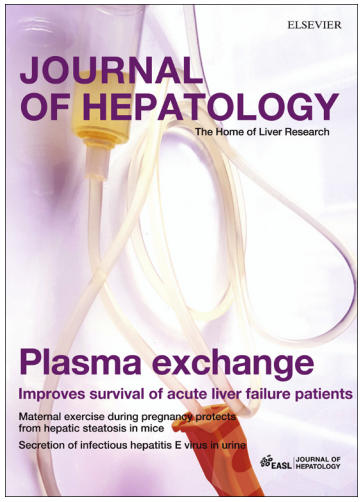
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Tenofovir disoproxil fumarate (TDF) versus TDF/emtricitabine (FTC) in lamivudine-resistant hepatitis B: a 5-year randomized study

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Title page

Tenofovir disoproxil fumarate (TDF) versus TDF/emtricitabine (FTC) in lamivudine-resistant hepatitis B: a 5-year randomized study

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Abbreviations: CHB, chronic hepatitis B; HBV: hepatitis B virus; HCC: hepatocellular carcinoma; LAM, lamivudine; HBeAg, hepatitis B e antigen; LAM-R, lamivudine resistant; ADV: adefovir dipivoxil; ETV, entecavir; ETV-R, entecavir resistant; TDF, tenofovir disoproxil fumarate; FTC, emtricitabine; HBsAg, hepatitis B surface antigen; ALT: alanine aminotransferase; ULN, upper limit of normal; CL_{Cr}, creatinine clearance; pol/RT, polymerase/reverse transcriptase; AE, adverse event; DEXA, dual energy X-ray absorptiometry; BMD, bone mineral density; SD, standard deviation; CI, confidence interval.

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Authors' contributions:

The study was designed and conducted according to the protocol by the sponsor (Gilead Sciences) in collaboration with the principal investigators. The sponsor collected the data, monitored the study conduct, and performed the statistical analyses. All authors were involved in data acquisition and analysis and interpretation of the data, contributed to drafting the manuscript and critical revision of the manuscript with regards to important intellectual content. All authors approved the final version of the manuscript prior to submission

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ABSTRACT

Background and aims:

Long-term treatment with tenofovir disoproxil fumarate (TDF) alone, or in combination with emtricitabine (FTC) is associated with sustained viral suppression in patients with lamivudine resistant (LAM-R) chronic hepatitis B (CHB).

Methods:

LAM-R CHB patients were randomised 1:1 to receive TDF 300 mg or FTC 200 mg and TDF 300 mg once daily in a prospective, double-blind, study. The proportion of patients with plasma hepatitis B virus (HBV) DNA <69 IU/mL (<400 copies/mL) at Week 96 (primary efficacy endpoint) was reported previously. Here we present Week 240 follow-up data.

Results:

Overall, 280 patients were randomised to receive TDF (n=141) or FTC/TDF (n=139), and 85.4% completed 240 weeks of treatment. At Week 240, 83.0% of patients in the TDF arm, and 82.7% of patients in the FTC/TDF treatment arm had HBV DNA <69 IU/mL (P=0.96). Rates of normal alanine aminotransferase (ALT) and normalised ALT were similar between groups (P=0.41, P=0.97, respectively). Hepatitis B e antigen loss and seroconversion at Week 240 were similar between groups, (P=0.41, P=0.67, respectively). Overall, six patients achieved hepatitis B surface antigen (HBsAg) loss and one patient (FTC/TDF arm) had HBsAg seroconversion by Week 240. No TDF resistance was observed up to Week 240. Treatment was generally well tolerated, and renal events were mild and infrequent

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(~8.6%). The mean change in bone mineral density at Week 240 was -0.98 % and -2.54% at the spine and hip, respectively.

Conclusions:

TDF monotherapy was effective and well tolerated in LAM-R CHB patients for up to 240 weeks.

Abstract word count: 250

LAY SUMMARY:

The goal of oral antiviral treatment for chronic hepatitis B (CHB) is to achieve and maintain undetectable HBV DNA levels. Treatment options with enhanced potency, and low risk of resistance development for patients infected with lamivudine resistant (LAM-R) HBV are required. Tenofovir disoproxil fumarate (TDF) monotherapy was effective and well tolerated without TDF resistance development in CHB patients with LAM-R, for up to 240 weeks.

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Introduction

The goal of oral antiviral treatment for chronic hepatitis B (CHB) is to achieve and maintain undetectable hepatitis B virus (HBV) DNA levels, thus slowing or preventing disease progression to cirrhosis and hepatocellular carcinoma (HCC) [1–3]. The first approval of lamivudine (LAM) in 1996 (by the European Medicines Agency) [4] was a major breakthrough in CHB management; however the subsequent rising rate of resistance development has limited the long-term effectiveness of this agent [5]. Lok and colleagues found that 65% of hepatitis B e antigen (HBeAg)-positive patients developed LAM-resistant (LAM-R) mutations after 5 years of treatment [5]. Despite this important limitation, LAM remains a widely prescribed antiviral agent for CHB, particularly in under-resourced countries due to its availability and low cost [6]. Therefore, there continues to be a need for newer treatments for LAM-R CHB [7].

Previous strategies to treat LAM resistance have included switching to adefovir dipivoxil (ADV) monotherapy or adding ADV to LAM or, more recently, switching to entecavir (ETV), but these have not proven to be highly effective [8–10]. Tenney and colleagues found that the cumulative probability of genotypic ETV resistance and resistance associated with breakthrough was 51% and 43%, respectively, in LAM-refractory patients who were switched to ETV monotherapy for up to 5 years [9]. Furthermore, Lee and colleagues demonstrated insufficient antiviral efficacy in LAM-R and entecavir-resistant (ETV-R) patients treated with combination therapy [8]. At 2 years, approximately 50% of patients treated with ADV in combination with a nucleoside analogue achieved complete virological suppression (44.3% with telbivudine plus ADV and 51.4% with ETV plus ADV) [8]. This highlights the need for

