

# Persistent Viremia in Human Immunodeficiency Virus/Hepatitis B Coinfected Patients Undergoing Long-Term Tenofovir: Virological and Clinical Implications

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Tenofovir (TDF) is considered the ideal treatment for patients coinfecting with human immunodeficiency virus (HIV) and hepatitis B virus (HBV). However, certain coinfecting patients exhibit incomplete viral suppression, with persistent, and sometimes transient, bouts of HBV replication. The reasons for this, including clinical effect, are unclear. A total of 111 HIV-HBV-infected patients undergoing TDF-containing antiretroviral therapy were prospectively followed. Serum HBV-DNA viral load, hepatitis surface (HBsAg) and e antigen (HBeAg) status were obtained at baseline and every 6-12 months. Amino acid (aa) changes on the polymerase gene were assessed using direct sequencing after nested polymerase chain reaction in patients with persistent viremia (PV). After a median of 74.7 months (interquartile range: 33.4-94.7), virological response (VR; <60 IU/mL) occurred in 96 of 111 (86.5%) patients. Of these, 86 of 96 (89.6%) remained completely undetectable during follow-up (stabilized VR). The remaining 10 of 96 (10.4%) patients had a transient blip of detectable HBV-DNA (transient PV), during which time 9 of 9 (100%) with available samples had detectable plasma TDF. Low-level PV (LL-PV; 61-2,000 IU/mL) was observed in 11 of 111 (9.9%) patients, the majority of which had detectable plasma TDF (8 of 9; 88.9%). High-level PV (>2,000 IU/mL) was rare (4 of 111; 3.6%) and was associated with nonadherence. At TDF initiation, patients with stabilized VR had significantly higher nadir CD4<sup>+</sup> count, compared to those with transient PV ( $P = 0.006$ ) or LL-PV ( $P = 0.04$ ). No consistent aa changes, other than those associated with lamivudine resistance, were observed in patients with persistent viremia. Importantly, HBeAg loss, HBeAg seroconversion, and HBsAg loss only occurred in patients with stabilized VR. Two patients with stabilized VR developed hepatocellular carcinoma and 2 with LL PV died, 1 of a liver-related cause. **Conclusion:** Suboptimal HBV control during TDF treatment has a negative effect on serological outcomes, but not necessarily clinical events. Immunoregulation may provide more insight into this phenomenon. (HEPATOLOGY 2014;00:000-000)

In patients infected with human immunodeficiency virus (HIV), hepatitis B virus (HBV) has emerged as a major cause of morbidity and mortality, accounting for substantial rises in cirrhosis, hepatocel-

lular carcinoma (HCC), and end-stage liver disease.<sup>1</sup> The incidence of these diseases can be greatly curbed by controlling HBV replication.<sup>2,3</sup> There are currently a variety of antiviral drugs available for suppressing

Abbreviations: aa, amino acid; aOR, adjusted odds ratio; ADV, adefovir; ALT, alanine transaminase; ARV, antiretroviral; AST, aspartate transaminase; CI, confidence interval; ETV, entecavir; FTC, emtricitabine; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HDV, hepatitis D virus; HIV, human immunodeficiency virus; HL-PV, high-level persistent viremia; IQR, interquartile range; LAM, lamivudine; LL-PV, low-level persistent viremia; LOWESS, locally weighted scatterplot smoothing; NA, nucleoside/nucleotide analog; PCR, polymerase chain reaction; Peg-IFN, pegylated interferon; PEIU, Paul Ehrlich Institute units; pol, polymerase; PV, persistent viremia; qHBeAg, quantification of hepatitis B e antigen; qHBsAg, quantification of hepatitis B surface antigen; RT, reverse transcriptase; TDF, tenofovir; VL, viral load; VR, virological response.

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HBV, such as lamivudine (LAM), adefovir (ADV), entecavir (ETV), and tenofovir (TDF). The lattermost antiviral is ideal for treating coinfecting patients as a result of its dual anti-HBV and -HIV activity as well as superior potency in suppressing HBV-DNA replication.<sup>4,5</sup> Coupled by the fact that no mutations associated with TDF resistance have been consistently described in both HBV- and HIV-HBV- infected patients over extensive follow-up,<sup>4,6</sup> it would then appear that optimal HBV suppression could be easily achieved in coinfecting patients.

However, recent data have emerged suggesting that some patients exhibit suboptimal virological suppression after long periods of antiretroviral (ARV) therapy, where serum HBV-DNA could transiently, and even persistently, replicate.<sup>7</sup> A number of reasons for persistent viremia have been broached in the literature. First, guidelines often point toward poor adherence as a major source of unfavorable virological outcomes,<sup>8</sup> which may likely be the case given previous research.<sup>6,7</sup> Second, HBV-specific T-cell response and intrahepatic T-cell activation are diminished in the presence of HIV, with incomplete reconstitution during prolonged ARV therapy.<sup>9,10</sup> Immunological features of HIV-HBV coinfection, including degree of immunosuppression, might explain why certain patients exhibit suboptimal HBV suppression. Third, data from *in vitro* studies suggest that certain amino acid (aa) substitutions on the polymerase (*pol*) gene, in combination with *precore* mutations, can give rise to TDF resistance,<sup>11,12</sup> despite strong clinical evidence against it.<sup>13,14</sup>

The aim of the present study was then to thoroughly evaluate various virological and clinical characteristics in explaining suboptimal virological suppression in HIV-HBV coinfecting patients undergoing extensive treatment with TDF-containing ARV therapy. We then develop our analysis by further exploring the effect of persistent viremia on HBV serological outcomes, which include two markers gaining substantial interest in predicting therapeutic success— hepatitis B surface antigen quantification (qHBsAg)

and hepatitis B e antigen quantification (qHBeAg).<sup>15</sup> Finally, we wished to determine whether patients experiencing persistent viremia are at risk of developing liver-related morbidity or mortality.

## Patients and Methods

**Patients.** Patients were selected from the French HIV-HBV Cohort Study.<sup>16</sup> Briefly, this prospective study recruited 308 patients from seven centers located in Paris and Lyon, France, during May 2002–May 2003. Patients were included if they had HIV-positive serology confirmed by western blotting and HBsAg-positive serology for at least 6 months. Data collection continued until 2010–2011.

