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Kinetics of hepatitis B surface antigen loss in patients with HBeAg-positive chronic hepatitis B treated with tenofovir disoproxil fumarate

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Abstract

Background & Aims—In a study of 266 chronic hepatitis B e antigen (HBeAg)-positive patients, 23 experienced hepatitis B surface antigen (HBsAg) loss with up to 5 years of tenofovir disoproxil fumarate (TDF) treatment. HBsAg kinetics in patients with and without HBsAg loss and predictors of HBsAg loss were evaluated.

Methods—HBsAg levels were quantified every 12 weeks. A multivariable regression analysis, involving prespecified baseline characteristics and on-treatment response parameters, was performed; a stepwise procedure identified independent predictors of HBsAg loss.

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Conflict of interest

Patrick Marcellin has received research grants from Hoffman-La Roche, Gilead Sciences, Inc., Bristol-Myers Squibb, Vertex Pharmaceuticals, Novartis Pharmaceuticals, Janssen/Tibotec, Merck, Boehringer Ingelheim, Abbott Laboratories, and Pfizer and has received financial compensation for consultancy and/or lecture activities from Hoffman-La Roche, Gilead Sciences, Inc., Bristol-Myers Squibb, Novartis Pharmaceuticals, Janssen/Tibotec, and Merck. Maria Buti has received research grants from Gilead Sciences, Inc. and served on advisory boards for Gilead Sciences, Inc. and Bristol-Myers Squibb. Zahari Krastev has received research grants from Gilead Sciences, Inc. Robert A. de Man has received research grants from Gilead Sciences, Inc. Stefan Zeuzem has received financial compensation for consultancy and/or lecture activities from Abbott Laboratories, Achillion, AstraZeneca, Bristol-Myers Squibb, Boehringer Ingelheim, Gilead Sciences, Inc., Idenix, Janssen-Cilag, Merck, Novartis Pharmaceuticals, Presidio, Roche, Santaris, and Vertex Pharmaceuticals. Robert Flisiak has received financial compensation for consultancy and/or lecture activities from Gilead Sciences, Inc. Selim Gurel serves on the advisory board for Bristol-Myers Squibb, Merck, and Roche. Geoffrey M. Dusheiko has received research grants and financial compensation for consultancy activities from Bristol-Myers Squibb and Gilead Sciences, Inc. E. Jenny Heathcote has no commercial relationships to disclose. Anuj Gaggar, John F. Flaherty, Jr., Lillian Lou, Benedetta Massetto, Lanjia Lin, Phillip Dinh, G. Mani Subramanian, and John G. McHutchison are employees and stockholders of Gilead Sciences, Inc.

Results—Among patients with HBsAg loss, 14 (61%), 1 (4%), 0 and 7 (30%) were genotypes A through D, respectively; 1 (4%) was genotype F. HBsAg loss was preceded by viral suppression (HBV DNA <29 IU/ml; n = 23) and HBeAg loss (n = 19). Among treated patients the strongest independent predictors of HBsAg loss were Caucasian race with genotype A/D and 4 years of infection (HR = 14.3, 95% confidence interval [CI] 4.7–43.4; $p < 0.0001$) and an HBsAg decline of $1 \log_{10}$ IU/ml at week 24 (HR = 13.7, 95% CI 5.6–33.7; $p < 0.0001$). Among TDF-treated patients, a reduction in HBsAg level of $1 \log_{10}$ by week 12 or 24 had a positive predictive value of 35%–45%, respectively, and a negative predictive value of 94%–97%, respectively.

Conclusions—HBsAg loss in HBeAg-positive patients receiving TDF involves a chronology of virologic and serologic responses; patients with HBV genotypes A or D and a rapid early decline in HBsAg are more likely to lose HBsAg.