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195 therapeutic alternatives with enhanced potency for patients infected with LAM-R
196 HBV.
197
198 We previously reported that tenofovir disoproxil fumarate (TDF) either alone or in
199 combination with emtricitabine (FTC) has the potential to suppress HBV DNA levels
200 in LAM-R CHB patients without resistance development up to Week 96 [11,12]. Here
201 we present the long-term efficacy and safety results, after 5 years of treatment with
202 TDF or FTC/TDF in LAM-R patients.
203
204 **Materials and methods**
205 **Patients**
206 This was a prospective, randomised, double blind, double-dummy, multi-centre 240-
207 week study (ClinicalTrials.gov ID NCT00737568) conducted in compliance with the
208 Declaration of Helsinki and informed consent regulations at each participating site.
209 Written informed consent was obtained from all study participants. The study design,
210 methods and statistical analyses have been previously published in detail [12]. All
211 authors had access to the study data and reviewed and approved the final
212 manuscript. Patients were ≥18 years of age and had confirmed CHB (defined as a
213 positive serum hepatitis B surface antigen [HBsAg] test for at least 6 months) with
214 plasma HBV DNA ≥3 $\log_{10}$ IU/mL. Patients were receiving LAM and were confirmed
215 to have LAM-R, defined as the presence of rtM204V/I ± rtL180M, at screening. In
216 addition, patients had alanine aminotransferase (ALT) <10 x upper limit of normal
217 (ULN), haemoglobin ≥10 g/dL and neutrophils ≥1000 mm^3. Patients were required to
218 have a creatinine clearance (CL_{Cr}) ≥50 mL/min (by the Cockcroft-Gault formula).

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Exclusion criteria included the following: patients with decompensated liver disease or HCC, or those co-infected with hepatitis C, hepatitis D, or HIV.

Study treatment

Patients were randomised 1:1, to receive either TDF 300 mg once daily or the fixed-dose combination of FTC 200 mg and TDF 300 mg once daily. To maintain blinding, each patient received identical placebo matched to the drug in the other treatment group. Randomisation was stratified by HBeAg and ALT level (≥2 times ULN range [43 U/L for males and 34 U/L for females] vs <2 times ULN).

Efficacy analyses

The primary efficacy endpoint was the proportion of patients with plasma HBV DNA <69 IU/mL (<400 copies/mL) at Week 96 [12]; a pre-defined follow-up analysis at Week 240 is reported here. Secondary efficacy endpoints at Week 240 included the proportion of patients with plasma HBV DNA <29 IU/mL (<169 copies/mL), the proportion of patients with normal or normalised (≤ULN) ALT, the proportion of patients with HBeAg loss or seroconversion to anti-HBe (HBeAg-positive patients), and the proportion of patients with HBsAg loss or seroconversion. The incidence of virological breakthrough (confirmed HBV DNA ≥69 IU/mL after achieving <69 IU/mL, or a confirmed ≥1 $\log_{10}$ IU/mL increase from nadir) and the development of drug-resistant mutations (conducted annually in subjects with HBV DNA ≥400 copies/mL) were evaluated by the last time point on treatment. Population sequencing of HBV polymerase/reverse transcriptase (pol/RT), in vitro phenotypic analyses, and viral genotype were determined as previously described [11].

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244 **Safety analyses**

245 Safety analyses up to Week 96 have been described previously [12]. Briefly, adverse
246 events (AEs) and laboratory tests were assessed at regular intervals (every 1–3
247 months) and included pre-specified renal safety parameters of confirmed change
248 from baseline in serum creatinine of ≥0.5 mg/dL, decrease in CL_{Cr} <50 mL/min, and
249 serum phosphorus <2 mg/dL. Dual energy X-ray absorptiometry (DEXA) scans of the
250 hip and spine were performed at baseline and then at 24-week intervals up to Week
251 96 and at 48-week intervals up to Week 240 and percentage change in mean bone
252 mineral density (BMD) at the hip and spine was calculated.
253
254 **Statistical analysis**
255 Statistical analyses have been reported previously [12]. In addition, multivariate
256 analysis was used to identify factors associated with the achievement of normal ALT
257 at Week 240. The primary efficacy and safety analyses were performed on the full
258 analysis set which included all patients who were randomised and received at least
259 one dose of study medication. Patients who withdrew post-randomisation and prior
260 to receipt of any study medication were excluded. The final enrolled sample size of
261 280 provides at least a 90% power to detect a difference of 20% between the
262 treatment groups.
263
264 **Results**
265 **Study patients**
266 A total of 752 patients were screened and 280 were randomised to receive TDF
267 (n=141) or FTC/TDF (n=139) at 62 study sites in North America, Europe and New
268 Zealand between October 2008 and January 2010 (Fig. 1). The most frequent

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reasons for screening failure were HBV DNA levels below the entry threshold, lack of documented LAM resistance or not receiving LAM concurrent with time of screening. A total of 239 (85.4%) patients completed 240 weeks of treatment (TDF, 121/141 [85.8%]; FTC/TDF, 118/139 [84.9%]. Reasons for discontinuation were similar between groups (Fig. 1).

Baseline demographics and disease characteristics were generally balanced between the two treatment groups as previously described [12]. Briefly, the majority of the patients enrolled were male (75%) and Caucasian (61%). Thirty-four percent of patients were Asian. At baseline the mean age (standard deviation, SD) was 46.7 (13.6) years. Almost half (47.5%) of those enrolled were HBeAg-positive and the predominant HBV genotypes were A (22%), C (19%), and D (45%). Baseline mean (SD) HBV DNA and ALT values were 5.70 (1.90) IU/mL, and 79 (122) U/L, respectively, and mean (SD) CL_{Cr} was 93.0 (22.7) mL/min. Baseline mean (SD) spine and hip BMD were 1.1 (0.19) g/cm^2 and 1.0 (0.15) g/cm^2, in the TDF and FTC/TDF groups respectively and 33.8% (91/269) and 22.1% (59/267), and 7.1% (19/269) and 1.1% (3/267) patients had T scores consistent with either osteopenia (>2.5 to <-1) or osteoporosis (≤-2.5). Mean (SD) CL_{Cr} was 93.0 (22.7) mL/min, respectively. The duration of previous LAM treatment was similar between groups (mean: 3.8 years).