We retained individuals from a previous analysis of 143 patients undergoing a TDF-containing ARV regimen.<sup>15</sup> Additionally, we decided to only include patients with  $\geq 12$  months of follow-up, because virological response in patients undergoing nucleoside/nucleotide analog (NA) therapy may require extensive periods of follow-up to be fully evaluated,<sup>17</sup> particularly in HIV-HBV coinfection. As a result, 13 patients were excluded. Second, follow-up was right censored for patients who discontinued TDF use or had pegylated interferon (Peg-IFN) intensification, resulting in the exclusion of, respectively, 7 and 5 patients because of short follow-up (<12 months). Finally, patients with baseline hepatitis C virus (HCV)- or hepatitis D virus (HDV)-positive serology had been excluded in the previous analysis. However, we additionally excluded patients with incident HCV infection ( $n = 2$ ) or with HCV/HDV infection later discovered during follow-up ( $n = 5$ ). Thus, 111 patients were included in the present analysis. All patients provided written informed consent to participate in the cohort and the protocol was approved by the Pitié-Salpêtrière Hospital Ethics Committee (Paris, France) in accord with the Declaration of Helsinki.

**Quantification of Serum Viral Loads.** HBV-DNA viral loads (VLs) were obtained at TDF treatment initiation and every 6–12 months during

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follow-up. Commercial polymerase chain reaction (PCR)-based assays were used to quantify HBV-DNA VL (COBAS AmpliPrep/COBAS TaqMan HBV Test; detection limit: 12 or 38 IU/mL; COBAS AmpliCor HBV Monitor Test; detection limit: 60 IU/mL; Roche Diagnostic Systems, Meylan, France). Because of varying detection limits, we decided to define undetectable HBV-DNA at the highest threshold (<60 IU/mL). HIV-1 VLs were measured using either a branched-DNA (Quantiplex 3.0; detection limit: 50 copies/mL; Bayer Diagnostics, Cergy Pontoise, France) or real-time PCR technique (COBAS AmpliPrep/COBAS TaqMan HIV-1 Test; detection limit: 40 copies/mL; Roche Molecular Systems).

**HBV Replication Profiles.** HBV replication profiles were defined *a priori* based on HBV-VL at the end of follow-up, where patients were initially classified on whether or not they had detectable HBV-VL. Among those with undetectable HBV-VL, patients were divided into two subgroups based on previous research<sup>7</sup>: stabilized virological response (VR; achieving and/or constantly maintaining HBV-DNA <60 IU/mL) or transient persistent viremia (PV; achieving <60 IU/mL, intermittently >60 IU/mL thereafter). Among those with detectable HBV-VL, patients were divided into two subgroups based on HBV-VL levels established from previous guidelines<sup>18</sup>: low-level persistent viremia (LL-PV; 60-2,000 IU/mL) or high-level persistent viremia (HL-PV; >2,000 IU/mL).

**Plasma TDF Concentrations.** Plasma TDF was measured using ultra performance liquid chromatography combined with tandem mass spectrometry (lower limit of quantification: 5 ng/mL; Waters Corporation, Milford, MA),<sup>19</sup> with the intent of assessing treatment adherence. Plasma concentrations were only performed in patients with PV. For patients with LL-/HL-PV, TDF was quantified at the last study visit. For patients with transient PV, TDF was quantified at the visit during which the blip in HBV replication occurred. If there was a prolonged blip (i.e., more than one consecutive visit with detectable HBV-VL), TDF concentrations were determined at the visit with the highest HBV-VL. To determine whether TDF concentrations were sufficient, we also quantified TDF levels in randomly selected patients with undetectable HBV-DNA (n = 34) at various visits during follow-up and compared them to patients with PV.

**Genotypic Analysis.** At baseline, L-nucleoside-associated *pol* gene mutations at positions rt173, rt180, and rt204 and *precore* mutations at nucleotide 1896 were determined using DNA chip technology (bioMérieux, Marcy l'Etoile, France).<sup>20</sup> During

follow-up, genotypic analysis was performed in patients with PV. Samples were obtained at baseline and at the same visits used to quantify plasma TDF. aa substitutions of *pol* were examined by direct sequencing after nested PCR amplification of the reverse transcriptase (RT) encoding region, between aa 42-309, using the ANRS consensus method adapted from a previous work.<sup>21</sup> Sequences were analyzed using a sequence editing program (Seqscape; Applied Biosciences, les Ulis, France) and were further submitted to the "Genotypic prediction of HBV drug resistance" tool (Geno2pheno[HBV] 2.0; available at: <http://hbv.bioinf.mpi-inf.mpg.de>).

**Serostatus and Quantification of Serological Markers.** Qualitative hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), and antibodies were detected at baseline and every yearly visit using a commercial enzyme-linked immunoassay (Diasorin, Antony, France).

Antigen levels were quantified at baseline and every 6-12 months during treatment. qHBsAg was performed using the Architect HBsAg assay (Abbott Laboratories, Rungis, France).<sup>22</sup> qHBeAg was determined using the Architect assay (with Architect i2000 analyzer) during the first 3 years of follow-up and the Elecsys HBeAg assay thereafter (with Modular E170 analyzer; Roche Diagnostics). Using a previously designed protocol,<sup>23</sup> signals from each assay were converted to Paul Ehrlich Institute units (PEIU)/mL. qHBeAg results from the Architect assay were then again converted into Elecsys units by an HIV-HBV-specific linear regression function. In other words, all qHBeAg units were expressed in PEIU/mL as if quantified from the Elecsys assay. Full details on the calculation of conversion factors are provided in the Supporting Methods (see the Supporting Information).

**Assessing Liver-Related Parameters.** Alanine transaminase (ALT) and aspartate transaminase (AST) were evaluated at baseline and every 6 months using standard methods. Baseline liver fibrosis levels were estimated using the noninvasive score FibroMeter, as detailed elsewhere,<sup>24</sup> and converted into Metavir equivalents. Liver biopsies were available in 64 patients at some point during therapy (18 with two biopsies) and evaluated using Metavir classification.<sup>16</sup> Any liver-related and non-liver-related clinical event was confirmed with the treating physician.