Keywords

Chronic hepatitis B; Hepatitis B surface antigen; Tenofovir disoproxil fumarate

Introduction

The ultimate goal of antiviral treatment for patients with chronic hepatitis B (CHB) infection is to prevent disease progression to cirrhosis, hepatocellular carcinoma and liver-related mortality. For most patients this requires lifelong treatment with oral antiviral agents. In a small subset of patients who receive oral antiviral treatment, loss of hepatitis B surface antigen (HBsAg), with or without seroconversion, can occur [1,2]. Sustained HBsAg loss is associated with a complete and definitive remission of CHB activity and improved long-term outcome [1–4]. Studies with interferon therapy have demonstrated that up to 8% of hepatitis B e antigen (HBeAg)-positive patients may achieve this end point; however, loss of HBsAg and seroconversion to anti-HBs with long-term oral antiviral treatment have not been well characterized [3–5].

Tenofovir disoproxil fumarate (TDF) has demonstrated efficacy superior to adefovir dipivoxil (ADV) at 48 weeks in controlled trials in HBeAg-positive and -negative patients, as well as good safety and tolerability, with no resistance detected through 5 years of treatment [6–8]. In a study of HBeAg-positive patients, a total of 23 patients (10% by Kaplan-Meier [KM] percentage estimate) had confirmed HBsAg loss with up to 5 years of TDF treatment [6]. Here, we provide a detailed summary of the baseline and on-treatment characteristics of these patients with HBsAg loss in comparison to those who did not experience HBsAg loss with TDF treatment. The temporal sequence of clinical and laboratory events associated with HBsAg response in individual patients as well as the predictors of HBsAg loss are presented.

Patients and methods

Study descriptions

Study 103 (NCT00116805) evaluated the safety and efficacy of TDF 300 mg once daily in patients with HBeAg-positive CHB. The study design and methodology have been previously described [6–8]. Patients were randomized 2:1 to TDF or ADV for a 48-week

double-blind phase, after which both treatment groups were eligible to receive open-label TDF for up to an additional 7 years.

HBsAg assessments

The presence of HBsAg was qualitatively assessed by the AUSZYME monoclonal enzyme immunoassay (EIA) (Abbott Laboratories, Abbott Park, IL) and loss of HBsAg was defined as a negative test. Seroconversion to anti-HBs was determined in patients with loss of HBsAg by the AUSAB EIA (Abbott Laboratories, Abbott Park, IL). In addition, levels of HBsAg (IU/ml) in serum were quantified every 12 weeks by the ARCHITECT assay (Abbott Laboratories, Abbott Park, IL), having a lower detection limit of 0.05 IU/ml (linear range 0.05–250 IU/ml). Quantitative HBsAg levels were analysed retrospectively on samples stored at -70°C . All samples were initially analysed without dilution; if the result was >250 IU/ml, the sample was diluted 1:500. If the result was $>125,000$ IU/ml, further dilution at 1:999 was performed. For results $>249,750$ IU/ml, a value of 249,751 IU/ml was used.

Statistical analyses

Descriptive statistics (median and range for continuous variables, frequency and percentage for categorical variables) were used to summarize baseline demographics and disease characteristics for patients with and without HBsAg loss. The Fisher's exact and Wilcoxon rank sum tests were used to compare categorical and continuous variables, respectively. The Kaplan-Meier methodology was used to summarize the probability of patients achieving HBsAg loss. The Kaplan-Meier methodology was also used to characterize the sequence of events: HBV DNA <29 IU/ml, HBeAg loss, HBeAg seroconversion, HBsAg loss, and HBsAg seroconversion. Cox proportional hazard models were used to elucidate significant predictors of HBsAg loss, both at baseline and on-treatment. Predictors found significant in the univariate analyses were used in a stepwise multivariate analysis to identify significant predictors. Two sets of analyses were performed: one on all ($n = 23$) patients and another only for those initially randomized to and continued on TDF ($n = 16$). For each set of analyses, one analysis was performed on baseline factors, while a second analysis was performed on both baseline and on-treatment factors. Positive and negative predictive values for ultimate HBsAg loss based on initial declines of quantitative HBsAg levels were also generated. The time profiles of changes in serum alanine aminotransferase (ALT), quantitative HBsAg, and HBV DNA levels for patients with HBsAg loss were also presented graphically.

