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Five year efficacy and safety of tenofovir-based salvage therapy for patients with chronic hepatitis B who previously failed LAM/ADV therapy

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CHB	Chronic Hepatitis B
NA	Nucleot(s)ide analogs
LAM	Lamivudine
ADV	Adefovir dipivoxil
MDR	Multi-drug resistant
TDF	Tenofovir disoproxil
HBeAg	Hepatitis B e antigen
anti-HBs	Hepatitis B surface antibody
VBT	Virological breakthrough

LLOQ	Lower Limit of Quantification
PBS	Polymerase Sequence
Wt	Wild type
LLOD	Lower limits of detection
VIDRL	Victorian Infectious Diseases Reference Laboratory
HCC	Hepatocellular carcinoma
TACE	Transarterial chemoembolisation
NHL	Non-Hodgkins lymphoma
eGFR	estimated glomerular filtration rate
ULN	Upper limit of normal
NGS	Next generation sequencing

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ABSTRACT

Background: Multidrug resistant HBV continues to be an important clinical problem. The TDF-109 study demonstrated that TDF±LAM is effective salvage therapy through 96 weeks for LAM resistant patients who previously failed ADV add-on or switch therapy. We evaluated the 5 year efficacy and safety outcomes in patients receiving long-term TDF±LAM in the TDF-109 study.

Methods: 59 patients completed the first phase of the TDF-109 study and 54/59 were rolled over into a long-term prospective open-label study of TDF±LAM 300mg daily.

Results: Results are reported at the end of year 5 of treatment. At year 5, 75% (45/59) had achieved viral suppression by intent-to-treat analysis. Per protocol assessment revealed 83% (45/54) were HBV DNA undetectable. Nine patients remained HBV DNA detectable, however 8/9 had very low HBV DNA levels (<264IU/ml) and did not meet virological criteria for virological breakthrough (VBT). One patient experienced VBT, but this was in the setting of documented non-compliance. The response was independent of baseline LAM therapy or mutations conferring ADV resistance. Four patients discontinued TDF, one patient was lost to follow-up and one died from hepatocellular carcinoma.

Conclusions: Long-term TDF treatment appears to be safe and effective in patients with prior failure of LAM and a suboptimal response to ADV therapy. These findings confirm that TDF has a high genetic barrier to resistance is active against multidrug resistant HBV, and should be the preferred oral anti-HBV agent in CHB patients who fail treatment with LAM and ADV.

Abstract Word Count:239

Key Words:Hepatitis B, Viral Hepatitis, Hepatology, Antiviral Therapy

- Long-term antiviral therapy for hepatitis B is effective however may be complicated by resistance.
- This is a 5-year prospective, multicenter study which examined the effectiveness of tenofovir salvage therapy in patients who had previous failure to lamivudine and adefovir with high rates of genotypic resistance.
- 83% of patients achieved an undetectable viral load at year 5 of treatment, and proven to be safe with minimal side-effects.
- This study confirms that tenofovir should be the preferred antiviral therapy in patients with prior failure of or resistance to lamivudine and adefovir.

INTRODUCTION/BACKGROUND

Chronic hepatitis B (CHB) is a major global public health issue, infecting more than 240 million people¹ and ranked as the tenth leading cause of death worldwide². Untreated CHB may result in life-threatening sequelae such as acute or fulminant hepatitis, cirrhosis, hepatocellular carcinoma or liver failure, which occur in up to 15 to 25% of patients³. Advances in antiviral therapy have transformed the treatment of CHB in the last two decades. Nucleot(s)ide analogs (NA) are highly effective in reducing the viral load, however treatment with the first generation of drugs approved, such as lamivudine (LAM) and adefovir dipivoxil (ADV), readily lead to drug resistance. Up to 70% of patients develop resistance after 4 years of treatment on LAM⁴ while 30% of treatment naïve patients develop ADV resistance at 5 years⁵.

A major issue in the current management of CHB has been to develop effective therapy for patients with multi-drug resistant (MDR) HBV. Of the second-generation drugs, Tenofovir disoproxil (TDF) is considered the most effective salvage therapy for patients who have failed LAM. This is because LAM resistance mutations predispose to the development of entecavir resistance but do not confer resistance to TDF. However, there is limited information regarding the efficacy of TDF therapy in patients who have failed both LAM and ADV. A

particular concern with the use of TDF in this population is that the presence of ADV resistance mutations reduces sensitivity to TDF in vitro and thus might predispose to breakthrough in patients receiving long-term treatment^{6,7}.