**Statistical Analysis.** Risk-factor analysis for undetectable HBV-DNA over time was carried out using random-effects logistic regression, accounting for within-patient variance. Time-varying factors were assessed by including the product of TDF treatment

**Table 1. Determinants of Undetectable HBV-DNA**

	Univariable		Multivariable <sup>†</sup>	
	OR (95% CI)	P Value	aOR (95% CI)	P Value
Age at baseline, per year	1.06 (1.01-1.11)	0.03	1.05 (0.98-1.12)	0.15
Gender, females vs. males	4.22 (1.13-15.77)	0.03		
High HBV-endemic zone	2.70 (1.00-7.32)	0.05		
AIDS-defining event	0.83 (0.37-1.89)	0.7		
Nadir CD4 <sup>+</sup> cell count ( $\sqrt{\text{mm}^3}$ ) at baseline	1.38 (0.73-2.63)	0.3		
CD4 <sup>+</sup> cell count, ( $\sqrt{\text{mm}^3}$ )				
At baseline	1.42 (0.74-2.73)	0.3		
During follow-up*	1.03 (1.00-1.07)	0.08	1.03 (0.99-1.06)	0.12
Undetectable HIV-RNA	5.35 (3.15-9.07)	<0.001	1.81 (0.83-3.94)	0.14
HIV-RNA, per log <sub>10</sub> copies/mL	0.32 (0.23-0.45)	<0.001		
Duration of ART (per year) at baseline	0.95 (0.85-1.06)	0.4		
HBV infection duration (per year) at baseline	1.03 (0.96-1.10)	0.4		
Baseline HBV-DNA, per log <sub>10</sub> IU/mL	0.64 (0.54-0.75)	<0.001	0.65 (0.50-0.85)	0.002
qHBsAg, per log <sub>10</sub> IU/mL*	0.96 (0.92-0.99)	0.04		
HBeAg positive				
At baseline	0.10 (0.04-0.22)	<0.001		
During follow-up*	0.71 (0.58-0.87)	0.001	0.74 (0.61-0.89)	0.001
TDF duration, per month	1.14 (1.11-1.17)	<0.001	1.45 (1.19-1.77)	<0.001
Previous LAM treatment	1.77 (0.50-6.26)	0.4		
Baseline LAM resistance mutations, N = 87	0.88 (0.37-2.10)	0.8		
Baseline <i>precore</i> mutations, N = 89	0.99 (0.39-2.50)	0.9		
Cumulative LAM duration, per year*	0.99 (0.98-1.00)	0.16		
Cumulative FTC-duration, per year*	0.98 (0.96-1.00)	0.04		
Concomitant LAM/FTC*	0.97 (0.92-1.03)	0.3		
ALT, >35 IU/mL				
At baseline	0.29 (0.13-0.67)	0.004		
During follow-up*	0.95 (0.92-0.99)	0.02	0.96 (0.93-0.99)	0.03
AST, >35 IU/mL				
At baseline	0.53 (0.24-1.14)	0.11		
During follow-up*	0.97 (0.93-1.00)	0.08		

\*Time-dependent covariate.

<sup>†</sup>Adjusted for age at baseline, CD4<sup>+</sup> cell count during follow-up, undetectable HIV-RNA, baseline HBV-DNA, HBeAg-positive status during follow-up, time (duration with TDF), and ALT levels during follow-up. Information on how the model was constructed can be found in the Supporting Results.

Abbreviations: AIDS, acquired immune deficiency syndrome; aOR, adjusted OR; ART, antiretroviral therapy.

duration and the covariable of interest, including its individual components, to the model. All risk factors with  $P \leq 0.2$  in univariable analysis were used to create a predictive, multivariable model. A forward step-wise selection process was then performed, removing any covariable with a  $P$  value greater than the above-described threshold upon adjustment.

In patients with detectable HBV-DNA at baseline, undetectable HBV-DNA rates at 12 and 24 months, including 12-month change in HBV-DNA, were evaluated. qHBsAg and qHBeAg during treatment were initially examined by locally weighted *scatterplot* smoothing (LOWESS) plot, whereas 12-month change, 12-month change  $>1.0$  log<sub>10</sub> in IU/mL (qHBsAg) or PEIU/mL (qHBeAg), and maximum change (defined as the nadir decrease from baseline) were also evaluated. Average HIV-RNA log<sub>10</sub> copies/mL levels during follow-up (while using 50 copies/mL as a uniform detection threshold) and percentage of visits with undetectable HIV-RNA VLs overall were also summarized.

Comparisons between HBV replication profiles were performed for all clinical parameters at baseline and during follow-up using Kruskal-Wallis' test for continuous variables and Pearson's chi-square test or Fisher's exact test for categorical variables, unless otherwise stated. In the subset of patients with liver biopsies, liver fibrosis levels were compared between HBV replication groups using a mixed-effect linear regression adjusted for TDF duration, while accounting for within-patient correlation for those with two biopsies.

All statistical analyses were performed using Stata software (v12.1; StataCorp LP, College Station, TX), and significance was determined using a  $P$  value  $<0.05$ .

## Results

**Baseline Description of the Study Population.** At TDF initiation, 78 (70.3%) patients were HBeAg positive and 92 (82.9%) had detectable HBV-DNA with a median HBV-DNA VL at 5.19 log<sub>10</sub> IU/mL

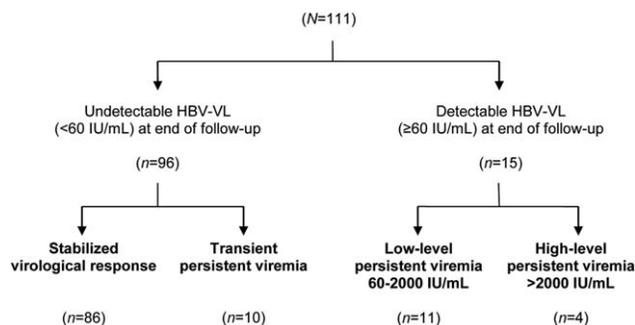


Fig. 1. Flow chart of HBV replication profiles.

(interquartile range [IQR]: 3.16-7.01). All patients had previously initiated ARV therapy (median duration: 6.6 years; IQR, 4.2-8.5), thus median CD4<sup>+</sup> cell count was at 396/mm<sup>3</sup> (IQR, 292-552) and 62 (55.5%) had undetectable HIV-RNA. Of the 101 patients (91.0%) with previous LAM exposure, median LAM duration was 50.3 months (IQR, 31.2-71.2) at TDF initiation. Patients initiated TDF in combination with LAM (n = 82; 73.9%), alone (n = 24; 21.6%), with emtricitabine (FTC; n = 3; 2.7%), or with LAM plus FTC (n = 2; 1.8%).