Results

Over a median of 240 weeks (range 4–264 weeks) of treatment, 23 patients, 16 initially randomized to TDF, and 7 to ADV (10% by Kaplan-Meier estimate, 95% confidence interval [CI] 6.8% to 14.7%) achieved HBsAg loss. 18 of these patients, 11 randomized to TDF and 7 to ADV, (8% by Kaplan-Meier estimate, 95% CI 5.1% to 12.5%) also experienced anti-HBs seroconversion. Of 7 patients, with HBsAg loss over 5 years and initially randomized to ADV, none achieved loss of HBsAg until switching to TDF treatment after week 48 (Fig. 1A). In contrast, of 16 patients with HBsAg loss over 5 years and

initially randomized to TDF, 5 patients achieved HBsAg loss, including 2 with seroconversion on or before week 48 of treatment. Of the 23 patients with HBsAg loss, 20 discontinued study treatment (TDF) and entered treatment-free follow-up while 3 patients continued on-treatment through week 240. Among 20 patients entering treatment-free follow-up, 12 remained on study through week 240, while 8 patients discontinued early (6 for seroconversion, 1 withdrew consent, and 1 was lost to follow-up) and were no longer available for further follow-up assessments. All but 1 patient had continued HBV DNA suppression (<29 IU/ml) off-treatment; 1 patient had seroreversion with a transient rise in HBV DNA to 343 IU/ml and subsequently resuppressed HBV DNA and lost HBsAg again after TDF was reinstituted. Further, for patients with results below the limit of quantification of the HBV DNA assay (<29 IU/ml), results reported as “target not detected” were seen in 4/20 (20%) and 14/20 (70%) at the time of TDF discontinuation and at week 240 or at the last available study visit, respectively.

Six of 23 patients with HBsAg loss (1 initially randomized to ADV and 5 to TDF) had paired liver biopsy data available at baseline and year 5, including 3 patients with baseline cirrhosis (Ishak fibrosis score of 6 in all instances). At year 5, by Ishak scoring, of the 3 patients who were non-cirrhotic at baseline, 1 patient each improved by 1 and 2 points, while 1 patient had no change in the fibrosis score. Of the 3 cirrhotic patients, 2 showed a 3-point improvement in the fibrosis score, while 1 had no change at year 5. These results were generally comparable to those for the entire cohort with available histology data at year 5 [6].

Baseline characteristics for patients with HBsAg loss ($n = 23$) in comparison to the other patients who did not achieve HBsAg loss ($n = 243$) are summarized in Table 1. Overall, significant differences were found between groups with regard to race, baseline HBsAg and HBV DNA levels, patient-reported duration of HBV infection, and HBV genotype (Table 1). In comparison to those without HBsAg loss, a higher percentage of patients with HBsAg loss were Caucasians (91%). Of note, there were no Asian patients with HBsAg loss, despite 36% (96/266) of the overall study population being of Asian descent (32% and 62% of whom were genotypes B and C, respectively). At baseline, patients with HBsAg loss had higher plasma levels of hepatitis B virus (HBV) DNA and higher serum HBsAg titres, in comparison to those without HBsAg loss. Genotypes A ($n = 14$; 61%) and D ($n = 7$; 30%) were most common among patients with HBsAg loss, and in contrast, no patients with genotype C, and only 1 patient each with genotypes B and F experienced HBsAg loss.

The chronology of virologic and serologic responses in patients with HBsAg loss is presented in Fig. 1B. Virologic suppression (HBV DNA <29 IU/ml) was achieved prior to HBsAg loss in all patients (median [range] of 280 [54–1113] days). Serologically, 19 out of 23 patients had loss of HBeAg before or at the time of confirmed HBsAg loss (median [range] of 223 [0–1093] days), 1 patient with HBsAg loss had loss of HBeAg that occurred at the last visit and could not be confirmed, and 3 patients had confirmed loss of HBeAg after loss of HBsAg (Fig. 1B). In 18 out of 22 patients with both confirmed HBeAg loss and HBsAg loss, seroconversion to anti-HBe preceded the loss of HBsAg (median [range] of 141 [0–1093] days); 2 patients achieved anti-HBe seroconversion after HBsAg loss, while 2 had HBeAg loss without seroconversion (Fig. 1B). In the 23 patients with HBsAg loss, the

magnitude of change in HBsAg levels from baseline to week 240 or the last available visit was greater, compared to those without HBsAg loss (mean [SD] change -4.9 [0.5] vs. -0.8 [0.9] $\log_{10}$ IU/ml, respectively).

