not detected), the proportion of patients with HBsAg seroconversion to anti-HBs at week 48, the change from baseline in fibrosis as assessed by FibroTest (BioPredictive, Paris, France) at week 48, the incidence of drug-resistant mutations in patients who had HBV DNA of 69 IU/mL or higher at week 48, and the proportion of patients with ALT normalisation at week 48. A patient was determined to have ALT normalisation if he or she had ALT greater than the ULN (43 U/L for men <68 years, 35 U/L for men >68 years, 34 U/L for women <68 years, and 32 U/L for women >68 years, by central laboratory normal range, or 30 U/L for men and 19 U/L for women by the American Association for the Study of Liver Diseases [AASLD] normal range) at baseline but within normal range at a post-baseline visit. The entry criteria for ALT (>60 U/L for men and >38 U/L for women) was based on the requirement for ALT values to be at least two times the ULN by AASLD criteria. Adverse events and graded laboratory abnormalities were also assessed.

Statistical analysis

Sample sizes of 576 for the tenofovir alafenamide group and 288 for the tenofovir disoproxil fumarate group were calculated to have 84% power to establish non-inferiority with a margin of 10% at a one-sided significance level of 0·025. The non-inferiority margin was based on results from a phase 3 trial comparing tenofovir disoproxil fumarate with adefovir dipivoxil in patients with chronic HBV infection.[22] In that trial, 69% of patients with HBeAg-positive HBV receiving tenofovir disoproxil fumarate had HBV DNA less than 29 IU/mL at week 48 versus 9% of patients receiving adefovir dipivoxil. A 10% non-inferiority margin preserves at least 80% of the lower bound of the 95% CI for the difference between tenofovir disoproxil fumarate and adefovir dipivoxil (appendix p 6). This assumes the expected difference in proportion of patients with HBV DNA less than 29 IU/mL is zero and the proportion of patients with HBV DNA less than 29 IU/mL in the tenofovir disoproxil fumarate group is 69% (more information on the non-inferiority margin is given in the appendix p 6). Safety and efficacy were

assessed in the full analysis set, which was defined as all patients who were randomly assigned and received at least one dose of study drug. We also assessed the primary efficacy endpoint in a prespecified per-protocol analysis set, which was defined as all patients in the full analysis set except those who did not have week 48 HBV DNA data for any reason other than discontinuation due to lack of efficacy, those who received ongoing therapy with any of the prohibited medications, and those with adherence rate for active study drug up to the week 48 visit below the 2·5th percentile. For the primary endpoint and the secondary efficacy endpoints involving proportions, missing data were handled using the missing equals failed approach. The baseline stratum-weighted difference in the proportions between the groups and its 95% CI were calculated based on stratum-adjusted Mantel-Haenszel proportions, where stratification factors include baseline HBV DNA concentration (≥8 $\log_{10}$ IU/mL *vs* <8 $\log_{10}$ IU/mL) and oral antiviral treatment status (treatment-naive *vs* treatment-experienced). To control for type I error in the assessment of the primary efficacy endpoint and key secondary safety and efficacy endpoints, the hypothesis testing was done in a sequential order as follows. The primary hypothesis of non-inferiority of tenofovir alafenamide relative to tenofovir disoproxil fumarate was tested first. If non-inferiority was established, multiplicity adjustments were done for the following key secondary safety and efficacy endpoints with a fallback procedure in the sequential order with prespecified weighted two-sided α levels: percent change in hip bone mineral density (weight=0·4, α=0·02), percent change in spine bone mineral density (weight=0·2, α=0·01), serum creatinine (weight=0·4, α=0·02), treatment-emergent proteinuria (weight=0, α=0), HBeAg loss and seroconversion (weight=0, α=0; appendix p 7). During the study an independent data monitoring committee reviewed the safety results on five occasions (about every 6 months). SAS version 9.2 (SAS Institute Inc) was used for all analyses. This study is registered with ClinicalTrials.gov, number NCT01940471.