**Determinants of Undetectable HBV-DNA.** Median follow-up was 74.7 months (IQR, 33.4-94.7). Risk factors of undetectable HBV-DNA VL over time are given in Table 1. Increased baseline HBV-DNA levels, longer duration of HBeAg-positive serology over time, and higher ALT levels over time were factors with significantly lower odds of undetectable HBV-DNA VL, whereas longer TDF duration was significantly associated with higher odds. Of note, neither cumulative LAM duration nor FTC duration were associated with undetectable HBV-DNA VL in multivariable analysis (adjusted odds ratio [aOR]/year = 1.00, 95% confidence interval [CI]: 0.99-1.01; aOR/year = 0.98, 95% CI: 0.97-1.01, respectively).

**Distribution and Description of HBV Replication Profiles.** Undetectable HBV-VL at the end of follow-up occurred in 96 (86.5%) patients, with 86 (77.5%) remaining completely undetectable during treatment (stabilized VR; Fig. 1). Among them, HIV-RNA was undetectable for a median 90.0% of study visits (IQR, 75.0%-100.0%). In the other 10 patients, detectable HBV replication was observed after a median of 8.8 months (range, 4.5-29.5) after having achieved undetectable HBV-VL (transient PV), during which time median peak HBV-DNA VL was 1,155 IU/mL (range, 68-7,162). Only 2 of these patients had a concomitant peak in qHBsAg during blip in HBV replication (Supporting Fig. 1). At the same time, plasma HIV-RNA was undetectable in 6 (60%) patients, low in 3 (30%;

HIV-RNA VL range: 116-1,953 copies/mL), and fairly high in the remaining patient (HIV-RNA VL: 139,189 copies/mL).

In patients with detectable HBV-VL at the end of follow-up, LL-PV was observed in 11 (9.9%) patients, of whom 3 had achieved HBV VL <60 at least once during follow-up. In this group, median HBV-DNA VL was 111 IU/mL (range, 78-860) at the last visit. Meanwhile, HIV-RNA VL was undetectable for 7 (63.6%) patients and high for the remaining 4 (36.4%; range HIV-RNA VL: 3,421-590,000 copies/mL). HL-PV was found in 4 (3.6%) patients, among whom HBV-DNA ranged from 3.33 to 5.00 log<sub>10</sub> IU/mL at the last visit. Only 1 patient achieved an undetectable HBV-VL during the course of follow-up. At the last visit, HIV-RNA was undetectable for 2 patients, whereas the remaining 2 had low HIV-RNA VLs (1,070 and 1,100 copies/mL).

**Baseline Characteristics and HBV Replication Profiles.** Table 2 describes the baseline characteristics of patients between profile groups. Because of small numbers of patients, LL-PV and HL-PV groups were combined for analysis. Among those with detectable baseline HBV-DNA, median HBV-DNA was higher for those with stabilized VR and LL-/HL-PV profiles than transient PV ( $P = 0.09$  and  $0.01$ , respectively). Patients with stabilized VR, as compared to all PV profiles, were significantly more likely to be HBeAg negative ( $P = 0.001$ ) and have higher CD4<sup>+</sup> nadir count ( $P = 0.004$ ). Finally, stabilized VR and transient PV profiles had significantly lower baseline ALT/AST levels, when compared to LL-/HL-PV ( $P < 0.04$ ).

**On-Treatment Virological and Serological Characteristics per HBV Replication Profile.** In patients with detectable baseline HBV-DNA, median time until undetectable HBV-VL was not different between patients with stabilized VR versus transient PV profiles (12.6 vs. 23.2 months, respectively; Fig. 2A). In HBeAg-positive patients, it would appear that qHBeAg decline was faster in patients with stabilized VR and LL-/HL-PV profiles (Fig. 2B). These profile groups also had slightly steeper 12-month  $\Delta$ qHBeAg, compared to transient PV profiles, with more patients exhibiting  $>1.0$  log<sub>10</sub> 12-month PEIU/mL decreases (Table 3); however, there were no significant differences. Overall, faster 12-month decline in qHBsAg (Fig. 2C; Table 3) was also observed among patients with stabilized VR and LL-/HL-PV profiles versus transient PV ( $P = 0.15$  and  $P = 0.04$ , respectively). Importantly, HBeAg-loss, HBeAg seroconversion, and HBsAg loss only occurred in patients with stabilized VR.