Dynamics of serum HBsAg levels in relation to ALT values

The dynamics of change in HBV DNA, ALT, and HBsAg levels are illustrated for each individual patient with HBsAg loss in Fig. 2A and B. Of note, 10 out of 23 (43%) patients experienced a marked, transient increase in serum ALT (i.e., >5 – 10 times the upper limit of normal range [43 U/L for males, 34 U/L for females]), usually within the first 96 weeks of treatment. These rises in ALT levels generally preceded loss of HBsAg; however, it cannot be concluded that this phenomenon is causative of HBsAg loss, as similar on-treatment flares were also observed in patients without HBsAg loss (data not shown).

Kinetics of HBsAg decline in relation to viral genotype

The impact of HBV genotype on HBsAg decline and HBsAg loss was further explored and is shown in Fig. 3. Regardless of genotype, or initial treatment assignment (TDF or ADV), patients without HBsAg loss showed only slight initial declines in HBsAg levels (median decrease of $0.5 \log_{10}$ IU/ml at week 12), with a subsequent plateau over 240 weeks of continuous treatment. In contrast, steeper initial declines in HBsAg levels were clearly evident for genotype A and D patients, the genotype B patient, and the genotype F patient (data not shown) who achieved HBsAg loss.

Predictors of HBsAg loss among patients initially randomized to TDF

Given the overall slower kinetics of HBsAg decline with ADV compared with TDF (Fig. 2A and B), we investigated baseline and on-treatment factors for patients initially randomized to TDF who experienced HBsAg loss ($n = 16$) and all patients who experienced HBsAg loss ($n = 23$). In the univariate model for patients initially randomized to TDF, a number of factors were significantly associated with loss of HBsAg (Table 2A). Highly significant baseline factors included HBV genotype (A/D vs. B/C), Caucasian race, baseline level of HBsAg and HBV DNA, and baseline Knodell necroinflammatory score. On-treatment factors of significance included loss of HBeAg by week 24 of treatment, decline in serum HBsAg level of at least $1 \log_{10}$ IU/ml or decline in HBsAg level of $\geq 75\%$ at either week 12 or week 24 (Table 2B). In the multivariate analysis, significant individual baseline predictors of HBsAg loss were HBsAg level, the composite of Caucasian race with genotype A or D, a reported duration of HBV infection of ≥ 4 years, and the Knodell necroinflammatory score (Table 2C). Moreover, significant independent factors most significantly associated with HBsAg loss included HBV genotype (A or D), loss of HBeAg at week 24, and decline in HBsAg level of at least $1 \log_{10}$ IU/ml or $\geq 75\%$ at week 24 (Table 2D). We therefore explored the predictive value of various dichotomous levels of HBsAg reduction (0 – 0.5 , 0.5 , $1.0 \log_{10}$ IU/ml; 0 – 50% , 50% , $\geq 75\%$) at week 12 or week 24 with the ultimate achievement of HBsAg loss (Table 3). A lack of appreciable decline in HBsAg levels ($< 0.5 \log_{10}$) at week 12 or 24 had a very high negative predictive value (NPV $>90\%$); in contrast, a $\geq 1 \log_{10}$ decline in HBsAg level by week 12 or 24 had the highest positive predictive value (PPV), 34.8% and 44.8% respectively, for progressing to HBsAg loss.

Discussion

Loss of HBsAg and seroconversion to anti-HBs is an important goal of therapy in patients with CHB infection. Unfortunately, loss of HBsAg occurs infrequently in CHB patients, treated long-term with oral antiviral agents [5,9,10]. This report provides an analysis of the characteristics and predictive factors associated with HBsAg loss during long-term treatment with TDF of HBeAg-positive patients. Although individual patient responses varied, definitive trends were observed regarding the chronology of the virologic and serologic responses that accompany loss of HBsAg in this patient population. Finally, when predictive factors were explored by multivariate analysis, the $\log_{10}$ IU/ml decline in HBsAg levels by week 24 was a strong independent predictor of ultimate HBsAg loss.