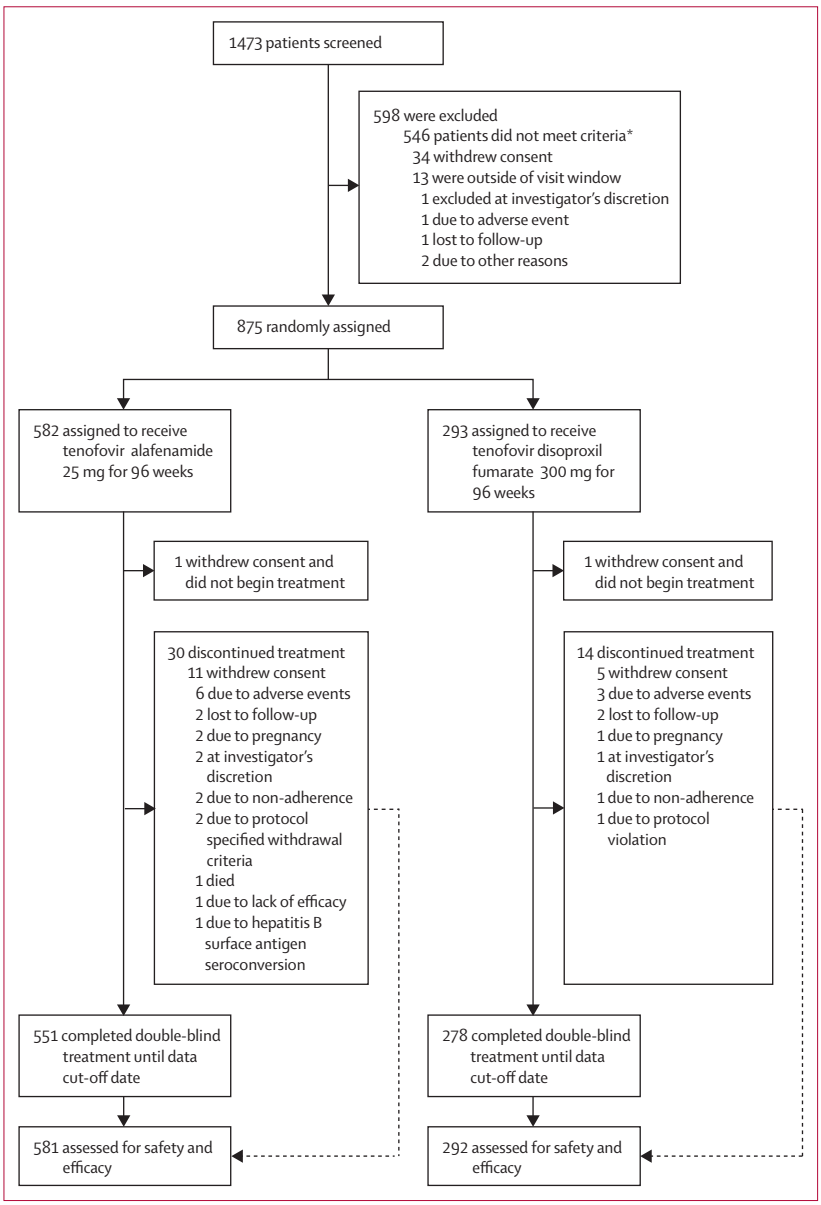

Figure 1: **Trial profile**
*The most common reasons for not meeting screening criteria were hepatitis B e antigen status, and HBV DNA or alanine aminotransferase concentrations.

Role of the funding source

The study funder oversaw trial management, data collection, and statistical analyses. The first draft of the report was prepared by a medical writer employed by the funder. The corresponding author had full access to all data in the study and had final responsibility for the decision to submit for publication.

Results

Of the 1473 patients screened between Sept 11, 2013, and Dec 20, 2014, 875 eligible patients were randomly assigned and 873 received treatment: 581 received tenofovir alafenamide and 292 received tenofovir disoproxil fumarate (figure 1, appendix p 8). The groups were balanced overall in baseline characteristics (table 1). Most patients were Asian (482 [83%] in the tenofovir alafenamide group and 232 [79%] in the tenofovir disoproxil fumarate group). 432 (49%) patients were enrolled in east Asian countries, 157 (18%) in European countries, 137 (16%) in North America, 20 (2%) in Australia, 17 (2%) in New Zealand, and 110 (13%) in India. The most common HBV genotype was C (455 [52%] patients in both groups), followed by genotype D (134 [23%] in the tenofovir alafenamide group and 63 [22%] in the tenofovir disoproxil fumarate group), then genotype B (100 [17%] in the tenofovir alafenamide group and 48 [16%] in the tenofovir disoproxil fumarate group). About a quarter of patients

	Tenofovir alafenamide 25 mg (n=581)	Tenofovir disoproxil fumarate 300 mg (n=292)
Age (years)	38 (11)	38 (12)
Sex		
Female	210 (36%)	103 (35%)
Male	371 (64%)	189 (65%)
Race		
Asian	482 (83%)	232 (79%)
White	96 (17%)	53 (18%)
Other*	3 (1%)	7 (2%)
Body-mass index (kg/m^2)	23·8 (4·14)	24·1 (4·00)
HBV DNA (log_{10} IU/mL)	7·6 (1·34)	7·6 (1·41)
HBV genotype		
A	39 (7%)	25 (9%)
B	100 (17%)	48 (16%)
C	303 (52%)	152 (52%)
D	134 (23%)	63 (22%)
E	2 (<1%)	1 (<1%)
F	3 (1%)	2 (1%)
Unknown	0	1 (<1%)
HBeAg positive†	567 (98%)	288 (99%)
ALT (U/L)	117 (105·1)	125 (128·2)
ALT concentration >ULN by central laboratory criteria‡	537 (92%)	268 (92%)
ALT concentration >ULN by AASLD criteria	572 (98%)	290 (99%)
Cirrhosis		
Yes	41 (7%)	24 (8%)
No	376 (65%)	189 (65%)
Unknown	164 (28%)	79 (27%)
eGFR by CG (mL/min)	113·7 (27·8)	112·5 (29·3)
Serum creatinine (mg/L)	0·81 (0·17)	0·82 (0·16)
Bone mineral density DXA status		
Normal at hip	385/569 (68%)	196/284 (69%)
Normal at spine	324/569 (57%)	170/284 (60%)
Bone mineral density by DXA (g/cm^2)		
Hip	0·96 (0·14)	0·96 (0·14)
Spine	1·06 (0·16)	1·06 (0·16)
FibroTest scores	0·34 (0·23)	0·32 (0·22)

Data are n (%), n/N (%), or mean (SD). HBV=hepatitis B virus. HBeAg=hepatitis B e antigen. ALT=alanine aminotransferase. ULN=upper limit of normal. AASLD=American Association for the Study of Liver Diseases. eGFR by CG=estimated glomerular filtration rate by Cockcroft-Gault. DXA=dual energy x-ray absorptiometry. *Other races include Black or African American, Native Hawaiian or Pacific Islander, and unspecified. †18 patients who were positive for HBeAg at screening were negative for HBeAg at baseline; loss of HBeAg was confirmed in 14 of the 18 patients. ‡The central laboratory (Covance Laboratories) did laboratory analyses in the USA (Indianapolis, IN), Europe (Geneva, Switzerland), and east Asia (Singapore).

Table 1: **Baseline characteristics**

had been previously treated for HBV with one or more oral nucleos(t)ide antiviral drugs (151 [26%] patients in the tenofovir alafenamide group *vs* 77 [26%] patients in the tenofovir disoproxil fumarate group). Of the 228 patients who had previously received treatment with oral nucleos(t)ides, the most common were entecavir (117 [13%]), lamivudine (84 [10%]), and tenofovir disoproxil fumarate (70 [8%]; appendix p 9). Median duration of exposure to masked study drug at the time of the present analysis was 57 weeks (IQR 48–72) in both groups. The change from baseline in HBV DNA by visit is shown in the appendix (p 10).