Early data from Van Bommel showed that with follow-up of 1 year, TDF sustainably suppressed viraemia in patients who failed treatment with LAM and ADV⁸. We have also previously shown that in a group of MDR patients, TDF was effective in reducing the viral load after 2 years of treatment⁹. However it is becoming increasingly clear that NA therapy is required lifelong for many patients and this raises important issues regarding the long-term efficacy and safety of these drugs as well as the possible impact of pre-existing or evolving resistance mutations. In particular, concerns have been raised that although 96 weeks of salvage therapy with TDF is effective for the vast majority of MDR patients, preselection of ADV resistance mutations may eventually reduce TDF efficacy¹⁰.

Therefore, the aim of this study was to evaluate the virological outcomes and safety after 5 years of TDF±LAM salvage therapy for patients enrolled who had all previously failed ADV treatment in the setting of LAM resistance⁹. We present the overall outcome data, as well as detailed sub-analyses according to the presence of NA-resistance variants.

PATIENTS AND METHODS

Patients

The TDF-109 study was an investigator-initiated, prospective, multi-centre, open-label study of TDF±LAM rescue therapy in 60 patients who had failed both LAM and ADV treatment. Patients were recruited from eight tertiary referral hospitals in Victoria and New South Wales, Australia. Adult patients were considered for the study if they satisfied all the following criteria: chronic hepatitis B, previous failure of LAM therapy; current treatment with ADV for at least 6 months and the presence of significant persistent viraemia (defined at 5log₁₀copies/ml in HBeAg positive patients or 4log₁₀copies/ml in HBeAg

negative patients) or breakthrough viraemia. Patients were excluded if they had: previously used TDF or other antiviral agents within 6 months, co-infections with hepatitis C, D, HIV, evidence of hepatocellular carcinoma (HCC), decompensated liver disease, significant comorbidities, pregnant, breastfeeding or unwilling to use contraception during the study. Patients satisfying the criteria were offered participation, screened, and had a baseline visit scheduled within 30 days of screening.

Consent was obtained from all the patients and this study was conducted with the approval of the Austin Health human research ethics committee.

At study entry 38/60 (63%) of patients were switched from ADV to TDF monotherapy and 22/60 (37%) were switched from ADV+LAM to TDF+LAM combination therapy. Sixteen patients who were initially treated with TDF monotherapy and had an HBV DNA >351IU/ml at 24 weeks had LAM added as per protocol and continued this combination long-term. Treatment outcomes at year 1 and 2 have previously been reported⁹.

At the completion of year 2, all patients were invited to roll-over into the current long-term follow-up study. Patients continued treatment with TDF 300mg±LAM 100mg and were reviewed at 12-weekly intervals from years 2-5. At each study visit, blood was taken for serum biochemistry, HBsAg, anti-HBs, HBeAg, anti-HBe and HBV DNA level. In addition, the HBV polymerase gene was sequenced from the baseline serum sample and then yearly in those with viral load >1000IU/ml. Antiviral drug resistance sequencing was also performed at the time of virological breakthrough (VBT), defined by an increase from the HBV DNA nadir of >1 log or the detection of HBV DNA in a patient who had previously been HBV DNA undetectable.

The primary end point of the study was the number of patients who achieved an undetectable plasma HBV DNA level at year 5 (lower limit of quantification (LLOQ <20IU/ml). Secondary endpoints included:

- i) number of patients who achieved an undetectable plasma HBV DNA level at years 3 and 4 (LLOQ <20IU/ml).
- ii) rate of HBV DNA decline and virological response analyzed according to the baseline HBV polymerase sequence: wild type (wt), L-nucleoside (rtM204I/V), acyclic phosphonate (rtN236T), shared (rtA181T/V), and double (rtA181T/V+rtN236T) resistance pathways¹¹
- iii) number of patients who experienced VBT and/or genotypic resistance
- iv) number of HBeAg-positive patients who achieved HBeAg seroconversion
- v) safety outcomes.