Why do some patients lose HBsAg while others (the majority) do not despite similar levels of suppression of HBV replication? Why is this phenomenon only rarely observed in HBeAg negative patients [6,11,12]? The chronological sequence of events we observed, namely HBV DNA suppression, HBeAg loss, and then HBsAg loss is not an unexpected finding [12], and may reflect virion clearance from the liver or clearance of infected hepatocytes. Most patients who lost HBsAg experienced pronounced reductions in HBsAg levels in the first 6 months of treatment, suggesting a susceptibility to either rapid downregulation of HBV replication, or hepatocyte lysis in patients primed to lose HBeAg and HBsAg. HBsAg loss was more common in patients with higher baseline concentrations of HBsAg, which was surprising as the reverse is true for interferon-treated patients [13].

The magnitude of HBsAg decline as a predictor of HBsAg loss has also been observed with other treatments for HBV infection [11,12,14–16]. Although not as well characterized, transient elevations in ALT values as an indication of loss of HBsAg and potential anti-HBs seroconversion have also been previously noted during the natural course of HBV infection, and also with treatment for HBV infection [17,18]. These observations reflect the association of HBsAg decline with the development of a vigorous immune response, biochemically evidenced by an ALT “flare” or rise in serum ALT level, which in turn leads to immune clearance of infected liver cells [17]. HBsAg loss is not associated with the eradication of HBV infection since HBV DNA can be detected after spontaneous or treatment induced HBsAg loss [19,20]. However, HBsAg loss does mean improved clinical outcome with no risk of reactivation of the liver disease in immunocompetent patients. In our population, nearly all patients who discontinued TDF had HBV DNA <29 IU/ml maintained through week 240 or the last available study visit. The majority of these patients had full viral suppression as indicated by “no target detected” PCR assay results.

Our data do not provide an explanation for the apparent greater likelihood of HBsAg loss in patients with genotype A, or the cellular mechanisms of clearance in these patients. The same observation has been made in patients treated with interferon [21]. These data suggest that the effector limbs of the immune response in patients with varying genotypes require further study [22]. Interestingly, HBsAg loss seems to occur earlier under TDF treatment, whereas it can occur years after the interruption of treatment with interferon [5,23]. These observations argue for studying the effects of the combination of interferon with TDF as a means to accelerate the decline of HBsAg levels and increase the rate of HBsAg loss.

Data on HBsAg loss during long-term treatment with other oral antiviral agents are limited, although it has long been recognized that some patients lose HBsAg following antiviral therapy [9–12,20,24]. A retrospective analysis of a randomized phase 3 study of entecavir vs. lamivudine in HBeAg-positive patients found that at 96 weeks of treatment, HBsAg loss was seen in 5% of patients who received entecavir and in 3% of patients randomized to receive lamivudine [25]. After up to 5 years of entecavir treatment, 2/145 (1.4%) HBeAg-positive patients lost HBsAg [9]. During 3 years of telbivudine treatment, HBsAg loss was observed in 6% of patients [16]. Conversely, 1 year of pegylated interferon- α therapy has been associated with HBsAg loss in 11% and 12% of patients, respectively, at 3 and 5 years after treatment cessation, in HBeAg-positive as well as in HBeAg-negative patients [14,26]. Notably, our finding of 10% HBsAg loss in HBeAg-positive patients after 5 years of TDF treatment is consistent with these prior results using pegylated interferon- α therapy. Although varied, these results suggest a common theme, as also evidenced in the present trial, that an initial rapid decline in HBsAg level is significantly associated with the eventual loss of HBsAg, and in most instances, subsequent seroconversion to anti-HBs.