	Tenofovir alafenamide 25 mg (n=581)	Tenofovir disoproxil fumarate 300 mg (n=292)	Difference in proportions (95% CI)	p value
HBV DNA <29 IU/mL	371 (64%)	195 (67%)	–3·6% (–9·8 to 2·6)	0·25
HBeAg loss*	78/565 (14%)	34/285 (12%)	1·8% (–3·0 to 6·5)	0·47
HBeAg seroconversion*	58/565 (10%)	23/285 (8%)	2·1% (–2·0 to 6·3)	0·32
HBsAg loss†	4/576 (1%)	1/288 (<1%)	0·4% (–1·1 to 1·8)	0·52
HBsAg seroconversion†	3/576 (1%)	0	0·5% (–0·7 to 1·7)	0·22
Normalised ALT by central laboratory normal range‡	384/537 (72%)	179/268 (67%)	4·6% (–2·3 to 11·4)	0·18
Normalised ALT by AASLD normal range§	257/572 (45%)	105/290 (36%)	8·7% (1·8 to 15·6)	0·014

Data are n (%) or n/N (%) unless otherwise stated. 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. *Among patients who were seropositive for HBeAg and negative for, or missing, anti-HBe at baseline. †Among patients who were seropositive for HBsAg and negative for, or missing, anti-HBs at baseline. ‡Among patients with ALT at baseline above the central laboratory normal range. §Among patients with ALT at baseline above the AASLD defined normal range.

Table 2: **Primary and secondary efficacy endpoints**

371 (64%) of 581 patients receiving tenofovir alafenamide had HBV DNA less than 29 IU/mL at week 48, compared with 195 (67%) of 292 patients receiving tenofovir disoproxil fumarate (adjusted difference –3·6% [95% CI –9·8 to 2·6]; p=0·25). Because the lower bound of the two-sided 95% CI of the difference in the rate of response was greater than the prespecified –10% margin, tenofovir alafenamide met the primary endpoint of non-inferiority to tenofovir disoproxil fumarate (table 2). Results in the prespecified per-protocol analysis set were consistent with those of the primary analysis in showing that tenofovir alafenamide was non-inferior to tenofovir disoproxil fumarate in antiviral efficacy (appendix p 12).

Of the 873 patients treated, 307 did not achieve HBV DNA less than 29 IU/mL at week 48. Of these, 271 had treatment failure (HBV DNA ≥29 IU/mL at 48 weeks): 183 (31%) of 581 patients receiving tenofovir alafenamide and 88 (30%) of 292 patients receiving tenofovir disoproxil fumarate. Of the 183 patients receiving tenofovir alafenamide who had HBV DNA of 29 IU/mL or higher at week 48, 41 (22%) had HBV DNA concentrations between 29 IU/mL and less than 69 IU/mL and 142 (78%) had HBV DNA concentrations of 69 IU/mL or higher. Of the 88 patients receiving tenofovir disoproxil fumarate who had HBV DNA of 29 IU/mL or higher at week 48, 19 (22%) had HBV DNA between 29 IU/mL and less than 69 IU/mL, and 69 (78%) had HBV DNA of 69 IU/mL or higher. Most of the patients who had HBV DNA of 29 IU/mL or higher at week 48 were viraemic (≥29 IU/mL) throughout all 48 weeks of treatment.