**Table 2. Baseline Characteristics of HBV Replication Profiles**

Characteristics	Stabilized VR (n = 86)	Transient PV (n = 10)	LL-/HL-PV <sup>‡</sup> (n = 15)	P Value <sup>§</sup>
<b>Demographics</b>				
Gender, male/female (% male)	76/10 (88.4)	8/2 (80.0)	15/0 (100)	NS
Age, years*	41 (36-48)	42 (35-45)	38 (33-45)	NS
BMI, kg/m <sup>2</sup> *	22.6 (20.9-24.0)	23.3 (22.3-26.2)	22.2 (20.5-23.4)	NS
Born in high HBV endemic zone <sup>†</sup>	18 (20.9)	2 (20.0)	1 (6.7)	NS
<b>HIV characteristics</b>				
AIDS-defining event <sup>†</sup>	23 (26.7)	5 (50.0)	5 (33.3)	NS
Detectable HIV-RNA <sup>†</sup>	33 (38.8)	6 (60.0)	5 (33.3)	2
HIV-RNA <sup>‡</sup> , log <sub>10</sub> copies/mL*	3.37 (2.57-4.12)	4.56 (3.66-4.84)	4.06 (2.08-4.81)	ns
CD4 <sup>+</sup> cell count, per mm <sup>3</sup> *	400 (324-576)	185 (86-532)	460 (219-514)	1
Nadir CD4 <sup>+</sup> cell count, per mm <sup>3</sup> *	238 (136-321)	54 (30-179)	124 (8-309)	1
Duration of prior cART therapy, years*	6.3 (3.5-8.6)	6.8 (4.2-7.8)	6.9 (6.0-8.4)	ns
<b>Viral hepatitis characteristics</b>				
Known HBV infection duration, years*	7.5 (3.0-12.0)	9.7 (3.7-11.0)	6.5 (2.8-9.1)	NS
Prior LAM exposure <sup>†</sup>	78 (90.7)	9 (90.0)	14 (93.3)	NS
Detectable HBV-DNA <sup>†</sup>	70 (81.4)	7 (70.0)	15 (100)	NS
HBV-DNA <sup>‡</sup> , log <sub>10</sub> IU/mL*	5.07 (3.18-7.15)	2.95 (2.82-5.44)	6.58 (4.58-7.28)	3
HBeAg positive <sup>†</sup>	54 (62.8)	10 (100)	14 (93.3)	1,2
qHBeAg <sup>¶</sup> , PEI U/mL*	497 (126-790)	367 (81-701)	363 (171-714)	NS
qHBsAg, log <sub>10</sub> IU/mL*				
HBeAg <sup>+</sup>	4.74 (4.33-5.06)	4.66 (3.66-5.09)	4.79 (4.20-5.26)	NS
HBeAg <sup>-</sup>	3.44 (2.84-3.65)	—	3.51	NTP
ALT, IU/L*	45 (30-83)	40 (22-62)	71 (51-149)	2,3
AST, IU/L*	41 (27-61)	28 (24-52)	70 (40-85)	2,3
F3-F4 fibrosis <sup>#</sup> , N = 98 <sup>†</sup>	19 (24.1)	0	3 (23.1)	NS
LAM-resistant mutations, N = 78 <sup>†</sup>	46 (79.3)	4 (66.7)	13 (92.9)	NS
Precore mutations, N = 80 <sup>†</sup>	11 (16.7)	2 (25.0)	5 (33.3)	NS

\*Median (IQR).

<sup>†</sup>Number (%).<sup>‡</sup>These groups were combined for analysis, yet the following significant differences were observed when only including the LL-PV group: BMI, versus transient PV,  $P = 0.04$ ; nadir CD4<sup>+</sup> cell count, versus stabilized VR,  $P = 0.04$ .<sup>§</sup>Significance determined using Kruskal-Wallis' test for continuous variables and Pearson's chi-square test or Fisher's exact test for categorical variables. Significant differences ( $P < 0.05$ ) between HBV replication profile groups were indicated as follows: 1, stabilized VR and transient PV; 2, stabilized VR and LL-/HL-PV; 3, transient PV and LL-/HL-PV.<sup>‡</sup>Among patients with detectable HIV-RNA or HBV-DNA viral loads.<sup>¶</sup>Among HBeAg-positive patients.<sup>#</sup>Estimated using the noninvasive biochemical FibroMeter score.

Abbreviations: cART, combination antiretroviral therapy; NS, no significant differences between groups; NTP, no test performed.

**Genotypic Characteristics of Patients With Persistent Viremia.** Table 4 gives a complete description of the genotypic findings among patients with transient PV. Four of the six patients with available baseline sequences had LAM resistance mutations (rtM204V,  $n = 3$ ; rtV173V/L+rtL180M+rtM204V:  $n = 1$ ). At the time point where transient HBV replication occurred, the majority of mutations were associated with LAM resistance, with other aa changes observed at positions rtL217R ( $n = 2$ ) and rtR274W ( $n = 1$ ).

Among those with LL-PV (Table 5A), 3 of 4 patients with available baseline sequences had the triple rtV173L+rtL180M+rtM204V LAM resistance mutation, whereas 1 patient also harbored an HBV strain with aa changes at position rtL217R. During TDF treatment, no changes from baseline sequences were observed in these patients. Among those with HL-PV

(Table 5B), all had LAM resistance mutations at baseline with other aa changes at positions rtL80I ( $n = 1$ ) and rtL217R ( $n = 1$ ). No other aa changes were noted during follow-up, except in 1 patient with a change at position rtV173L.

**TDF Levels During PV.** Among patients with transient PV, all 9 with available plasma samples had detectable TDF concentrations (median, 62 ng/mL; IQR, 54-67). In patients with LL-PV, 8 of 9 patients with available samples had TDF detected (median, 89 ng/mL; IQR, 82-121). Finally, in patients with HL-PV, 2 of 3 with available samples had plasma TDF  $< 5$  ng/mL, whereas the remaining patient was considered as having low TDF levels (30 ng/mL), according to the interval between last drug intake and sampling. Patients exhibiting PV did not have significantly different TDF concentrations, when compared to randomly