In our analysis, loss of HBsAg was more likely to occur in patients who were Caucasian, and was not observed to occur in patients of Asian descent. HBV genotype is likely to play a role in this observation, as loss of HBsAg was more common in patients with genotypes A and D, both of which are more commonly found in non-Asian populations [10,27]. Conversely, genotypes B and C were not as well represented in our cohort of patients with HBsAg loss despite constituting a reasonably high proportion of the overall study population (13% genotype B, and 26% genotype C). Indeed, when the kinetics of HBsAg decline were evaluated by baseline genotype, the steepest declines were observed with genotypes A and D. Furthermore, consistent with previous studies [12,28], in our analysis, a reduction in HBsAg levels of $-1 \log_{10}$ IU/ml at week 24 has a very high NPV (97%) and modest PPV (45%; Table 3) for ultimate HBsAg loss.

In conclusion, 10% of HBeAg-positive patients treated long-term with TDF experienced loss of HBsAg and 8% had seroconversion to anti-HBs. The few patients who achieved loss of HBsAg were mostly genotypes A and D and all demonstrated steeper initial declines in serum HBsAg titres. These results are encouraging as they help to inform future studies, including immune-based approaches to enhance rates of HBsAg loss in chronic HBV patients.

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Abbreviations

CHB chronic hepatitis B

HBsAg	hepatitis B surface antigen
HBeAg	hepatitis B e antigen
TDF	tenofovir disoproxil fumarate
HBV	hepatitis B virus
KM	Kaplan-Meier
EIA	enzyme immunoassay
CI	confidence interval
ALT	alanine aminotransferase
NPV	negative predictive value
PPV	positive predictive value

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Fig. 1. Time to loss of HBsAg (Kaplan-Meier estimate) and corresponding chronology of virologic and serologic responses in 23 HBeAg-positive patients who achieved HBsAg loss
(A) Time to loss of HBsAg by initial blinded treatment assignment. TDF-TDF and ADV-TDF correspond to subjects randomized to receive TDF or ADV, respectively, for the first 48 weeks of double-blind treatment, followed by open-label TDF through week 240. (B) Time to first confirmed virologic (HBV DNA <29 IU/ml) and serologic (HBeAg loss and seroconversion, and HBsAg loss and seroconversion) responses through week 240. A confirmed event was defined as an event observed in two or more consecutive visits (on- or off-treatment). (This figure appears in colour on the web.)

Fig. 2. Dynamics of change in plasma HBV DNA concentrations ($\log_{10}$ copies/ml), serum ALT levels (U/L), and serum HBsAg levels ($\log_{10}$ IU/ml) over 240 weeks

(A) Changes in HBV DNA (triangles), ALT (diamonds), and HBsAg (circles) levels in the 16 HBeAg-positive patients initially randomized to TDF (TDF-TDF). (B) Changes in the 7 HBeAg-positive patients initially randomized to ADV (ADV-TDF) who achieved HBsAg loss. (This figure appears in colour on the web.)

Fig. 3. Kinetics of HBsAg decline in relation to viral genotype

Included are results for 22 HBeAg-positive patients (genotype A [n = 14], B [n = 1], and D [n = 7] – closed circles) who achieved HBsAg loss and 224 HBeAg-positive patients (genotype A [n = 45], B [n = 34], C [n = 68], and D [n = 77] – open circles) who did not achieve loss of HBsAg over 240 weeks.

Table 1

Baseline clinical and demographic characteristics.