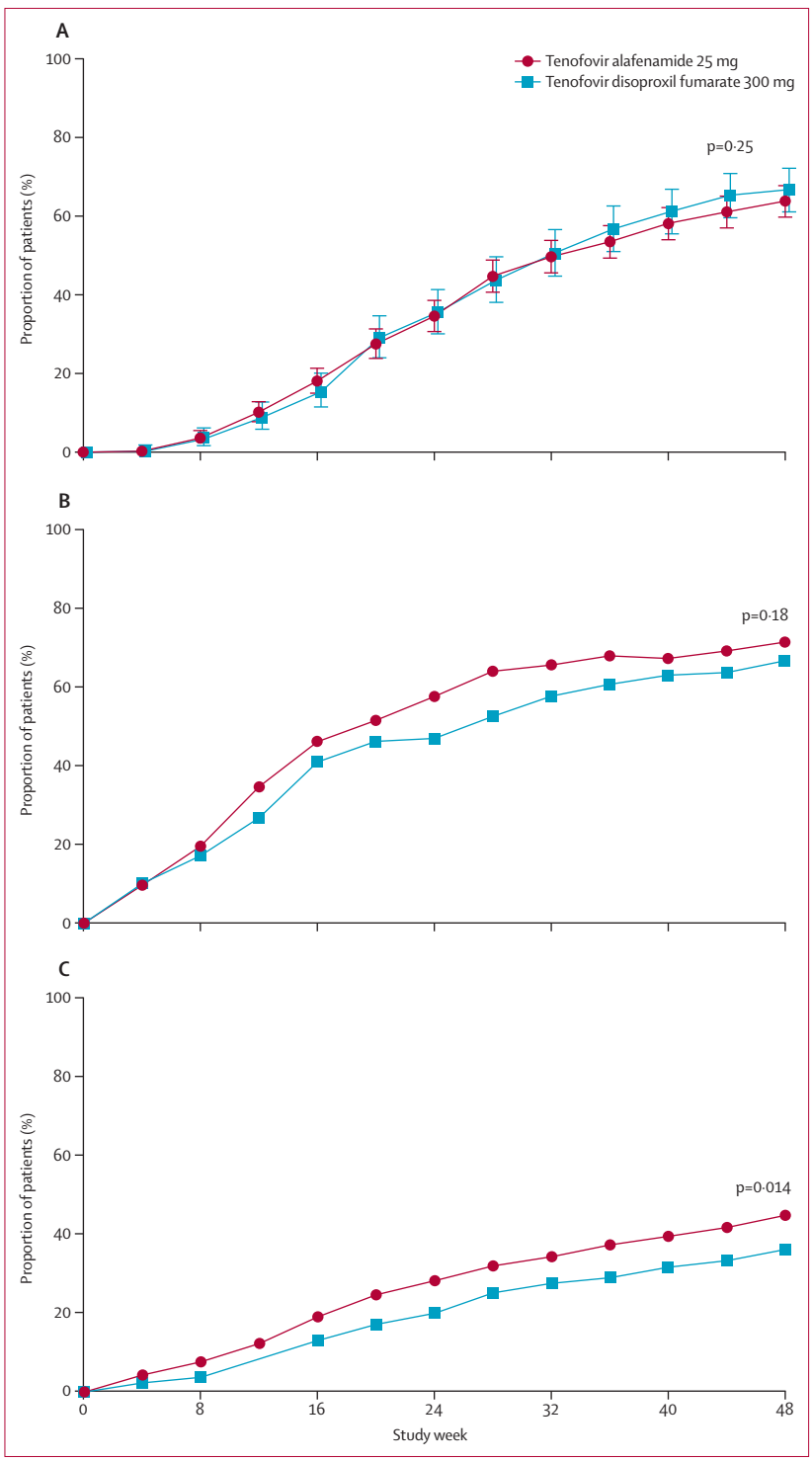

Figure 2: **Viral suppression and alanine aminotransferase (ALT) normalisation by visit week**
(A) Proportion of patients with HBV DNA less than 29 IU/mL by study visit. Bars are 95% CI. (B) Proportion of patients achieving ALT normalisation by central laboratory (Covance Laboratories) criteria (≤43 U/L for men and ≤34 U/L for women <69 years of age; ≤35 U/L for men and ≤32 U/L for women >69 years of age) by study visit. (C) Proportion of patients achieving ALT normalisation by American Association for the Study of Liver Diseases criteria (≤19 U/L for women and ≤30 U/L for men) by study visit.

Nine patients discontinued treatment due to an adverse event (six [1%] of those receiving tenofovir alafenamide and three [1%] of those receiving tenofovir disoproxil fumarate), one patient receiving tenofovir alafenamide had missing data, one patient in the tenofovir alafenamide group discontinued treatment due to lack of efficacy, and 25 patients (19 [3%] receiving tenofovir alafenamide and six [2%] receiving tenofovir disoproxil fumarate) discontinued treatment for other reasons (eg, withdrawal of consent, loss to follow-up, protocol-specified reason, protocol violation, investigator's decision).

Patients in both groups had small mean decreases in FibroTest scores at week 48. The group receiving tenofovir alafenamide had a significantly greater decrease in FibroTest score than the group receiving tenofovir disoproxil fumarate (–0·07 *vs* –0·04, with a least-squares method difference of –0·03, 95% CI –0·04 to –0·01; p=0·007), but both the overall reduction and the difference between the two groups are of unclear clinical significance.

78 (14%) of 565 patients receiving tenofovir alafenamide had loss of HBeAg at week 48 compared with 34 (12%) of 285 patients receiving tenofovir disoproxil fumarate. There was no relation between HBeAg loss and ALT flare (defined as confirmed serum ALT greater than two times the baseline value and greater than ten times the ULN, with or without associated symptoms). Of the seven patients with ALT flare during treatment, only two had HBeAg loss. In both cases, the ALT flare was within the first 8 weeks of treatment, while the HBeAg loss occurred later, at week 24 in one patient and at week 48 in the other. HBeAg seroconversion at week 48 was experienced by 58 (10%) patients receiving tenofovir alafenamide and 23 (8%) patients receiving tenofovir disoproxil fumarate. Rates of HBsAg loss and seroconversion were very low in both groups. Four (1%) of 576 assessable patients receiving tenofovir alafenamide and one (<1%) of 288 assessable patients receiving tenofovir disoproxil fumarate had HBsAg loss at week 48. Three of the patients with HBsAg loss had genotype D HBV (all in the group receiving tenofovir alafenamide) and two had genotype A HBV (one in each group). HBsAg seroconversion at week 48 occurred in three (1%) patients receiving tenofovir alafenamide and no patients receiving tenofovir disoproxil fumarate.

384 (72%) of 537 patients receiving tenofovir alafenamide, as compared with 179 (67%) of 268 patients receiving tenofovir disoproxil fumarate had ALT above the ULN at baseline and had normal ALT at week 48 of treatment by central laboratory criteria; the difference was not significant (4·6%, 95% CI –2·3 to 11·4; p=0·18; figure 2B, C). However, using laboratory criteria recommended by the AASLD (≤30 U/L for men and ≤19 U/L for women), a significantly higher proportion of patients receiving tenofovir alafenamide achieved normalised ALT than those receiving tenofovir disoproxil fumarate: 257 (45%) of 572 *vs* 105 (36%) of 290, a difference of 8·7% (95% CI 1·8 to 15·6; p=0·014).

The mean percent decrease in hip bone mineral density from baseline to week 48 for patients receiving tenofovir alafenamide (–0·10%, 95% CI –0·29 to 0·09) was significantly less than the reduction for patients receiving tenofovir disoproxil fumarate (–1·72%, –2·02 to –1·41); the adjusted difference of 1·62% (1·27 to 1·96) was statistically significant (p<0·0001; figure 3A). Similarly, the mean percent decrease in spine bone mineral density from baseline to week 48 was –0·42% (95% CI –0·66 to –0·17) for patients receiving tenofovir alafenamide, which was significantly less than the mean percent decrease of –2·29% (–2·67 to –1·92) in patients receiving tenofovir disoproxil fumarate; the adjusted difference of 1·88% (1·44 to 2·31) was statistically significant (p<0·0001; figure 3B).