Efficacy

Virological response

The proportion of patients achieving the study endpoint of plasma HBV DNA <69 IU/mL (<400 copies/mL) at Week 240 was similar between treatment groups (TDF,

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117/141 [83.0%]; FTC/TDF, 115/139 [82.7%], P=0.96) (Table 1). Patients receiving either TDF or FTC/TDF maintained similar treatment responses through to Week 240. At all time points during the study the proportion of patients achieving HBV DNA levels <69 IU/mL was similar between treatment groups (Fig. 2). The proportion of patients achieving HBV DNA levels of <29 IU/mL (<169 copies/mL) at Week 240 was also similar between groups (P=0.93, Table 1), and also did not differ at any time-points during the study.

The mean changes in HBV DNA from baseline through to Week 240 were similar between treatment groups (P=0.65, Table 1). When stratified by HBeAg status there was no significant difference (P=0.33 [HBeAg-positive], P=0.32 [HBeAg-negative]) between treatment groups although the mean HBV DNA decline was greater in HBeAg-positive patients than in HBeAg-negative patients (Table 1). However, HBeAg-positive patients had higher mean (SD) baseline HBV DNA levels than HBeAg-negative patients; 7.37 (±1.7) $\log_{10}$ copies/mL vs 5.64 (±1.7) $\log_{10}$ copies/mL.

Biochemical response

The percentage of patients with ALT values above the normal range was similar between treatment groups at baseline [12] and the percentage of patients who achieved ALT normalisation at Week 240 was similar between groups (P=0.41) (Table 1). Factors associated with lack of achievement of normal ALT at Week 240 were high baseline ALT levels (P<0.01) and high baseline BMI in the overall population (P=0.03) as well as in female patients (P=0.03). Additional information on all factors that were analysed are provided in Table S1 of the Supplementary Appendix.

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Serological response

By Week 240, 29 (21.80%) patients achieved HBeAg loss and 15 (11.3%) patients achieved HBeAg seroconversion with similar rates between treatment groups (P=0.41 and P=0.67, respectively, Table 1). When rates of HBeAg seroconversion were assessed according to baseline ALT levels, seven patients with baseline ALT ≥2 x ULN (TDF, 4; FTC/TDF, 3) and eight patients (TDF, 4; FTC/TDF, 4) with ALT <2 x ULN at baseline achieved HBeAg seroconversion. Overall, six patients (2%) [TDF, 2; TDF/FTC, 4], achieved HBsAg loss by Week 240 of which three patients [TDF, 1; TDF/FTC, 2] were HBeAg positive at baseline and three patients [TDF, 1; TDF/FTC, 2] were HBeAg negative at baseline. Only one patient in the FTC/TDF treatment group experienced HBsAg seroconversion by Week 240 (Table 1).

Resistance surveillance

The presence of known drug resistance mutations at baseline has been previously reported [11]. Eleven patients (TDF, 4/141 [2.8%], FTC/TDF, 7/139 [5.0%]) were viraemic at Week 240 or their last study visit and qualified for sequence analysis of pol/RT. Of the 11 patients that qualified for sequence analysis, one qualified due to virological breakthrough. Sequence analysis results are shown in Fig. 3. Over half of these patients (7/11 [64%]) were non-adherent to study medication at the time of qualification (as assessed by measuring plasma tenofovir levels). Overall, sequence analysis demonstrated no sequence changes in four patients, unique polymorphic-site substitutions in two patients (including the patient with virological breakthrough), and conserved-site substitutions in three patients. Sequence analysis was not possible in two patients due to low viral load. Of the three patients who had

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conserved-site substitutions (rtA87G, rtM/I204I, rtI233V) two were non-adherent to study medication; none were associated with tenofovir resistance in vitro (fold change <2). No TDF resistance was observed through 240 weeks of treatment.

Impact of prior treatment and baseline resistance mutations on virological response

Given the number of patients with prior ETV exposure, ADV exposure, and ETV resistance mutations at baseline, an evaluation was performed to see if they had an impact on long-term treatment response. The decrease in mean HBV DNA levels was sustained up to Week 240 regardless of prior treatment exposure to ETV or ADV or resistance to ETV and the decline between patients with LAM-R and ETV-R, and LAM-R only was similar (Fig. 4).

Safety

General safety profile

The overall incidence of treatment-emergent AEs was similar between treatment groups (Table 2). The most frequent treatment-emergent AEs were nasopharyngitis (15.7%), headache (15.5%), fatigue (10.4%) and arthralgia (10.0%). Nine (3%) patients discontinued due to an AE (TDF, 4; FTC/TDF, 5). Four patients (two in each treatment group) experienced an on-treatment hepatic flare. Seven deaths were reported (TDF, 3; FTC/TDF, 4), none of which were considered related to study treatment (one case of each of the following: cardiac arrest, pneumonia, bladder cancer, pancreatic cancer, polytrauma, hepatoma and gastrointestinal bleeding).

Renal-related safety profile

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Overall two patients, both in the TDF group, had a confirmed increase in serum creatinine of ≥0.5 mg/dL (>44 µmol/L) from baseline. One subject who had a serum creatinine value of 0.9 mg/dL and CL_{Cr} of 80 mL/min at baseline, experienced a single episode of confirmed increase in serum creatinine to 1.4 mg/dL (CL_{Cr} of 55 mL/min) at Week 156; the event resolved within 4 weeks after stopping TDF. The other subject with a baseline serum creatinine value of 1.0 mg/dL and CL_{Cr} of 65 mL/min, experienced a single episode of confirmed increase in serum creatinine to 1.6 mg/dL (CL_{Cr} 39 mL/min) at Week 216; the patient was subsequently lost to follow up.