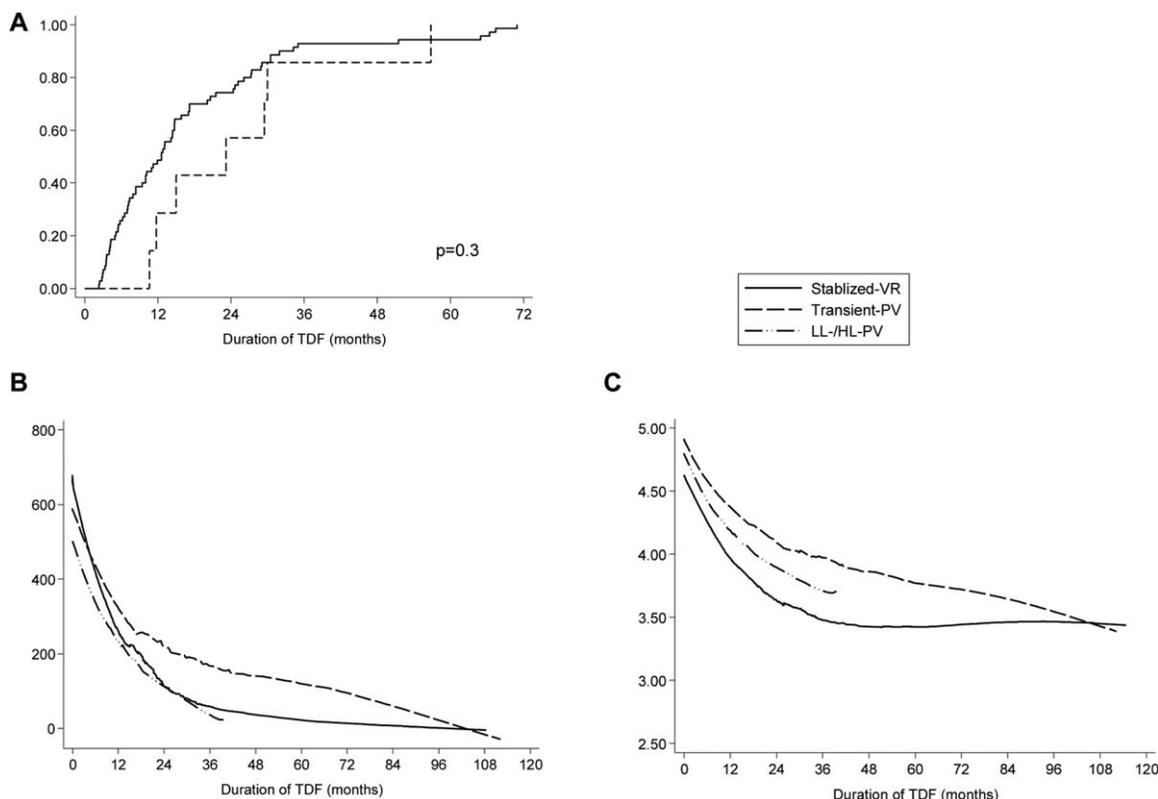


Fig. 2. Time to undetectable HBV-DNA, qHBeAg, and qHBsAg levels between HBV replication profiles. Cumulative probability of time to undetectable HBV-DNA is compared between patients with stabilized VR versus transient PV using a log-rank test (A). LOWESS plots are also given by HBV replication profile group for qHBeAg (PEIU/mL) in HBeAg-positive patients (B) and qHBsAg ( $\log_{10}$  IU/mL) in all patients (C). For the two latter plots, follow-up was truncated to 48 months in the combined LL-/HL-PV group. The low number of patients after this time point resulted in substantial variability, thus highly skewing the plot.

selected patients with undetectable HBV-DNA (median, 65 ng/mL; IQR, 42-100;  $P = 0.6$ ).

#### **Transaminases and Liver-Related Outcomes.**

Overall, almost all patients observed declines in ALT levels, with a median maximum decline at -19 (IQR, -53, -8). Median maximum ALT decline only tended to be stronger in LL-/HL-PV than the transient PV group ( $P = 0.08$ ; Table 3), which was expected given the high baseline transaminase levels in the LL-/HL-PV group. Otherwise, no other significant differences were observed in median maximum increase in ALT levels during follow-up or ALT levels at the last follow-up visit (Table 3).

No significant difference in Metavir equivalents (from the FibroMeter) was observed at baseline, yet no patient in the transient VR group had F3-F4 fibrosis (Table 2). In the subgroup with liver biopsies, taken after a median of 5.3 months of TDF (IQR, 0-27.7), there were no significant differences in Metavir fibrosis or activity level between stabilized VR and other PV groups: transient PV, 0.1 (95% CI: -0.7, 0.8;  $P = 0.9$ ) or -0.1 (95% CI: -0.6, 0.4;  $P = 0.8$ ); LL-/HL-PV, 0.5 (95% CI: -0.2, 1.2;  $P = 0.14$ ) or 0.4 (95% CI: -0.1, 0.9;  $P = 0.09$ ), respectively.

At TDF initiation, 3 patients had already experienced a severe liver-related event ( $n = 1$ , hepatic failure and  $n = 2$ , portal hypertension). Of these, 2 had stabilized VR and 1 had LL-PV. During TDF treatment, 2 patients developed HCC (both with stabilized VR replication profiles) and 2 died (both with LL-PV replication profiles). One death was the result of severe pneumonia, whereas the other was the result of a liver-related complication after the patient discontinued TDF therapy, resulting in an increase of HBV-DNA to 7.56  $\log_{10}$  IU/mL. No liver-related morbidity or mortality was observed in patients with HL-PV.

## **Discussion**

In this large, prospective study among patients on continuous TDF therapy for a median of 7 years, without ETV or Peg-IFN intensification, we demonstrate that 77.5% had optimal HBV suppression. In general, these patients were more likely to be either at later phases of HBV infection or have had more successful anti-HBV therapy preceding TDF initiation (higher proportion of HBeAg negative and lower ALT levels).

**Table 3. Clinical and Serological Characteristics of HBV Replication Profiles During Follow-up**

Characteristics	Stabilized VR (n = 86)	Transient PV (n = 10)	LL/HL PV <sup>†</sup> (n = 15)	P Value <sup>#</sup>
Total follow-up, months*	79 (33-95)	86 (45-96)	35 (23-40)	2,3
HBV-DNA <sup>†</sup>				
Partial VR	29 (41.4)	3 (42.9)	14 (93.3)	2,3
12-month change, log <sub>10</sub> IU/mL*	-3.16 (-4.92, -1.30)	-1.24 (-2.94, -0.24)	-2.18 (-4.28, -1.27)	NS
VR at 12 months <sup>‡</sup>	44 (62.9)	2 (28.6)	1 (6.7)	2
VR at 24 months <sup>‡</sup>	54 (80.6)	4 (57.1)	1 (8.3)	2,3
HIV-RNA				
Average HIV VL at last visit, log <sub>10</sub> copies/mL*	1.80 (1.71-1.99)	1.94 (1.74-2.38)	1.89 (1.63-3.00)	NS
% detectable HIV VLs during follow-up*	90.0 (75.0-100.0)	82.9 (70.0-88.9)	66.7 (0-100.0)	2
≥70% detectable HIV VLs during follow-up <sup>‡</sup>	68 (80.0)	7 (70.0)	7 (46.7)	2
qHBeAg <sup>§</sup>				
12-month change, PEI U/mL*	-260 (-669, -33)	-63 (-229, 0)	-216 (-487, -158)	NS
12-month change >1.0 log <sub>10</sub> <sup>‡</sup>	15 (27.8)	0	3 (21.4)	NS
Maximum change, log <sub>10</sub> PEI U/mL*	-2.03 (-2.70, -1.25)	-1.51 (-1.62, -0.82)	-1.02 (-1.36, -0.50)	2
qHBsAg				
12-month change, log <sub>10</sub> IU/mL*	-0.33 (-0.80, -0.06)	-0.14 (-0.32, -0.06)	-0.29 (-0.78, -0.23)	3
12-month change >1.0 log <sub>10</sub> <sup>‡</sup>	18 (20.9)	0	3 (20.0)	NS
Maximum change, log <sub>10</sub> IU/mL*	-0.77 (-1.44, -0.32)	-0.81 (-1.48, -0.54)	-0.93 (-1.40, -0.24)	NS
Serological response <sup>  </sup>				
HBeAg loss <sup>‡§</sup>	20 (37.0)	0	0	1,2
HBeAg seroconversion <sup>‡§</sup>	5 (9.3)	0	0	NS
HBsAg loss <sup>‡</sup>	4 (4.7)	0	0	NS
ALT				
Maximum decrease from baseline, IU/mL*	-19 (-53, -9)	-13 (-42, 0)	-32 (-113, -10)	NS
Maximum increase from baseline, IU/mL*	10 (0, 35)	22 (0, 41)	4 (0, 71)	NS
Last follow-up visit, IU/mL*	31 (24-43)	33 (20-41)	43 (27-64)	NS