Biomarkers associated with bone resorption (C-type collagen sequence), formation (procollagen type 1 N-terminal propeptide, bone-specific alkaline phosphatase, and osteocalcin), and metabolism (parathyroid hormone) either improved or showed significantly smaller median percent increases at week 48 in patients receiving tenofovir alafenamide than in those receiving tenofovir disoproxil fumarate (appendix pp 13–17). Three patients receiving tenofovir alafenamide and one patient receiving tenofovir disoproxil fumarate experienced a fracture event; these patients all had a history of injury preceding the fracture. None of the fractures was considered to be related to the study drugs by the investigators, and none resulted in discontinuation of study drugs.

Patients in both groups had small mean increases in serum creatinine from baseline to week 48; the increase of 0·01 mg/dL (95% CI 0·00–0·02) in patients receiving tenofovir alafenamide was significantly smaller than the increase of 0·03 mg/dL (0·02–0·04) in patients receiving tenofovir disoproxil fumarate (p=0·02; figure 4). Patients receiving tenofovir alafenamide had a significantly smaller median decrease in estimated glomerular filtration rate than did patients receiving tenofovir disoproxil fumarate (–0·6 mL/min [IQR –8·4 to 7·8] *vs* –5·4 mL/min [–12·6 to 3·0], p<0·0001; appendix p 18). 158 (27%) of 577 patients receiving tenofovir alafenamide and 65 (23%) of 286 patients receiving tenofovir disoproxil fumarate had at least one graded event of proteinuria by dipstick during the study (p=0·21 for the difference between groups). Median percentage changes from baseline to week 48 in the markers of proximal tubular dysfunction urine retinol-binding protein to creatinine ratio and urine β_2-microglobulin to creatinine ratio were significantly smaller in patients receiving tenofovir alafenamide than in those receiving tenofovir disoproxil fumarate (p<0·001 for the differences at week 48; appendix p 19). Mean change in serum phosphate concentrations from baseline was minimal (–0·1 mg/dL [SD 0·53]) in patients receiving tenofovir alafenamide and there was no change in patients receiving tenofovir disoproxil fumarate. No patient in either group experienced a serious renal adverse event, a renal adverse event resulting in discontinuation of study drugs, an event of proximal tubulopathy (including Fanconi syndrome), or an adverse event of renal failure.

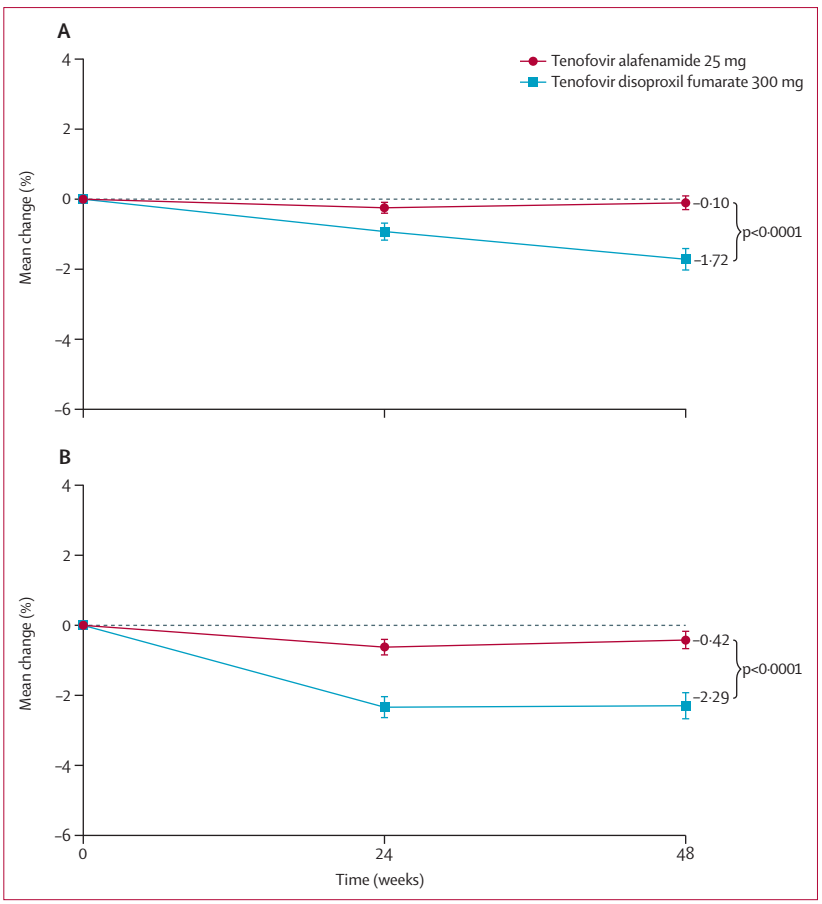

Figure 3: **Changes in bone mineral density**
(A) Mean percentage change in hip bone mineral density at weeks 24 and 48 of treatment. Bars are 95% CI. (B) Mean percentage change in spine bone mineral density at weeks 24 and 48 of treatment. Bars are 95% CI.

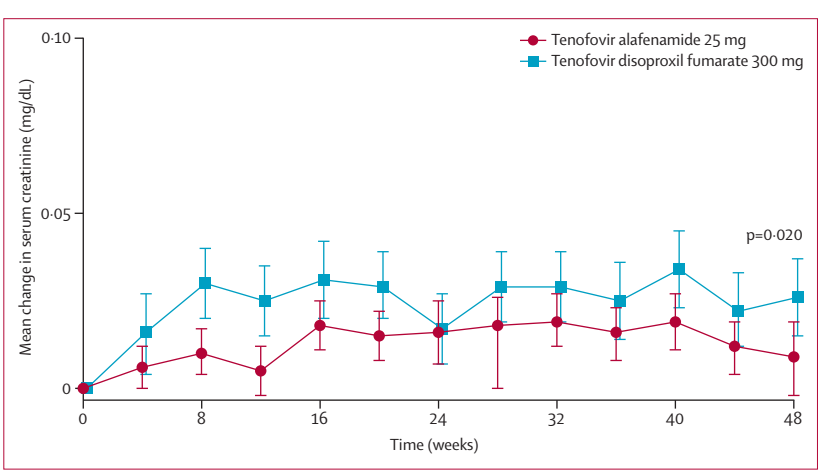

Figure 4: **Change in serum creatinine, by treatment group**
Mean change from baseline in serum creatinine (mg/dL) by study visit. Bars are 95% CI.