Nineteen patients (6.8%) had confirmed CL_{Cr} <50 mL/min (baseline CL_{Cr} values ranged from 49–86 mL/min in these subjects), of which 12 underwent dose modification, three discontinued study treatment, and four received the full dose of study treatment. Twelve of the 19 patients who had a confirmed CL_{Cr} <50 mL/min had a dose modification to every–other-day dosing, two of these patients discontinued TDF within 6 months after the start of the dose modification. Of the seven patients who did not have a dose modification, one patient (FTC/TDF arm) discontinued from the study drug within 6 months after the confirmed decrease in CL_{Cr}. Three patients (TDF, 2; FTC/TDF, 1) had experienced confirmed decreases in serum phosphate values <2.0 mg/dL (0.65 mmol/L), that were generally transient in nature, resolved without treatment, and were not associated with increases in serum creatinine or decreases in CL_{Cr}. (Table 2)

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Bone-related safety profile

Similar declines in BMD were observed for each treatment group that differed over time at the hip and spine observed (Fig. 5). At the spine the decline reached a nadir at Week 24 and subsequently stabilised over 240 weeks (Fig. 5A). For both groups combined, mean percentage change (SD) in BMD at the spine was -0.98 [4.8] % at Week 240 with a maximum decline of 1.8% seen at Week 24. At baseline, 59%, 34% and 7% of patients overall had BMD T scores in spine consistent with normal bone (≥-1), osteopenia (-1 to -2.5) and osteoporosis (≤-2.5), respectively. At Week 240, 53%, 37% and 10% had T scores in the normal, osteopenic and osteoporotic range, respectively.

Declines in hip BMD were progressive over the duration of the study; at Week 240 the mean % change (SD) in BMD was -2.54 [3.5] % (Fig. 5B). At baseline, 77%, 22% and 1% of patients overall had hip T scores consistent with normal bone, osteopenia and osteoporosis, respectively. At Week 240, 68%, 30% and 1% of patients had hip T scores that were consistent with normal bone, osteopenia and osteoporosis, respectively.

Overall, the proportion of patients with BMD T-scores consistent with osteopenia and osteoporosis increased from baseline to Week 240 by six percentage points for the spine (41% to 47%) and eight percentage points (23% to 31%) for the hip.

Ten fractures were reported in seven patients. In the TDF group, three patients experienced fractures; Grade 2 foot fracture (1), Grade 3 fractures of the ankle and fibula (1) and simultaneous Grade 2 fractures of the clavicle, scapula, and skull base

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(1). In the FTC/TDF group, four patients experienced fractures; Grade 3 clavicle fracture (1), Grade 1 ankle fracture (1), Grade 2 foot fracture (1), and Grade 2 humerus fracture (1). Information regarding trauma association was available for six of these patients; this indicated that all the fractures were trauma associated. None of the fractures resulted in dose modification, interruption or discontinuation of study treatment. All fractures were considered unrelated to study treatment.

Discussion

In this prospective, randomised, controlled, long-term trial, TDF monotherapy provided highly effective viral suppression in LAM-R patients. Combination therapy with FTC and TDF did not demonstrate any additional virological benefit. Over 5 years, one of the longest follow-up periods compared with previous trials in LAM-R patients, TDF treatment was well tolerated; renal events observed were generally mild, and decreases in BMD were seen.

Our findings are in accordance with those in published literature that have shown that TDF monotherapy is an appropriate treatment option for LAM-R patients in terms of virological response and prevention of developing additional resistance [13]. The HBV DNA suppression rates observed in this study are consistent with those reported at Week 96 [12] and showed that TDF monotherapy results in excellent rates of virological suppression in patients with LAM-R. These findings are similar to those reported in previous studies of TDF monotherapy and TDF combination therapy in LAM-R patients [13–15]. van Bömmel and colleagues [14] found that all LAM-R patients treated with TDF monotherapy achieved viral suppression at Week 48, and reported similar results more recently with 95% of LAM-R patients achieving

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HBV DNA suppression within a median of 3.5 months [15]. Importantly, the virological suppression rates observed in LAM-R patients treated with TDF monotherapy are higher than those reported in LAM-R patients treated with ETV or ADV combination therapy [16]. In the current study, 83% of TDF-treated patients with or without FTC achieved HBV DNA <69 IU/mL at Week 240, while only 36% of LAM-R patients treated with ETV and 51% of those treated with ETV + ADV for a median duration of 10.4–84.3 months achieved virological suppression [16].

Despite high rates of virological suppression approximately one-third of patients did not normalise ALT; however, this is consistent with our findings at Week 96 and also with ALT normalisation rates reported in other trials of FTC/TDF and ETV that included LAM-R patients [17–18]. The reasons for persistently elevated ALT in virally suppressed patients remain unclear but are likely multifactorial and could include other causes of liver disease, such as non-alcoholic fatty liver disease/steatosis [19–21].

In this study, modest rates of HBeAg loss and HBeAg seroconversion were observed. Although our findings are lower than those reported in treatment-naïve patients [22–23] they are consistent with studies of TDF and FTC/TDF in treatment-experienced CHB patients. Berg and colleagues reported rates of HBeAg loss in the range of 22%–24% in ADV-experienced patients [17], and Kuo and colleagues also reported HBeAg loss of 11% of LAM-R patients [24]. Rates of HBeAg seroconversion in our study were 12.3% in the TDF group and 10.3% in the FTC/TDF group, which are similar to those rates reported in LAM-refractory patients treated with ETV and in patients treated with LAM, TDF or ADV [25–26].

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Furthermore, a higher proportion of patients with baseline ALT ≥2 x ULN achieved HBeAg seroconversion compared with patients with lower baseline ALT, providing evidence to suggest that seroconversion is higher in patients with elevated ALT at baseline.

The low rates of HBsAg loss observed in this study are consistent with other studies evaluating the long-term treatment with oral antiviral agents in LAM-R patients [14,26,27]. In mostly treatment-naïve HBeAg-positive patients treated with TDF for up to 8 years, 11.8% of patients experienced HBsAg loss. Analysis of factors associated with HBsAg loss showed that Caucasian race, a shorter duration of HBV infection (≤4 years), baseline HBsAg level (not assessed in this trial), and presence of HBV genotypes A or D were associated with greater likelihood of HBsAg loss at 240 weeks [28]. We also observed low rates of HBsAg seroconversion (<1% overall) which are lower than those reported by Berg and colleagues in ADV-experienced patients treated with TDF or FTC/TDF followed for the shorter duration of 168 weeks [17].