\*Median (IQR).

<sup>†</sup>Among patients with detectable HBV-DNA at baseline.<sup>‡</sup>Number (%).<sup>§</sup>Among HBeAg-positive patients.<sup>||</sup>Determined at the end of follow-up. Three patients had lost HBeAg, but later reconverted back to HBeAg-positive serology. Likewise, 1 patient had lost HBsAg, but later reconverted to HBsAg-positive serology.<sup>#</sup>These groups were combined for analysis, yet the only significant difference when including the LL-PV group alone was in maximum ALT decline versus transient PV ( $P = 0.04$ ).<sup>#</sup>Significance determined using Kruskal-Wallis' test for continuous variables and Pearson's chi-square test or Fisher's exact test for categorical variables. Significant differences ( $P < 0.05$ ) between HBV replication profile groups were indicated as follows: 1, stabilized VR and transient PV; 2, stabilized VR and LL-/HL-PV; 3, transient PV and LL-/HL-PV.

Abbreviation: NS, no significant differences between groups.

Poor adherence is often considered the first cause of persistent viremia to suspect among patients undergoing NA therapy with a high genetic barrier to resistance.<sup>8</sup> Although 18 of the 21 patients with persistent viremia and available samples had detectable plasma TDF at comparable levels to those with undetectable HBV-DNA, it may not give the overall picture of adherence over longer periods of time. Some clues can be inferred from rates of detectable HIV-RNA, where adherence reaching as low as 75% for some ARV classes is associated with effective HIV control.<sup>25</sup> Assuming that overall rates of undetectable HIV-RNA greater than 70% represent optimal adherence (HIV-RNA replication being explained by other factors, such as HIV resistance to non-nucleoside RT inhibitors or protease inhibitors<sup>26</sup>), PV could then be the result of fairly poor adherence in an estimated 11 of 25

patients. Still, the optimal adherence level for HBV suppression has not been determined; nevertheless, preliminary data suggest it to be much higher than HIV, from anywhere above 95%-100%.<sup>7,27</sup>

Another potential reason of PV could be the genetic variability of HBV. The aa change at position rtL217R, which has already been identified with poor VR to ADV,<sup>28</sup> was observed in 4 patients. To the best of our knowledge, this specific mutation has not yet been phenotypically analyzed vis-à-vis tenofovir. A conserved site change at position rtR274W was also observed in 1 patient. A substitution located at the same position (rtR274Q/R) had been identified in 1 patient from the GS-US-174-0103 Study as the result of TDF discontinuation.<sup>29</sup> Interestingly, a highly mixed group of quasi-species was also found in this patient, whereas phenotypic evaluation of rtR274Q revealed a TDF-

**Table 4. Genotypic Findings of Patients With Transient PV**

ID	Other anti-HBV Treatment During FU*	TDF Duration at Blip	HBV-DNA ( $\log_{10}$ IU/mL)		TDF (ng/mL)	Genotype	Genotypic Findings ( <i>pol</i> Gene)	
			Baseline	Blip			Baseline	Changes at Blip
119	LAM	23.7	2.82	3.41	26	A	rtM204V	rtV173L, rtL180M
145	LAM→FTC	46.2	2.44	1.83	62	G	rtM204V	n/a
210	LAM→∅	35.2	<1.78	3.67	67	A	n/a	rtV173L, rtL180M, rtM204V, rtL217R
301	LAM→FTC→∅	67.2	5.57	1.83	†	E	rtM204V	None
407	LAM→FTC→ADV	36.8	4.58	3.20	62	A	rtV173V/L, rtL180M, rtM204V	None
503	LAM→∅→FTC	28.8	2.93	2.06	73	n/a	n/a	n/a
534	LAM→∅	59.0	2.95	1.95	103	D	rtH126R, rtY135C	rtL217R <sup>‡</sup>
536	LAM→∅	16.8	<1.78	2.85	20	A	—	rtV173L, rtL180M, rtM204V
540	LAM→FTC	21.7	5.44	3.86 <sup>§</sup>	54	A/E	None	rtV173L, rtL180M, rtM204V
1134	LAM	19.5	<1.78	3.63	58	n/a	—	rtR274W

\*Bold represents treatment given before TDF initiation.

†This patient had HBV replication  $>10^6$   $\log_{10}$  IU/mL for the first 12 months of therapy, during which time plasma TDF was not detected. Plasma samples were not available at the visit where transient replication occurred.

‡Changes confirmed with a previous sample at 20.1 months of TDF therapy.

§This patient had a prolonged blip with three consecutive HBV-DNA levels at 3.06-3.86  $\log_{10}$  IU/mL.