Plasma HBV DNA levels

Plasma HBV DNA levels were measured by the Abbott Real Time HBV PCR assay or Roche TaqMan according to the local reference laboratory. The lower limits of detection (LLOD) for these assays are 15IU/ml and 20IU/ml, respectively.

HBV DNA population-based sequencing

HBV PBS was performed centrally at VIDRL, Melbourne. Reliable sequencing required a viral load of >1000IU/ml. HBV DNA was extracted from 200µl of patient serum using the QIAamp DNA MiniKit (QIAGEN, Valencia, CA, USA) according to the manufacturer's instructions. For amplification of POL, the sense primer was 1877a (nt1877–1996, CCTGCTGGTGGCTCCAGTTC) and the anti-sense primer 2996 (nt2996–3014, GCGTCAGCAAACACTTGGC) was used. The amplified HBV envelope/surface gene was purified using PCR purification columns (MO BIO Laboratories Inc. Carlsbad, CA, USA) and directly sequenced using Big Dye terminator Cycle sequencing (Ready Reaction Kit Version 3.1, Perkin-Elmer Cetus, Norwalk, CT, USA). HBV consensus sequences were constructed using the DNA sequence analysis program Seqscape (Applied Biosystems, Grand Island, NY USA). HBV genotype was determined using a web-

based program, SeqHepB (<http://www.seqvirology.com/genome7/index.htm>), by comparing the input sequence data with known HBV reference sequences¹².

Statistical methods

Continuous variables are expressed as median $\pm$ interquartile range unless specified. Categorical data are presented as a percentage(%). Intent-to-treat analysis included all patients as originally allocated (n=59) while per-protocol analysis included only those patients who completed the treatment at year 5 (n=54), as five patients dropped out. Survival analysis was used to analyse predictors of time to HBV DNA undetectability, using Cox proportional hazards regression on R software (RStudio Team (2015), Integrated Development Environment to R, Boston MA). P values <0.05 were considered statistically significant.

RESULTS

Patients

Of the 60 patients enrolled into the original study and who received TDF $\pm$ LAM therapy, one discontinued treatment at day 10 due to a rash. Therefore 59 patients reached 2 years of treatment⁹. The demographics and hepatitis B status of the patients who continued in the extension study are shown in Table 1. All 59 patients were followed beyond 2 years, however five did not complete 5 years of follow-up. Of these patients, one died from HCC at 27 months, two were lost to follow up (at 2 and 3.7 years) and two discontinued TDF at year 2; one ceased drug due to their concerns about drug use in pregnancy and the other due to severe intractable nausea. One of these 5 patients was still HBV DNA positive at the time of withdrawal (at year 3 with a viral load of 44IU/ml), while the other 4 were already at or below LLOQ.

Treatment outcomes

Virological suppression

At commencement of the extension study (i.e. after 2 years of treatment) the percentage of patients with HBV DNA levels below LLOQ was 64% (38/59). At years 3, 4 and 5, per protocol the percentage of those with HBV DNA at LLOQ was 70% (41/58), 76% (41/54) and 83% (45/54) respectively (Figure 1). By intent-to-treat analysis, including the study dropouts, 75% (45/59) had undetectable HBV DNA at year 5. The median HBV DNA viral load was less than the LLOQ at year 2 and this was unchanged through to completion of the study at year 5 (Figure 2).

Of those who reached LLOQ, the median time to undetectability was 15 months (range 1 to 51 months). Table 2 lists the time to HBV DNA LLOQ according to patient's baseline resistance patterns. In patients with rtA181T/V containing HBV at baseline, there was a tendency for longer time to achieve undetectable HBV DNA (24 months for rtA181T/V versus 10.5 months for wt, $p=0.23$).

A survival analysis was then performed to evaluate the effects of the following parameters on time to undetectability: baseline viral load, genotype, baseline mutations, age at commencement of TDF, gender and baseline HBeAg status (Table 3). The two significant independent predictors of time to undetectability were baseline HBV DNA load ($p<0.001$) and HBV genotype (A<B, C<D), A>D ($p=0.012$).

As shown in Table 2, nine patients who completed the study (9/54=17%) had serum HBV DNA levels >20IU/ml at year 5. However, the median viral load at year 5 for these 9 patients was only 89IU/ml (range 23–264IU/ml excluding one non-compliant patient whose HBV DNA peaked at 19453IU/ml and became DNA negative 6 months later). The details of the virological responses in these patients are shown in Figure 3. Six had previously become HBV DNA negative (at

time-points week 12 (two patients), week 24, year 1, 2.5 and 2.75 respectively), whilst in three patients' HBV DNA had never fallen below LLOQ throughout the 5 years. Of the six who previously had HBV DNA below LLOQ, three became HBV DNA undetectable with further follow up beyond year 5, suggesting possible non-compliance, while three patients had persistent low level viraemia through to 5 years.

The demographic details and the sequencing results of the patients with persistent viraemia at year 5 and the decline in their HBV DNA levels over the 5 years are listed in table 4. In the six patients who had previously been HBV DNA negative, three had wt virus, one M204I/V, one N236T, and one A181T/V+N236T resistance mutations at baseline.

Of the three persistently viraemic patients in whom HBV DNA levels never fell below the LLOQ, two had wt virus and one had the N236T mutation identified at baseline (Table 4). All three patients started with high viral loads, greater than 7logIU/ml, had slower decline of their HBV DNA (Figure 3) and had sufficient viraemia for sequencing at year 1 and 2 but not beyond this time-point. One patient with wt HBV had selected out for rtA181T/N236T at year 1 which persisted into year 2. In the remaining two, HBV DNA sequencing was unchanged from baseline. Note that the HBV DNA level at last follow-up was too low to permit POL sequencing studies at last follow-up in these patients. Thus of the nine patients who were HBV DNA positive at year five, four never had evidence of resistance changes identified in their HBV DNA samples, whilst five were infected with HBV displaying mutations associated with reduced response to ADV or TDF. Therefore, resistance changes were not more common than in the overall population (Table 1).

In regards to the one patient with VBT, his HBV DNA had initially become undetectable at week 24. Over the 4.5 years, his HBV DNA level did fluctuate, (range from undetectable – 3.3logIU/ml), however at the conclusion of the study, at year 5, it suddenly increased to 19453IU/ml. Prior to this, his HBV DNA had

been undetectable for 9 months. His HBV DNA was sequenced, and it remained unchanged from his baseline sequence (M204I). He admitted to being non-compliant to TDF+LAM and agreed to restart his AVT. His HBV viral load decreased to 66IU/ml in 3 months and became undetectable 3 months later.

HBeAg and HBsAg seroconversion

Thirty-nine patients were HBeAg positive at baseline. The remaining twenty-one patients were HBeAg negative and anti-HBe positive. By year 5, a total of eleven patients had lost HBeAg (11/39=28%) but none developed anti-HBe. All remain on NA therapy. One patient achieved HBsAg seroclearance at week 204 but anti-HBs remains undetectable through to year 5, and the patient remains on TDF+LAM.

Clinical outcomes

TDF was well tolerated in all 54 patients. No patients developed liver decompensation. However, despite viral suppression, three developed HCC, with one patient dying at 28 months. This patient was infected with genotype A virus whilst the other 2 were genotype B. These two patients had successful locoregional treatment with no HCC recurrence and continued on TDF. One patient had a history of HCC diagnosed before commencement of TDF, and was treated with two rounds of transarterial chemoembolisation (TACE). He was in remission until month 30 of the study, when he developed HCC recurrence and was treated with repeat TACE. His disease is currently in remission. The other was diagnosed with HCC at week 156, which was downsized with TACE. He subsequently underwent a successful liver resection. Both these patient's HBV DNA was undetectable pre-diagnosis of HCC and remained DNA undetectable at 5 years.

One patient was diagnosed with diffuse large B cell non-Hodgkins lymphoma (NHL) at week 156 and was treated with 6 cycles of chemotherapy. This patient had reached HBV DNA LLOQ at week 120 and remained HBV DNA undetectable until week 168. Subsequently, HBV DNA became detectable with ongoing low level fluctuating viraemia (range undetectable - 62IU/ml) until the study endpoint. His ALT also fluctuated and peaked at 105IU/ml at week 216 but fell to 48IU/ml by year 5.

One patient discontinued NA due to concerns about possible effects of TDF on fertility. His HBV DNA was below LLOQ from week 8 of TDF. He had a rapid rebound in HBV DNA viral load up to 7.6log₁₀IU/ml once TDF was ceased. However, interestingly this patient had a progressive decline in viral load off therapy, and had an undetectable viral load from year 4.