An important finding from this study is that no patient developed TDF resistance after 240 weeks of continuous treatment, even in patients harbouring baseline resistance mutations. The lack of resistance to TDF through 5 years of continuous treatment is striking considering the high rates observed in studies of other antiviral agents used for a similar duration. Tenney and colleagues reported a cumulative probability of genotypic ETV resistance of about 50% in LAM-refractory patients who were switched to ETV monotherapy [9]. Whilst Ze et al. reported rates of resistance development in 14% of LAM-R patients retreated with LAM + ADV and of 72% in

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ETV-treated patients [29]. These studies highlight the need for treatment options which do not confer a risk of resistance development in LAM-R patients, particularly in regions such as Asia, where LAM is commonly used [30]. The absence of viral resistance observed in this study has important clinical implications because patients who do not develop resistance may have a lower risk of disease progression [17,31].

Overall, 240 weeks of TDF as monotherapy or in combination therapy was well tolerated and there were few discontinuations due to AEs, which is consistent with the findings of other studies of TDF treatment in both treatment-naïve and treatment-experienced patients [17,32,33]. Given that TDF is renally metabolised there is a potential for kidney-specific toxicity and TDF-associated increased risk of tubulopathy [34]. Importantly, only 8.57% of patients overall experienced abnormalities in renal laboratory parameters and patients who experienced confirmed reductions in CL_{Cr}<50 mL/min were managed by dose modification. Phosphate levels in all patients who experienced decreases during treatment had normalised at Week 240 without dose modification, which suggests that the effects of TDF on renal function are not a substantial concern, although regular monitoring is required.

This is the first study to prospectively evaluate changes in BMD from baseline using serial DEXA scans in an adult CHB population for 240 weeks. Interestingly, the percentage change in spine BMD reached a nadir at Week 24 and subsequently stabilised over 240 weeks whilst the percentage change in hip BMD progressively decreased up to Week 240. Although direct comparison with other studies is not possible, the declines in BMD we report are consistent with those reported at the hip

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in studies evaluating TDF/LAM and efavirenz in HIV-infected subjects. Although there was an increase from baseline to Week 240 in the proportion of patients with BMD T-scores at the spine and hip consistent with osteopenia and osteoporosis, there was a low occurrence of fractures observed in the study overall and therefore the clinical significance of this, if any, is unknown. It would be useful to determine the role of nutrition and other factors in the BMD analyses. However, vitamin D and calcium supplementation was not a protocol requirement of our study and vitamin D levels were not assessed. Therefore, additional studies are required to determine the effect of these factors on any TDF-related effects on BMD.

In this study the degree of fibrosis and/or the presence of cirrhosis in patients was assessed at screening using the patient's medical history. However liver biopsies were not taken and non-invasive markers of fibrosis (FibroTest and Fibroscan) were not assessed so we are unable to draw any conclusions about the impact of long-term treatment on fibrosis stage. Our study is also limited by the small proportion of patients who had previously been treated with ADV monotherapy and these patients were not evenly distributed between treatment groups. As only 2% of patients had ADV resistance (rtA181T/C/G/S/V) at baseline and no patients harboured rtN236T, it is not possible to infer any effect of TDF in patients with ADV resistance. Similarly, as the number of patients with a complex resistance profile was small, the results from this study cannot be applied to patients with multiple nucleoside-nucleotide resistance.

In conclusion, our long-term results demonstrate that TDF alone for the treatment of CHB is effective and well tolerated in LAM-R patients. There was no evidence of an

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additional treatment benefit from combination therapy with FTC/TDF. Importantly, 240 weeks of continuous therapy with TDF alone, or in combination with FTC, was not associated with resistance development. TDF is, therefore, an effective and well-tolerated treatment option for CHB patients with resistance to lamivudine.

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FIGURE LEGENDS

Fig. 1: CONSORT flow diagram

FTC, emtricitabine; TDF, tenofovir disoproxil fumarate.

*Investigator's discretion (n=6), withdrew consent (n=5), safety/tolerability/efficacy reasons (n=3), lost to follow-up (n=3), protocol violation (n=2), study discontinued at site (n=1). †Investigator's discretion (n=5), withdrew consent (n=6), safety/tolerability/efficacy reasons (n=4), lost to follow-up (n=3), protocol violation (n=3).

Fig. 2: Percentage of patients with HBV DNA <400 copies/mL (69 IU/mL) by visit.

Full analysis set. Missing patients were classed as failures. Error bars represent 95% confidence intervals.

FTC, emtricitabine; TDF, tenofovir disoproxil fumarate.

Fig. 3: Sequence results for qualifying TDF and FTC/TDF patients.

Patients who were viraemic at Week 240 or their last study visit qualified for sequence analysis of pol/RT.

FTC, emtricitabine; pol/RT, polymerase/reverse transcriptase; TDF, tenofovir disoproxil fumarate.

Fig. 4: Mean viral load declines.

Mean HBV DNA (log_{10} cp/mL) in patients with and without entecavir (ETV) exposure (A), ETV resistance (B), or adefovir dipivoxil (ADV) exposure (C) up to Week 240.

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ADV-E; adefovir exposure, ETV-E; entecavir exposure; FTC, emtricitabine; TDF, tenofovir disoproxil fumarate.

Fig. 5: Mean percentage change in bone mineral density (BMD).

Dual energy X-ray absorptiometry (DEXA) scans of the spine (A) and hip (B) were performed at baseline and then at regular intervals up to Week 240. Error bars represent 95% confidence intervals (CIs).

FTC, emtricitabine; TDF, tenofovir disoproxil fumarate.

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718 **Table 1: Virological, biochemical, and serological responses at Week 240 (full**

719 **analysis set, missing equal to failure)**

Characteristic	TDF (n=141)	FTC/TDF (n=139)	P value*
Virological responses			
HBV DNA <69 IU/mL (<400 copies/mL)	117 (83.0)	115 (82.7)	0.96
Overall, n (%)	56/67 (83.6)	56/66 (84.8)	0.84
HBeAg-positive, n/N (%)	61/74 (82.4)	59/73 (80.8)	0.80
HBeAg-negative, n/N (%)			
HBV DNA <29 IU/mL (<169 copies/mL)	115 (81.6)	114 (82.0)	0.93
Overall, n (%)			
HBV DNA, change from baseline in log_{10} copies/mL			
Overall, mean (SD)	-4.18 (1.8)	-4.26 (2.0)	0.65
HBeAg-positive, mean (SD)	-5.02 (1.7)	-5.12 (1.6)	0.85
HBeAg-negative, mean (SD)	-3.39 (1.6)	-3.46 (2.0)	0.92
Biochemical responses			
ALT normalisation			
Overall†, n/N (%)	51/79 (64.6)	59/83 (71.1)	0.41
ALT normal			
Overall, n/N (%)	101/141 (71.6)	100/139 (71.9)	0.97
Serological responses			
HBeAg loss			

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Overall, n/N (%)	16/65 (24.6)	13/68 (19.1)	0.41
HBeAg seroconversion Overall‡, n/N (%)	8/65 (12.3)	7/68 (10.3)	0.67
HBsAg loss Overall, n/N (%)	2/141 (1.4)	4/139 (2.9)	0.40
HBsAg seroconversion Overall, n/N (%)	0	1/139 (0.7)	0.32

720 *P-values were from a stratified CMH test controlled for randomisation strata and a
721 two-sided Wilcoxon rank sum test for categorical data and continuous data,
722 respectively.
723 †The denominator is the N of subjects with abnormal ALT at baseline
724 ‡The denominator is the N of subjects that are HBeAg positive
725 ALT: alanine aminotransferase; CMH: Cochran-Mantel-Haenszel; FTC:
726 emtricitabine; HBeAg: hepatitis B e antigen; HBsAg; hepatitis B surface antigen;
727 HBV: hepatitis B virus; SD: standard deviation; TDF: tenofovir disoproxil fumarate
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730 **Table 2. Safety profile up to Week 240.**

Parameter	TDF (n=141)	FTC/TDF (n=139)	Total (N=280)
Any treatment-emergent AE, n (%)	110 (78.0)	110 (79.1)	220 (78.6)
Grade 3 or 4 AEs, n (%)	16 (11.3)	24 (17.3)	40 (14.3)
Treatment-emergent SAE related to study drug*	0	1 (0.7)**	1 (0.4)
AEs leading to study drug discontinuation	4 (2.8)	5 (3.6)	9 (3.2)
Deaths, n (%)	3 (2.1)	4 (2.9)	7 (2.5)
On-treatment hepatic flare, n (%)	2 (1.4)	2 (1.4)	4 (1.4)
Hepatocellular carcinoma	2 (1.4)	1 (0.7)	3 (1.1)
Serum creatinine ≥0.5 mg/dL (>44 µmol/L) above baseline, n (%)	2 (1.4)	0	2 (0.7)
CL_{Cr} <50 mL/min, n	10 (7.1)	9 (6.5)	19 (6.8)
Serum phosphate <2.0 mg/dL (0.65 mmol/L), n (%)	2 (1.4)	1 (0.7)	3 (1.1)

731 *AE/SAE judged by the investigator to be related to study drug; **ALT elevation

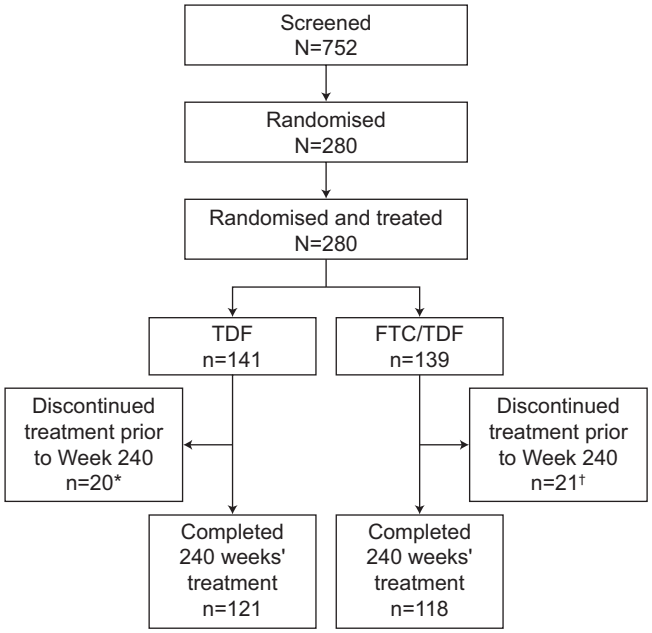
732 AE, adverse event; ALT, alanine aminotransferase; CL_{Cr}; creatinine clearance; FTC,

733 emtricitabine; SAE, serious adverse event; TDF, tenofovir disoproxil fumarate