Abbreviations: FU, follow-up; ∅, no other anti-HBV treatment; n/a, HBV strain not sequenced, thus data are unavailable; —, virological data were not obtained for this visit.

sensitive strain. Our study group has also identified a TDF-treated patient, not included in the present study, who harbored the possible TDF-resistance mutation, rtA194T, yet exhibited controlled HBV suppression.<sup>20</sup>

Taken together, we found no dominant virological characteristic on the *pol* gene, at both baseline and follow-up, that was consistently associated with persistent replication. Wider ranges of sequencing would be

**Table 5. Genotypic Findings of Patients With LL-PV and HL-PV**

ID	Other Anti-HBV Treatment During FU*	TDF Duration	HBV-DNA ( $\log_{10}$ IU/mL)		TDF (ng/mL)	Genotype	Genotypic Findings ( <i>pol</i> Gene)	
			Baseline	End of FU			Baseline	Changes During FU
<b>A. LL-PV</b>								
105	LAM	24.3	8.47	2.16	80	A	—	n/a
152	LAM→FTC	81.2	8.30	2.00	<5	A	—	n/a
204	LAM→FTC	56.4	6.88	1.92	142	A	—	n/a
410	LAM→∅→FTC	37.0	4.58	1.94	99	A	rtV173L, rtL180M, rtM204V, rtL217R	None <sup>†</sup>
502	LAM→∅	35.1	7.85	1.89	93	A	—	n/a
1110	LAM→∅	39.6	6.26	2.09	84	G	—	n/a
1131	LAM	33.4	2.94	2.93	85	A	None	None <sup>†</sup>
1140	LAM	18.9	2.61	1.95	205	D	rtV173L, rtL180M, rtM204V	None <sup>†</sup>
1155	LAM	36.6	5.06	2.81	57	A	rtV173L, rtL180M, rtM204V	None <sup>†</sup>
<b>B. HL-PV</b>								
208	LAM→LAM/FTC	96.6	6.88	4.94	—	A	rtL180M <sup>‡</sup> , rtM204G	None
1006	LAM	38.9	5.60	3.86	30	A	rtL180M, rtM204V	rtV173L
1022	LAM	24.8	6.58	5.00	<5	A	rtV173L/V, rtL180M, rtM204V, rtL217R	None
1143	LAM	15.2	3.68	3.33	<5	E	rtL80I, rtV173L, rtL180M/V, rtM204V	None

Two patients were excluded because they had no available sample for testing (n = 2).

\*Bold represents treatment given before TDF initiation.

†Information obtained from previous samples during TDF therapy with higher HBV-DNA viral load.

‡Mutation was no longer apparent at the end of follow-up.

Abbreviations: FU, follow-up; ∅, no other anti-HBV treatment; n/a, HBV strain not sequenced, thus data are unavailable; —, virological/pharmacological data were not obtained for this visit.

necessary in determining whether mutations on the *preS/S* and *preC/C* regions of the HBV genome are also associated with ongoing replication. Moreover, one cannot exclude that PV may favor the occurrence of compensatory mutations, especially considering that higher variability of HBV quasi-species has been previously observed with poor response.<sup>30</sup>

Interestingly, patients with transient PV had significantly lower CD4<sup>+</sup> cell counts, compared to other replication groups, whereas nadir CD4<sup>+</sup> cell count was significantly lower among patients with PV overall. Because adaptive cellular mechanisms are intrahepatically impaired in HIV-HBV coinfection,<sup>10</sup> it could be hypothesized that the loss of HBV-specific immunological control in the liver might have elicited bouts of transient or low-level replication. Any changes in genetic variability observed during follow-up would likely be mere reflections of the quasi-species originating from a persistent reservoir, such as within hepatocytes. Of course, more-exhaustive data would be needed to address this hypothesis. It should be noted that nadir CD4<sup>+</sup> cell count was not associated with undetectable HBV-DNA in univariable analysis. Yet, this analysis does not differentiate detectable HBV-DNA during normal treatment response versus PV, underscoring the importance of defining HBV replication groups.

Much ambiguity surrounds the clinical effect of persistent HBV replication in HIV-HBV coinfecting patients. Data from the REVEAL-HBV study observed that among patients with HBV replication consistently at <10<sup>4</sup> copies/mL, the incidence rate of HCC was very low, at 73.4 per 100,000 person-years.<sup>2</sup> This association could be considered no different in coinfection, given the lack of increased risk in HCC between HIV-HBV- and HBV-infected patients.<sup>31,32</sup> Indeed, inasmuch as our data were limited by the low incidence of clinical events, we could observe no evidence indicating that PV *per se* was linked to increased liver-related morbidity and mortality. Nevertheless, there was a clear link between consistently undetectable HBV-DNA and HBeAg loss, HBeAg seroconversion, and HBsAg loss, all of which are ideal therapeutic endpoints and correspond to decreased viral activity and improved liver-related outcomes.<sup>17</sup> This is in line with a previous study of HBV-monoinfected, NA-treated patients, where HBsAg loss only occurred in patients who were able to achieve HBV-DNA <300 copies/mL.<sup>33</sup>

One important and disconcerting observation was the significantly shorter duration of follow-up among patients with LL-/HL-PV, when compared to other groups. When matching follow-up of patients with

LL-/HL-PV to those with stabilized VR or transient PV in a sensitivity analysis (Supporting Methods), we observed that 12.5% of the latter patients were reclassified as having some form of LL-/HL virological persistence. Defining persistent replication at time points with short follow-up appears to be prone to misclassification. These changes did not substantially modify the analysis, especially with respect to serological endpoints (Supporting Tables 1-3); however, the 9 patients newly classified as HL-PV had characteristics typical of coinfecting patients at risk of delayed response,<sup>34</sup> including high HBV-DNA and rather high increases in transaminase levels. This puts into question previous studies where detectable VL at 6-12 months is used as the hallmark of nonresponse or delayed response,<sup>7,30,34</sup> even as the majority of these patients eventually achieve VR.<sup>35</sup>

Our study has several limitations that need to be addressed. First, the threshold for transient HBV-DNA replication was particularly low and HBV VLs were only evaluated every 6-12 months. Patients with stabilized VR profiles could have been misclassified. Second, qHBeAg was obtained by two different assays and rendered similar using a post-hoc approach. Some bias could have been generated for certain measures, especially the 12-month change in qHBeAg. Finally, we did not record HIV resistance mutations during follow-up, which may have helped explain certain instances during which HIV-RNA was detectable.

In conclusion, PV hinders important serological endpoints during TDF treatment in HIV-HBV coinfecting patients, yet this does not necessarily translate to increased risk in liver-related morbidity or mortality. No specific mutations on the *pol* gene were associated with PV, yet other genetic aspects of HBV need to be more thoroughly evaluated. Whether this phenomenon is caused by inadequate adherence remains to be concretely evaluated; nevertheless, our data suggest that roughly half of patients with PV were possibly the result of poor adherence. Other host factors, particularly immunoregulation, merit further investigation.

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## Supporting Information

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