Characteristics	With HBsAg loss (n = 23)	No HBsAg loss (n = 243)	p value [†]
Median (ranges) age, yr	35 (20, 64)	32 (18, 63)	0.261
Race, n (%)			
Caucasian	21 (91.3)	117 (48.2)	< 0.001
Asian	0	96 (39.5)	
Black	2 (8.7)	16 (6.6)	
Others	0	14 (5.8)	
Male, n (%)	18 (78.3)	165 (67.9)	0.356
Median (range) baseline HBsAg, log ₁₀ IU/ml		n = 241 *	<0.001
	5.1 (3.3, 5.4)	4.5 (1.0, 5.4)	
Median (range) baseline HBV DNA, log ₁₀ IU/ml	8.5 (6.9, 8.9)	8.1 (3.9, 10.2)	0.024
Median (range) baseline ALT, U/L	140 (50, 425)	109 (23, 964)	0.169
Knodell necroinflammatory score	n = 22	n = 238	0.492
0–3	0	14 (5.9)	
4–6	1 (4.5)	28 (11.8)	
7–9	13 (59.1)	135 (56.7)	
10–14	8 (36.4)	61 (25.6)	
Knodell fibrosis score	n = 22	n = 237	0.645
1	8 (36.4)	102 (43.0)	
3	8 (36.4)	90 (38.0)	
4	6 (27.3)	45 (19.0)	
Previous interferon experience, n (%)	3 (13.0)	40 (16.5)	>0.999
Previous lamivudine experience >12 wk, n (%)	1 (4.3)	8 (3.3)	0.563
Median (range) years positive for HBV [‡]	n = 21	n = 241	<0.001
	1.1 (0.6, 12.7)	4.4 (0.1, 49.5)	
HBV genotype, n (%)			<0.001
A	14 (60.9)	45 (18.5)	
B	1 (4.4)	34 (14.0)	
C	0	69 (28.4)	
D	7 (30.4)	78 (32.1)	

Characteristics	With HBsAg loss (n = 23)	No HBsAg loss (n = 243)	p value [‡]
E	0	5 (2.1)	
F	1 (4.4)	7 (2.9)	
Others/unable to genotype	0	5 (2.1)	

ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; IQR, interquartile range.

* Sample sizes are indicated if they differ from the total sample size in each group.

[‡] p values are from Fisher's exact test for categorical variables and Wilcoxon rank sum test for continuous variables.

[†] Patient reported.

Predictors for time to HBsAg loss over 240 weeks in HBeAg-positive patients treated with tenofovir disoproxil fumarate**Table 2**

(A) Significant baseline predictors for time to HBsAg loss through week 240 in HBeAg-positive patients (univariate analysis*). *Other factors considered in the analysis but not statistically significant were: age, ALT, body weight, body mass index, gender, previous interferon experience, previous lamivudine experience, previous exposure to HBV therapy, and baseline cirrhosis (present vs. not present). Grey boxes = does not apply. ADV, adefovir dipivoxil; ALT, alanine aminotransferase; CI, confidence interval; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HR, hazard ratio; TDF, tenofovir disoproxil fumarate. (B) Significant on-treatment predictors for time to HBsAg loss through week 240 in HBeAg-positive patients (univariate analysis*). *Other baseline factors considered but not statistically significant: HBV DNA <69 IU/ml at weeks 12 and 24, normal ALT levels at weeks 12 and 24, having HBeAg loss at week 12. ADV, adefovir dipivoxil; ALT, alanine aminotransferase; CI, confidence interval; HBV, hepatitis B virus; HBsAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HR, hazard ratio; TDF, tenofovir disoproxil fumarate. (C) Significant baseline predictors for time to HBsAg loss through week 240 in HBeAg-positive patients (multivariate analysis*). *Baseline factors that were significant in a univariate analysis were included in a stepwise multivariate analysis. Grey boxes = does not apply. CI, confidence interval; HBsAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HR, hazard ratio. (D) Significant baseline and on-treatment predictors for time to HBsAg loss through week 240 in HBeAg-positive patients (multivariate analysis*). *Baseline and on-treatment factors that were significant in univariate analysis were included in a stepwise multivariate analysis. Grey boxes = does not apply. ADV, adefovir dipivoxil; CI, confidence interval; HBsAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HR, hazard ratio; TDF, tenofovir disoproxil fumarate.