	Tenofovir alafenamide 25 mg (n=581)	Tenofovir disoproxil fumarate 300 mg (n=292)
Patients with any adverse event	398 (69%)	192 (66%)
Deaths	1 (<1%)	0
Patients with adverse event leading to study drug discontinuation	6 (1%)	3 (1%)
Patients with grade 3 or 4 adverse events	27 (5%)	11 (4%)
Patients with serious adverse events	22 (4%)	12 (4%)
Adverse events occurring in ≥5% of patients in any treatment group		
Upper respiratory tract infection	51 (9%)	22 (8%)
Nasopharyngitis	56 (10%)	16 (5%)
Headache	42 (7%)	22 (8%)
Cough	37 (6%)	19 (7%)
Fatigue	33 (6%)	14 (5%)
Diarrhoea	27 (5%)	15 (5%)
Upper abdominal pain	19 (3%)	15 (5%)
Patients with grade 3 or 4 laboratory abnormalities*	187 (32%)	96 (33%)
Grade 3 or 4 laboratory abnormalities in ≥1% of patients in any treatment group*		
Absolute neutrophil count <750 cells per μL	7 (1%)	1/286 (<1%)
Alanine aminotransferase >5 × ULN	62 (11%)	36 (13%)
Aspartate aminotransferase >5 × ULN	20 (3%)	19 (7%)
Amylase >2 × ULN	9 (2%)	7/287 (2%)
Creatine kinase ≥10 × ULN	18 (3%)	10 (3%)
Fasting LDL cholesterol >300 mg/dL	23/560 (4%)	0/282
γ-glutamyl transferase >5 × ULN	3 (1%)	3 (1%)
Non-fasting glucose >250 mg/dL	16/574 (3%)	5/287 (2%)
Occult blood	49 (8%)	23/286 (8%)
Urine erythrocytes	42/516 (8%)	26/259 (10%)
Urine glucose	26 (5%)	3/286 (1%)

Data are n (%) or n/N (%). ULN=upper limit of normal. *Laboratory results are based on 577 patients for tenofovir alafenamide 25 mg and 288 patients for tenofovir disoproxil fumarate 300 mg, unless otherwise stated; values are n (%) of patients for any given abnormailty.

Table 3: **Adverse events**

18 (3%) of 581 patients receiving tenofovir alafenamide and nine (3%) of 292 patients receiving tenofovir disoproxil fumarate had HBV DNA less than 29 IU/mL (below the limit of detection) at week 48; HBV DNA concentrations over time during treatment are shown in the appendix.

The rates of viral suppression between treatment groups were similar when assessed by both the proportion of patients with HBV DNA below 29 IU/mL (figure 2A), or by mean $\log_{10}$ change from baseline in HBV DNA concentration (IU/mL; appendix 20). Additionally, differences in the proportion of patients with HBV DNA less than 29 IU/mL at week 48 were not significantly different between the predefined subgroups, including age (≥50 years *vs* <50 years), sex, race (Asian *vs* non-Asian), HBV genotype (A/D *vs* B/C), treatment status (naive *vs* experienced), baseline HBV DNA (≥8 $\log_{10}$ IU/mL *vs* <8 $\log_{10}$ IU/mL), baseline ALT (>ULN *vs* ≤ULN), and baseline FibroTest score (≥0·75 *vs* <0·75 [a value of 0·75–1·00 is consistent with Metavir fibrosis stage F4]; appendix p 13).

34 patients (22 [4%] of 581 patients receiving tenofovir alafenamide and 12 [4%] of 292 patients receiving tenofovir disoproxil fumarate) qualified for resistance testing. Of the 22 patients in the tenofovir alafenamide group, 14 had virological breakthrough (a confirmed HBV DNA concentration ≥69 IU/mL after achieving <69 IU/mL, or >1 $\log_{10}$ IU/mL increase in HBV DNA from nadir) at week 48 and eight were viraemic (≥69 IU/mL) at or after the week 24 visit. All 12 patients in the tenofovir disoproxil fumarate group who underwent resistance testing had virological breakthrough at the time of study discontinuation. Sequence changes were similar for tenofovir alafenamide and tenofovir disoproxil fumarate, and no resistance was detected in either treatment group. 15 (44%) of 34 patients (eight in the tenofovir alafenamide group and seven in the tenofovir disoproxil fumarate group) who qualified for resistance testing had been non-adherent to study treatment based on analysis of plasma tenofovir concentrations.

Seven patients who received tenofovir alafenamide and six patients who received tenofovir disoproxil fumarate participated in the intensive pharmacokinetics substudy. Patients receiving tenofovir alafenamide had an 89% lower mean systemic plasma tenofovir exposure over the 24 h dosing interval (AUC_{tau}) than did those receiving tenofovir disoproxil fumarate. Additionally, 7·6 times higher intracellular concentrations of the active phosphorylated metabolite tenofovir diphosphate in peripheral blood mononuclear cells were recorded for patients receiving tenofovir alafenamide than for those receiving tenofovir disoproxil fumarate.