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Figure 1

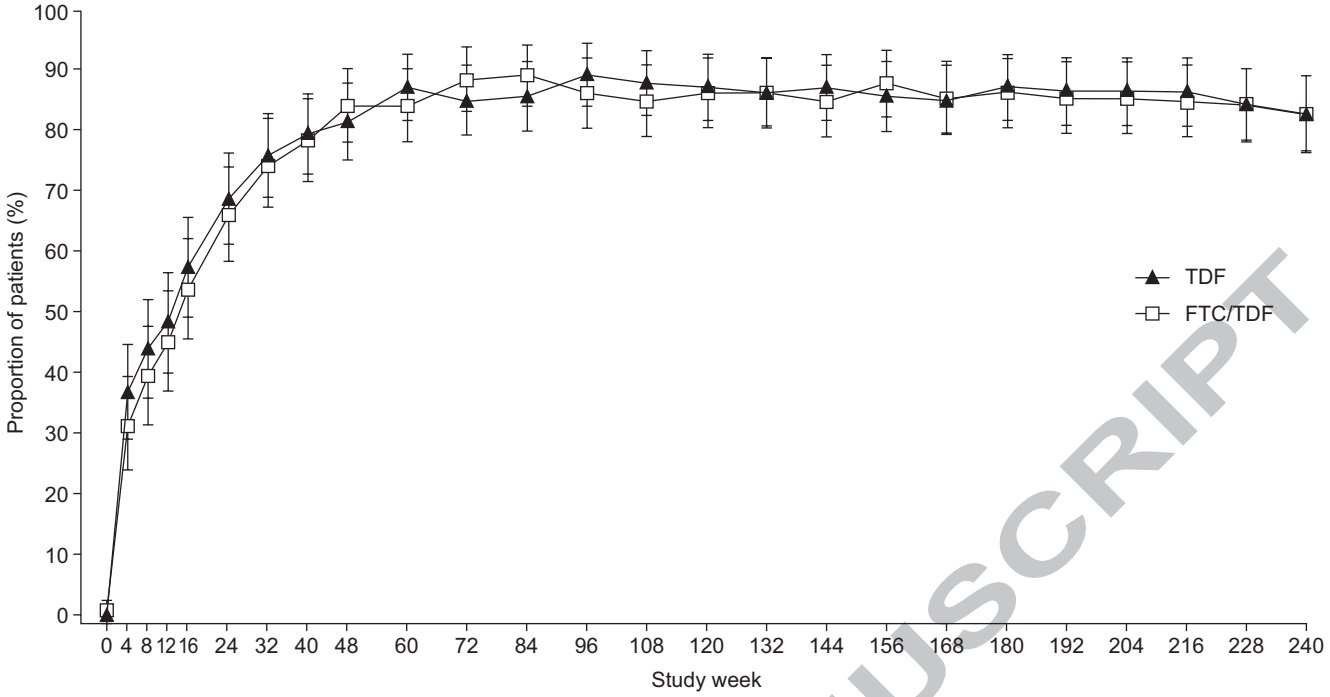
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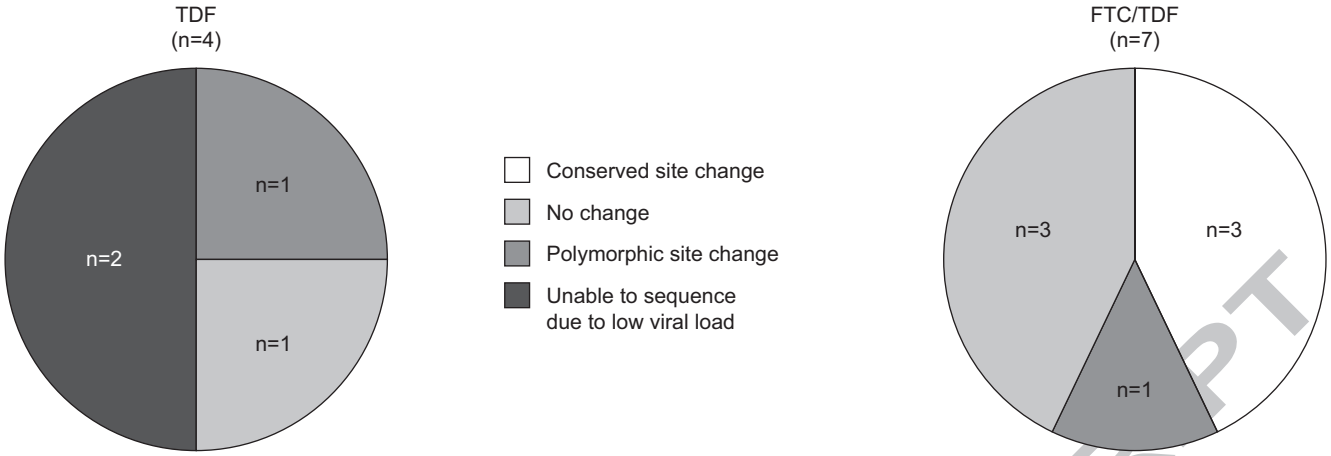
Figure 2



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Figure 3

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Figure 4

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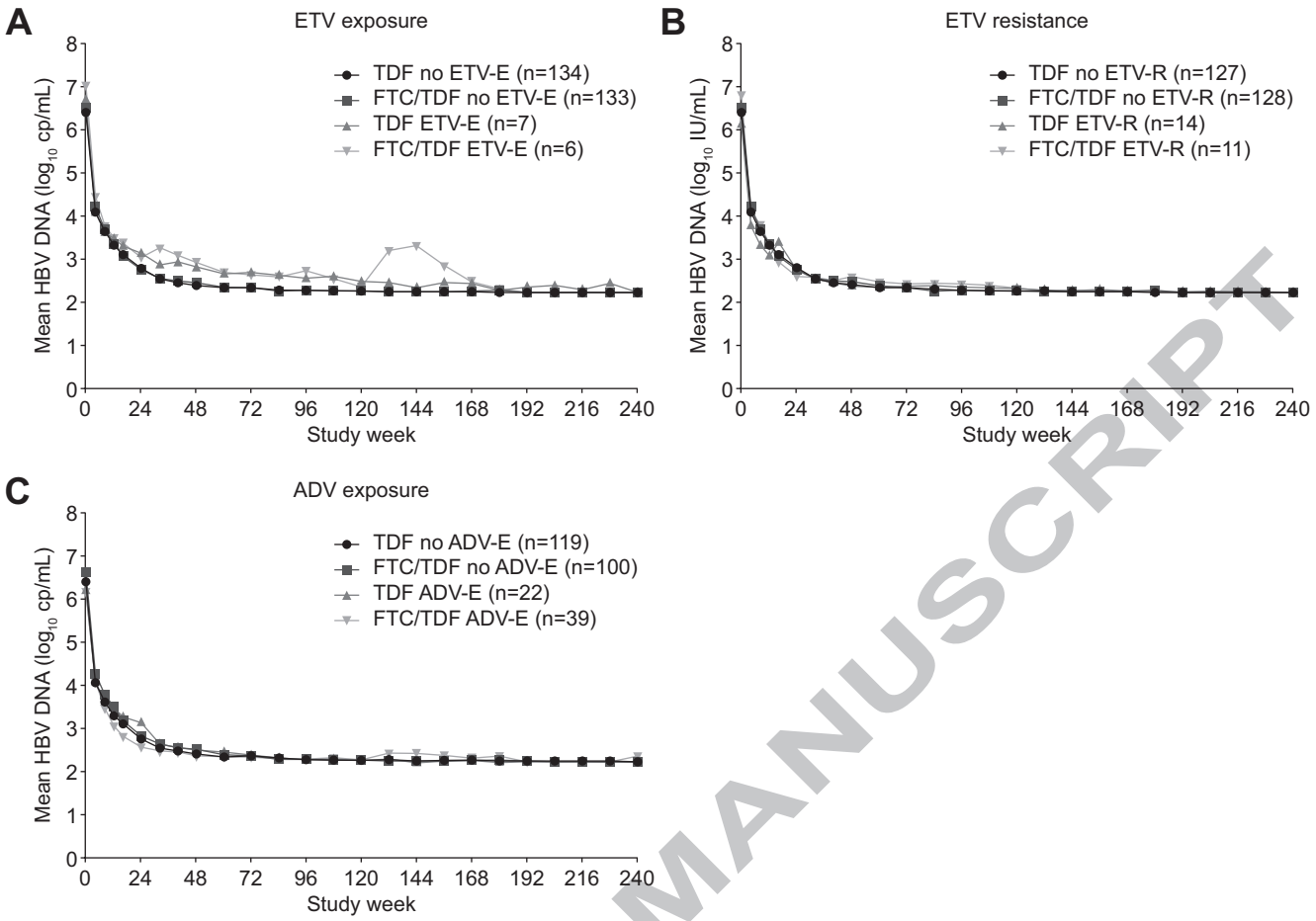
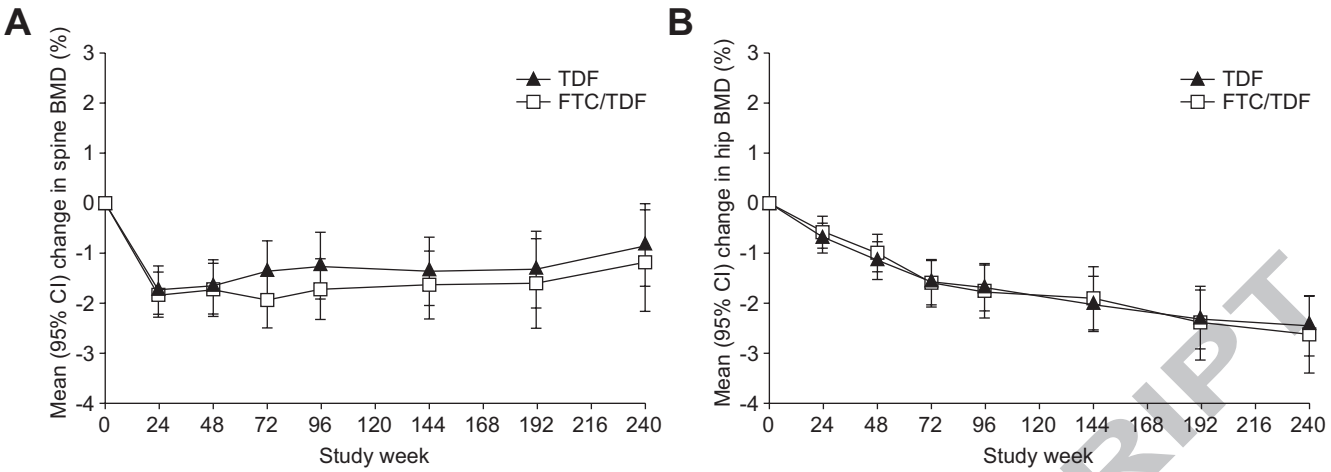