Renal function and other safety concerns

Serum creatinine remained stable in the majority of the participants over the course of the study at a median of 79µmol/L (range 45 – 121) at year 5, unchanged from the baseline median of 80µmol/L (range 48 – 116). Throughout the 5 years, only two patients had dose reduction to alternate days. The first was the patient who developed NHL. His renal function had deteriorated during chemotherapy, but then recovered and TDF was up titrated back to daily. The other patient had dose reduction from week 33 until year 5, according to estimated glomerular filtration rate (eGFR). His renal function was within normal limits prior to starting TDF however it increased to 120µmol/L four weeks after commencing TDF and his eGFR decreased to 55mL/min. Once the TDF dose was reduced to alternate days, his renal function normalized and remained stable through to year 5.

Transient elevations in ALT to >2 times the upper limit of normal occurred in 5/54 (9%) patients. Two were related to non-compliance with TDF and

associated with HBV DNA level elevation. Another patient had ALT >10 times the upper limit of normal (ULN) at year 1 associated with use of a herbal remedy, and a biopsy at that time suggested toxic drug reaction. His ALT never completely normalized and remained 1.5 – 5 times ULN throughout the 5 years. This might have reflected viral breakthrough due to non-compliance since his HBV DNA had abruptly risen to 4.2 log at year 5, having been undetectable, and became undetectable 6 months later. A raised ALT level, unrelated to HBV replication, was also observed in one of the patients with HCC .

There were no significant abnormalities in the other biochemical parameters during the study and no serious adverse events. As outlined above, only one patient died from HCC from the original 59 patients who entered the study.

DISCUSSION

This study has prospectively demonstrated that in patients with LAM resistance and failure to respond adequately to ADV, long-term TDF effectively suppressed HBV replication for five years. Despite being highly drug experienced, majority of the patients had a significant decline in HBV DNA levels with maintenance of DNA suppression from years two to five. No patient had primary non-response and none developed persistent breakthrough viraemia. Furthermore, long term TDF treatment in this population appeared safe, with few patients experiencing side effects and no patients showing any evidence of long-term decline in renal function.

The major complication of NA therapy is the development of drug resistant HBV. Incomplete virological response to ADV has been observed in patients with LAM-resistance hepatitis B virus infection¹³. Furthermore, the rtA181T and rtN236T HBV mutations both of which confer resistance to ADV, have been demonstrated to have reduced sensitivity to TDF in vitro^{6,7}. We and others have reported that patients switched to TDF with baseline genotypic ADV resistance are more likely

to have persistent viraemia compared to patients who are treatment naïve or without genotypic resistance^{9,13}. The process by which resistance mutations reduce TDF response is unknown, however a potential mechanism could be through the altered envelope (POL-ENV link), which could affect rates of clearance by the reticulo-endothelial system. This extension study has shown that despite a trend for those patients infected with the HBV rtA181T-containing mutation at baseline taking longer to achieve HBV DNA suppression, irrespective of their baseline resistant mutations, all compliant patients had maintenance of viral suppression.

Importantly, although a number of patients had detectable viral replication at year 5, apart from one patient who was non compliant (HBV DNA 19453IU/ml), all had very low levels of serum HBV DNA ranging from 23IU/ml to 263IU/ml and most of these patients had become HBV DNA undetectable at some stage. Mutations that have been linked to reduced sensitivity to TDF were detected in four of these nine patients, in three at baseline with one patient who was infected with wild-type virus at baseline, selecting for rtA181T+rtN236T resistance substitutions by year 1. These changes might have contributed to the ongoing replication at year 5 in the four patients, as this double substitution in the HBV polymerase has been associated with reduced sensitivity to TDF in vitro^{6,7}. However, none developed typical breakthrough viraemia¹¹. Thus, despite the cross resistance between ADV and TDF observed in vitro, TDF is effective in controlling viraemia in the long-term in patients with genotypic ADV resistance.

The phenomenon of persistent viraemia at low levels, has been described by recent studies. Regarded as 'viral blipping', the Asian race, LAM therapy, higher creatinine, HBeAg positivity and longer time to HBV DNA undetectability were associated with an increased risk of viral persistence, however this occurrence did not have a significant negative impact on clinical outcome¹⁴. Other studies have described treatment-experienced HBV-HBV co-infected patients with persistent HBV viraemia to be associated with lower CD4+ cell counts, suggesting there may be an immunological component to viral persistence^{15,16}.

It is possible that drug-resistant HBV quasispecies, not detected by the PBS method used in this study, affected the response to TDF in some patients. This technique only identifies quasispecies, which make up at least 20% of the virus population. Recent studies suggest that increased viral diversity is associated with reduced viral loads¹⁷ and also a reduced likelihood of virological response to treatment¹⁸. Using next generation sequencing (NGS) methodologies, would allow detection of smaller populations and could potentially be applied to this cohort to determine whether minor drug resistant quasispecies of HBV may affect treatment outcomes in those patients in whom only wild type virus was detected. However, NGS requires an HBV DNA load in serum of at least 1000IU/ml and very few patients were above this viral load from year 2 onwards.

It should also be noted that the three patients who remained viraemic throughout the 5 years, started with the highest viral loads at baseline ($>7.0 \log_{10} \text{IU/ml}$) and had progressive and sustained gradual decline of their HBV DNA throughout the study. This is in keeping with a previous study which found that patients with ADV resistance and with higher HBV DNA levels were less likely to achieve HBV DNA undetectability in response to TDF treatment¹³.

Interestingly, one HBeAg negative patient who discontinued TDF at year 2 had a rapid elevation in his HBV DNA level immediately after drug cessation. However, it subsequently declined and he became HBV DNA undetectable at year 4 without further antiviral therapy. He remained HBV DNA negative despite being persistently HBsAg positive. There are currently no consensus guidelines regarding the stopping of antiviral therapy in HBeAg negative patients who have remained HBV DNA negative during prolonged therapy. Although HBsAg seroclearance remains the ultimate treatment goal, the experience in this patient is in keeping with other studies which have shown that durable post treatment suppression of HBV DNA is achievable in a sub-set of patients¹⁹⁻²¹.

The study was limited by the fact that adherence was difficult to evaluate, as plasma and intracellular levels of TDF were not quantified. Second, few patients in the cohort underwent liver biopsy and none had Fibroscan recorded at baseline; thus any association between hepatic fibrosis and HBV DNA could not be investigated.

In conclusion, TDF treatment is safe and effective in patients with prior failure of LAM and a suboptimal response to ADV therapy. There was a steady decline in serum HBV DNA levels and maintenance of viral suppression at 5 years in all compliant patients in this study, irrespective of the detection of drug resistant mutants. These findings confirm that TDF has a high genetic barrier to resistance and should be the preferred oral anti-HBV agent in patients with CHB whom fail treatment with LAM and ADV.

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Contributors: PWA conceived the study. LYL conducted the extension study and coordinated the analysis and manuscript preparation. All the authors had input in to the study design, patient management or virological analysis and critical revision of the manuscript for intellectual content.

Table 1.

Patient demographics and characteristics at year 2.

	At Year 2
Total Patients	54
Males	72% (n = 39)
Age	47 (37.8 -54.4)
Viral Load at Baseline (Log IU/ml)	5.32 (4.81-6.72)
Viral Load at Year 2 (Log IU/ml)	1.18 (1.55-1.80)
Detectable HBV DNA	33% (n = 18)
Genotype	
A	5% (n = 3)
B	15% (n = 8)
C	55% (n = 30)
D	25% (n = 13)
Ethnicity	
Asian	70% (n = 38)
Caucasian	17% (n = 9)
Other	13% (n = 7)

HBeAg Positive	67% (n = 37)
Resistance Mutation at Baseline	
Wild-Type (none detected)	26% (n = 14)
L-nucleoside (rtM204I/V)	35% (n = 19)
Acyclic phosphonate (rtM204I/V)	9% (n = 5)
rtA181T/V containing	30% (n = 16)

Values are median (interquartile range) or percentage (%)

Table 2.

Analysis of median time to undetectable HBV DNA according to baseline resistance mutation.

Resistance Changes at Baseline	Amino Acid substitutions in the rt domain	Median time to undetectable (months)	Number Positive DNA at Year 5
Wildtype (None Detected)	-	10.5 (range 3-51)	36% (5/14)
L-Nucleoside (LAM/LdT)	M204I/V	12 (range 3-45)	5% (1/19)
Acyclic phosphonate (ADV)	N236T	6 (range 1-18)	40% (2/5)
181-Containing (LAM/LdT/ADV/TFV)	A181T/V +/- N236T	24 (range 3-48)	6% (1/16)

P = 0.23

Table 3.