Both treatments were well tolerated; most adverse events were mild-to-moderate in severity (table 3). Discontinuation of treatment due to adverse events was uncommon: six (1%) patients receiving tenofovir alafenamide and three (1%) patients receiving tenofovir disoproxil fumarate discontinued treatment due to one or more adverse events (appendix p 22). The most common adverse events overall were upper respiratory tract infection (51 [9%] of 581 patients receiving tenofovir alafenamide *vs* 22 [8%] of 292 patients receiving tenofovir disoproxil fumarate), nasopharyngitis (56 [10%] *vs* 16 [5%]), and headache (42 [7%] *vs* 22 [8%]; table 3). 22 (4%) patients receiving tenofovir alafenamide and 12 (4%) patients receiving tenofovir disoproxil fumarate experienced serious adverse events, none of which was deemed by the investigator to be related to study treatment. Serious adverse events that occurred in more than one patient were hepatocellular carcinoma (two patients receiving tenofovir disoproxil fumarate) and dizziness (two patients receiving tenofovir alafenamide; appendix p 23). No patient died during

treatment, but one patient with cirrhosis who received tenofovir alafenamide went into a coma on day 98 (week 14) and stopped treatment. She died 2 days later; the cause of death was cardio-respiratory arrest due to complications of H1N1 influenza.

Grade 3 or 4 laboratory abnormalities were similar in each treatment group (187 [32%] of 581 patients in the tenofovir alafenamide group and 96 [33%] of 292 patients in the tenofovir disoproxil fumarate group; table 3, appendix p 25). The most common grade 3 and 4 laboratory abnormalities were elevations in ALT (62 [11%] of 577 patients receiving tenofovir alafenamide and 36 [13%] of 288 patients receiving tenofovir disoproxil fumarate) and AST (20 [3%] patients receiving tenofovir alafenamide and 19 [7%] patients receiving tenofovir disoproxil fumarate). Three (<1%) patients receiving tenofovir alafenamide and four (1%) patients receiving tenofovir disoproxil fumarate experienced an ALT flare during treatment; these events occurred early in treatment (within the first 1–3 months) and all resolved without sequelae. In the tenofovir alafenamide group, 23 (4%) of 560 patients experienced grade 3 elevations in fasting LDL cholesterol (no patients had a grade 4 elevation); these were mostly isolated events in individuals with a history of dyslipidaemia, an elevated LDL concentration at baseline, or both. No patients receiving tenofovir disoproxil fumarate had a grade 3 or higher elevation in fasting LDL cholesterol.

Discussion

The findings of this large, randomised, phase 3 clinical trial establish that the antiviral efficacy of 48 weeks of treatment with tenofovir alafenamide is non-inferior to that of tenofovir disoproxil fumarate in patients with HBeAg-positive chronic HBV infection. Rates of viral suppression were similar between the two groups, as well as in prespecified groups. More patients receiving tenofovir alafenamide who had baseline ALT above the ULN by AASLD criteria achieved normalisation of ALT by week 48 than those receiving tenofovir disoproxil fumarate. The rates of HBeAg loss and seroconversion were similar with tenofovir alafenamide and tenofovir disoproxil fumarate treatment, and few patients in either treatment group experienced HBsAg loss throughout 48 weeks.

Although the rates of virological response were not significantly different between groups, the proportion of patients with HBV DNA less than 29 IU/mL at week 48 was numerically lower in patients receiving tenofovir alafenamide than in those receiving tenofovir disoproxil fumarate. This difference was not evident in the kinetics of viral decline (figure 2), nor in patients with the lowest (<8 $\log_{10}$ IU/mL) and highest (≥9 $\log_{10}$ IU/mL) concentrations of HBV DNA present at baseline (appendix p 28). The lack of a negative effect on other measures of efficacy, including biochemical and serological responses, suggests that the difference in the rates of viral suppression between the groups was not clinically relevant.

In view of the similarity of rates of viral suppression in the two treatment groups, the higher rate of ALT normalisation among patients receiving tenofovir alafenamide than among those receiving tenofovir disoproxil fumarate was unexpected. The fact that this difference was observable at all study timepoints after week 4 (figure 2B, C) and that the same effect was observed in a similarly designed trial of 425 patients with HBeAg-negative chronic hepatitis B indicate that it is not a chance result.[23] Further study will be required to understand this effect. Whether a more rapid ALT normalisation, which reflects a faster resolution of necroinflammation, will translate to a faster regression of liver fibrosis, will require longer term follow-up to confirm. Finally, the lower rates of HBeAg seroconversion we observed compared with those in previous studies in treatment-naive patients treated with tenofovir disoproxil fumarate[22] or entecavir[24] might be due in part to the lower baseline concentrations of HBV DNA and serum ALT, and a higher proportion of treatment-experienced patients included in the present trial.[15,25]

Both treatments appeared to be safe and well tolerated, with similar rates of adverse events, serious adverse events, and laboratory abnormalities. Only 1% of patients in both groups discontinued treatment due to adverse events. However, important differences between the treatment groups were noted in various bone and renal parameters after 48 weeks of therapy. These differences are relevant given that most patients with chronic HBV infection will require lifelong therapy, and are of particular importance for patients with comorbidities affecting bone and renal function, including the elderly.

Deterioration in hip and spine bone mineral density has been noted in HIV-infected patients receiving long-term treatment with tenofovir disoproxil fumarate,[26–28] and has more recently been described in patients receiving the drug for chronic HBV infection.[29] These reductions, although small (with reported rates of no more than 2% over 2 years), are relevant in light of results from a large 11-year cohort study done in Taiwan, in which patients with chronic HBV were shown to be at greater risk for developing osteoporosis than matched non-infected controls, even after correcting for confounding factors, such as age, sex, and the presence of comorbidities.[30] In our study, patients receiving tenofovir alafenamide had significantly smaller reductions in hip and spine bone mineral density at 48 weeks than did patients receiving tenofovir disoproxil fumarate, and a substantially lower percentage of patients receiving tenofovir alafenamide had bone loss of more than 3% at both hip and spine at week 48 (appendix p 29). Further support for the lesser impact of tenofovir alafenamide on bone is provided by its consistently minimal effect on markers of bone turnover: C-type collagen sequence, a marker of resorption, and the formation markers procollagen type 1 N-terminal propeptide, osteocalcin, and bone-specific alkaline

phosphatase (appendix). Given the similarities in patient demographics and baseline disease characteristics between groups, these differences appear to reflect a meaningful safety differential in bone parameters in favour of treatment with tenofovir alafenamide; however, longer term comparative treatment will be required to confirm the clinical relevance of these findings.

Tenofovir disoproxil fumarate over the long term has been linked to cases of kidney injury, including acute renal failure, proximal tubulopathy, and in rare instances, Fanconi syndrome.[31–36] Treatment with tenofovir disoproxil fumarate can also cause modest declines in glomerular filtration rate, which might be the result of subclinical tubular injury.[37,38] Previous studies in patients treated with tenofovir disoproxil fumarate for chronic hepatitis B for up to 8 years noted clinically relevant renal laboratory abnormalities in 2% of patients, with 3% requiring either treatment discontinuation or interruption due to a renal event.[14] In our study, patients receiving tenofovir alafenamide had a smaller mean increase in serum creatinine as well as less of a median decline in estimated glomerular filtration rate than did those receiving tenofovir disoproxil fumarate at week 48. Although rates of proteinuria by dipstick and the quantitative markers of urine protein to creatinine and urine albumin to creatinine ratios did not differ between treatments, patients receiving tenofovir alafenamide had smaller median percent changes in urine retinol-binding protein to creatinine ratio and β_2-microglobulin to creatinine ratio, both of which are considered more sensitive and specific indicators of renal tubular dysfunction.[39] With 48-week treatment, serious renal injury was rare in both groups; none of the patients treated with tenofovir alafenamide had a confirmed decline in estimated glomerular filtration rate below 50 mL/min compared with five (2%) patients in the tenofovir disoproxil fumarate group, and similar proportions of patients experienced confirmed declines in serum phosphate concentrations below 2 mg/dL.

This study has several limitations. Our eligibility criteria allowed enrolment of patients who might have been in the seroclearance phase of HBV infection, which could account for the patients who experienced HBeAg loss between screening and baseline. Although our sample size was large enough to demonstrate the non-inferiority in efficacy of tenofovir alafenamide to tenofovir disoproxil fumarate and significant differences in prespecified bone (hip and spine bone mineral density percent change) and renal (serum creatinine change) variables, the 48-week duration might not be long enough to conclusively show that patients receiving tenofovir alafenamide can be expected to have a lower incidence of clinically important but uncommon renal and bone events. None of the patients in our study population developed acute renal failure, Fanconi syndrome, or serious bone-related toxic effects during the 48-week double-blind period. Further follow-up is planned to assess whether the short-term improvements we observed in bone and renal variables will translate into a reduced incidence of bone and renal events in the long term. The study protocol was recently amended to extend the open-label follow-up with tenofovir alafenamide to year 8 to investigate its long-term efficacy and safety.

Contributors

HLYC, JFF, GMS, and JGM contributed to the study design. HLYC, SF, WKS, W-LC, C-YC, HJK, AJH, HLAJ, AC, TYOT, RM, EG, Y-SL, SKA, and KA served as investigators for this study. JFF, BM, AG, KMK, LL, and GMS contributed to the data interpretation. All authors contributed to the writing and review of the report.

Declaration of interests

HLYC serves on the advisory boards of Gilead Sciences, AbbVie, Bristol-Myers Squibb, and Roche, is a speaker for Gilead Sciences, AbbVie, Bristol-Myers Squibb, Roche, Novartis, and Echosens, and receives grants from Roche. SF is a consultant, adviser, and speaker for AbbVie, Bristol-Myers Squibb, Gilead Sciences, Janssen, MSD, and Roche, and is a consultant and adviser for GlaxoSmithKline. WKS serves on the advisory boards of Gilead and Bristol-Myers Squibb, and is a speaker for Gilead, AbbVie, Bristol-Myers Squibb, and Novartis. W-LC serves on the advisory boards of Gilead Sciences, Abbvie, Bristol-Myers Squibb, Roche, and PharmaEssentia, and is a speaker for Gilead Sciences, Bristol-Myers Squibb, Merck, and Roche. HLAJ receives grants from AbbVie, Bristol-Myers Squibb, Gilead Sciences, Innogenetics, Janssen, Medimmune, Medtronic, Merck, Novartis, and Roche, and is a consultant for AbbVie, Benitec, Bristol-Myers Squibb, Eiger Bio, Gilead Sciences, GlaxoSmithKline, Innogenetics, ISIS Pharmaceuticals, Janssen, Medimmune, Medtronic, Merck, Novartis, Roche, and Tekmira. EG receives research grants from Gilead Sciences, serves on the advisory boards of AbbVie, Boehringer Ingelheim, Gilead Sciences, Janssen, Novartis, Roche, and Tibotec, and is a speaker for Gilead Sciences, Novartis, Roche, and Tibotec. Y-SL serves on the advisory boards of Bayer, Bristol-Myers Squibb, and Gilead Sciences, receives grants from Bayer, Bristol-Myers Squibb, Gilead Sciences, and Novartis, and is a speaker for Bayer and Gilead Sciences. KA serves on the advisory boards of AbbVie, Achillion, Bristol-Myers Squibb, GlaxoSmithKline, Gilead, Intercept, Janssen, Merck, Novartis, and Roche, is a consultant for AbbVie, Achillion, Bristol-Myers Squibb, GlaxoSmithKline, Gilead, Intercept, Janssen, Merck, Novartis, and Roche, and receives grants from Bristol-Myers Squibb, Gilead, and Roche. JFF, BM, AG, KMK, LL, GMS, and JGM are employees and stockholders of Gilead Sciences. AJH, RM, SKA, AC, C-YC, HJK, and TYOT declare no competing interests.

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