Figure 5

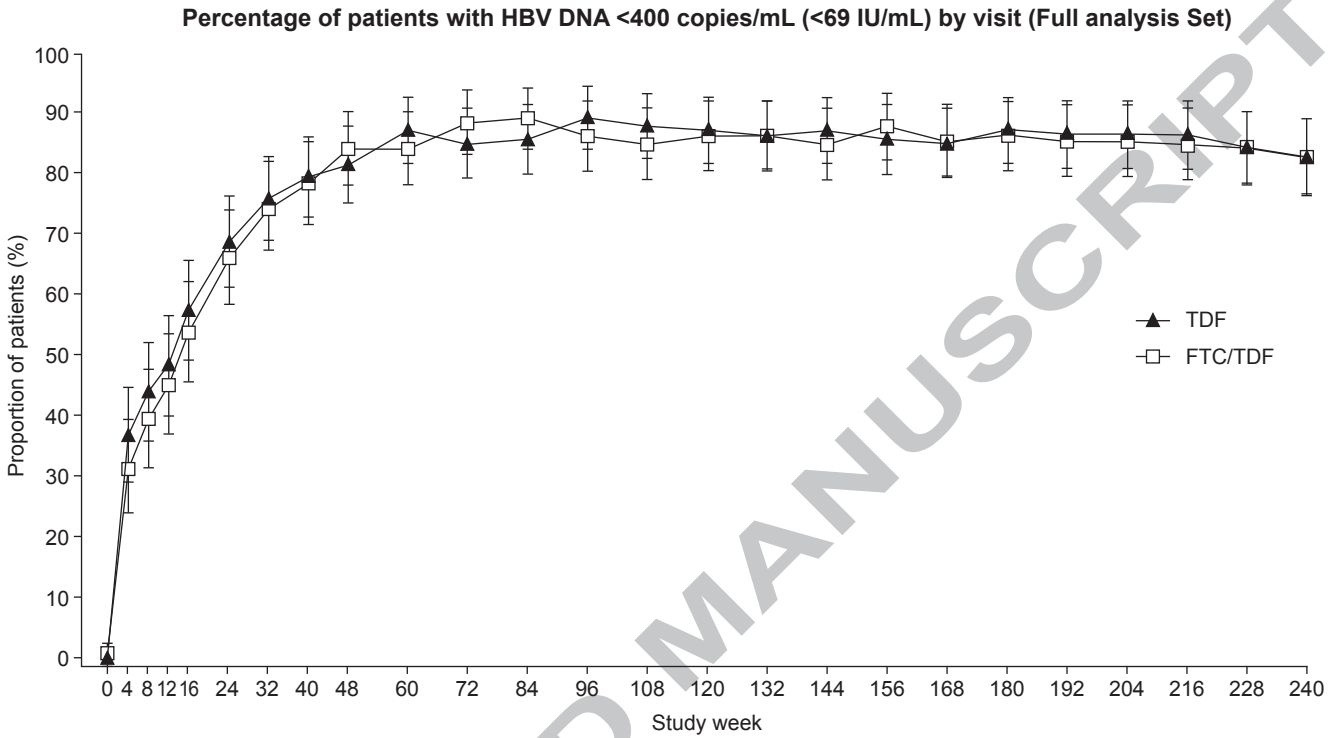
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*Graphical Abstract

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Missing patients were classed as failures.
Error bars represent 95% confidence intervals.

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