Factors predicting time to HBV DNA undetectability

Factors	Univariate Analysis Hazard ratio (95% CI); P value
Baseline viral load	0.619 (0.493, 0.777); 0.0000363
Genotype (Relative to genotype A, p = 0.012)	
B	0.3501 (0.104, 1.170); 0.088
C	0.2858 (0.101, 0.805); 0.0178
D	0.1385 (0.043, 0.444); 0.000871
Baseline Resistance Mutation (Relative to Acyclic phosphonate, p = 0.230)	
Double	1.374 (0.304, 6.212); 0.6796
L-nucleoside	2.860 (0.835, 9.829); 0.0952
Shared	2.061 (0.571, 7.445); 0.2697
Wildtype	1.439 (0.393, 5.265); 0.5822
Baseline HBeAg status (Relative to HBeAg negative)	1.777 (0.992, 3.184); 0.053
Gender (Relative to males)	1.648 (0.870, 3.124); 0.125
Age at commencement of TDF	1.015 (0.995, 1.036); 0.131

Table 4.

Patients' characteristics who remained HBV DNA positive at year 5

	107005	107017	108008	107007	107010	109003	102006	103001	104001
Nationality	Cambodian /Burmese	Sudanese	Macedonian	Asian	Asian	Caucasian	Caucasian	Asian	Tibetan
Genotype	C	D	D	B	C	D	D	C	D
HBeAg status	Positive	Positive	Negative	Positive	Positive	Positive until yr 4 No anti- HBe	Negative	Positive	Positive
POL sequence	N236T	wt	wt *	N236T	A181T/ N236T	wt	wt	wt	M204I/V
HBV DNA at Baseline (log IU/ml)	8	7.493	7.7	5.278	7.577	7	7.172	4.929	5.074

HBV DNA at week 96 (log IU/ml)	4.36	3.89	3.146	1.082	1.267	2.367	1.176	1.176	2.288
HBV DNA at week 240	2.16	2.42	2.17	2.16	1.36	1.50	1.74	1.62	4.289
Comments	Never reached LLOQ	Never reached LLOQ	Never reached LLOQ	LLOQ achieved by week 24 and had low viraemia at year 4 and 5	LLOQ achieved by year 3 however diagnosed & treated for NHL. Low level viraemia since year 4	LLOQ achieved by year 3, low level viraemia since year 4 and 5	LLOQ achieved by week 64 and had low level viraemia at year 4 and 5	LLOQ achieved by week 24 and had low level viraemia at year 5. Undetectable after year 5	LLOQ achieved by year 1 and had fluctuating low level viraemia. Undetectable after year 5

Note: POL sequence was at baseline. LLOQ = Lower limit of quantification.

*selected for rtA181T/N236T at year 1 & 2

Figure 1.

Red line denotes the cumulative percentage of TF109 patients achieving HBV DNA undetectability per protocol, per year, from baseline to year 5. Undetectability is defined as HBV DNA at the lower limit of quantification (LLOQ), <15 IU/ml for Abbott Real Time PCR assay or <20 IU/ml for Roche TaqMan assay. X axis denotes the time line in years, from the start of the study until completion of the study at year 5. Y axis denotes the percentage of patients at LLOQ.

Figure 2.

Blue line denotes median HBV DNA viral loads of the TDF 109 cohort per year, with error bars at 95% confidence interval. Red dotted line denotes the level of lower limit of quantification (LLOQ, which is <15 IU/ml for Abbott Real Time PCR assay or <20 IU/ml for Roche TaqMan assay).

Figure 3.

This graph illustrates individual viral load profiles of the nine TF109 patients who remained HBV DNA positive at the end of the study at year 5. Each colored line highlights a different patient. The numbers denotes the code given to each patient, depending on their site and individual patient numbers. Black line represents the level of lower limit of quantification (LLOQ, which is <15 IU/ml for Abbott Real Time PCR assay or <20 IU/ml for Roche TaqMan assay). X axis denotes the time line in years, from the start of the study until completion of the study at year 5. Y axis denotes the HBV DNA level in Log₁₀ IU/ml.

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