Variable	All patients [†] (n = 23/266)			TDF-TDF patients [‡] (n = 16/176)		
	HR	95% CI	p value	HR	95% CI	p value
Caucasian with genotype A or D, and 4 years of infection [§]	6.58	(2.71–15.99)	<0.0001	5.44	(1.98–14.98)	0.0010
HBsAg level (log ₁₀ IU/ml)	5.44	(2.13–13.89)	0.0004	4.18	(1.48–11.82)	0.0071
Duration of HBV infection, 4 years [§]	11.14	(2.59–47.82)	0.0012	8.40	(1.89–37.26)	0.0051
Race: Caucasian vs. non-Caucasian	9.69	(2.27–41.33)	0.0021	6.43	(1.46–28.30)	0.0138
Genotype: A/D vs. B/C	15.12	(2.03–112.44)	0.0080	9.97	(1.31–75.80)	0.0263
Knodell necroinflammatory score				1.47	(1.04–2.08)	0.0290
HBV DNA (log ₁₀ copies/ml)	1.71	(1.05–2.81)	0.0326	1.82	(1.01–3.30)	0.0467

B

Variable	All patients [†] (n = 23/266)			TDF-TDF patients [‡] (n = 16/176)		
	HR	95% CI	p value	HR	95% CI	p value
1 log ₁₀ IU/ml decline in HBsAg at week 12	6.12	(2.59–14.48)	<0.0001	8.34	(3.12–22.33)	<0.0001
1 log ₁₀ IU/ml decline in HBsAg at week 24	10.50	(4.62–23.85)	<0.0001	20.53	(6.59–63.96)	<0.0001
75% decline in HBsAg at week 12	5.96	(2.63–13.52)	<0.0001	10.77	(3.73–31.10)	<0.0001
75% decline in HBsAg at week 24	6.79	(2.88–16.03)	<0.0001	21.37	(4.85–94.13)	<0.0001
HBeAg loss at week 24	2.85	(1.12–7.22)	0.0276	4.66	(1.69–12.83)	0.0029

C

Variable	All patients [†] (n = 23/266)			TDF-TDF patients [‡] (n = 16/176)		
	HR	95% CI	p value	HR	95% CI	p value
HBsAg level (log ₁₀ IU/ml)	3.31	(1.22–8.99)	0.0191	4.86	(1.41–16.71)	0.0121
Caucasian with genotype A or D, and 4 years of infection [§]	7.63	(2.51–23.21)	0.0003	7.86	(2.39–25.82)	0.0007
Knodel necroinflammatory score				1.71	(1.15–2.55)	0.0086

D

Variable	All patients [†] (n = 23/266)			TDF-TDF patients [‡] (n = 16/176)		
	HR	95% CI	p value	HR	95% CI	p value
1 log ₁₀ IU/ml decline in HBsAg at week 24	13.69	(5.56–33.71)	<0.0001	5.232	(1.12–24.48)	0.0356
Caucasian with genotype A or D, and 4 years of infection [§]	14.26	(4.69–43.39)	<0.0001			
Genotype: A/D vs. B/C				18.459	(2.37–143.95)	0.0054
HBeAg loss at week 24				3.392	(1.13–10.19)	0.0296
75% decline in HBsAg at week 24				7.975	(1.12–56.81)	0.0382

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23 of 266 TDF-TDF and ADV-TDF patients had HBsAg loss through week 240.
‡ 16 of 176 TDF-TDF patients had HBsAg loss through week 240.
§ Patient reported.

Table 3

Exploratory measures of HBsAg decline at weeks 12 and 24 for HBeAg-positive patients on TDF and corresponding positive and negative predictive values for ultimate HBsAg loss.

Measure of decline in HBsAg level	Study time point	Percentage of patients (n/N)	Positive predictive value (%)	Negative predictive value (%)
0–0.5 log ₁₀ IU/ml	Week 12	55 (94/171)	6.4	85.7
	Week 24	39 (66/170)	0	83.7
0.5 log ₁₀ IU/ml	Week 12	26 (44/171)	25.0	95.3
	Week 24	34 (57/170)	29.8	100
1.0 log ₁₀ IU/ml	Week 12	13 (23/171)	34.8	93.9
	Week 24	17 (29/170)	44.8	97.2
0–50%	Week 12	42 (71/171)	5.6	87.0
	Week 24	24 (41/170)	0	86.8
50%	Week 12	39 (67/171)	19.4	96.2
	Week 24	48 (82/170)	20.7	100
75%	Week 12	21 (36/171)	30.6	95.6
	Week 24	28 (48/170)	31.3	98.4

HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen.