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Running title: TDF and PegIFN intensification in HIV-HBV

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ABSTRACT

In hepatitis B “e” antigen (HBeAg) positive patients with hepatitis B virus (HBV) mono-infection, intensification of nucleos(t)ide analogue treatment with pegylated-interferon (PegIFN) could help induce higher HBeAg-seroclearance rates. Our aim was to determine the long-term effect of adding PegIFN to tenofovir (TDF)-containing antiretroviral therapy on seroclearance in HBeAg-positive patients co-infected with the human immunodeficiency virus (HIV) and HBV. In this prospective matched-cohort study, forty-six patients with one-year PegIFN-intensification during TDF-containing antiretroviral therapy (TDF+PegIFN) were matched 1:1 to controls undergoing TDF without PegIFN (TDF) using a time-dependent propensity score based on age, CD4+ count, and liver cirrhosis status. Kinetics of HBeAg quantification (qHBeAg) and hepatitis B surface antigen quantification (qHBsAg) were estimated using mixed-effect linear regression and time to HBeAg- or HBsAg-seroclearance was modeled using proportional hazards regression. At baseline, previous TDF-exposure was a median 39.8 months (IQR=21.4-59.4) and median qHBeAg and qHBsAg levels were 6.9 PEIU/mL and 3.72 $\log_{10}$IU/mL, respectively (P>0.5 between groups). Median follow-up was 33.4 months (IQR=19.0-36.3). During intensification, faster average declines of qHBeAg (-0.066 versus -0.027 PEIU/mL/month, P=0.001) and qHBsAg (-0.049 versus -0.026 $\log_{10}$IU/mL/month, P=0.09) were observed in patients undergoing TDF+PegIFN versus TDF, respectively. After intensification, qHBeAg and qHBsAg decline was no different between groups (P=0.7 and P=0.9, respectively). Overall, no differences were observed in HBeAg-seroclearance (TDF+PegIFN=13.2 versus TDF=12.6/100 person•years, P=0.5) or HBsAg-seroclearance rates (TDF+PegIFN=1.8 versus TDF=1.3/100 person•years, P=0.7). In conclusion, PegIFN-intensification in HBeAg-positive co-infected patients did not lead to increased rates of HBeAg or HBsAg-clearance, despite faster declines of antigen levels while on PegIFN.

KEYWORDS: pegylated-interferon; treatment intensification; HIV; chronic hepatitis B infection; time-dependent propensity score.

INTRODUCTION

Chronic hepatitis B virus (HBV) infection is observed in roughly 10% of patients infected with the human immunodeficiency virus (HIV) and represents a major burden of disease [1,2]. HIV-HBV co-infection increases risk of developing liver fibrosis, cirrhosis, hepatocellular carcinoma (HCC), and liver-related death when compared to HIV-infection alone [3,4].

During HBV infection, patients remaining positive for hepatitis B “e” antigen (HBeAg) have the greatest risk of HCC and overall death [5,6]. Antiviral therapy aims to shift patients towards HBeAg-negative phases of chronic infection, where prognosis improves and viral activity in the liver decreases [7,8]. Antiretroviral therapy (ART) containing tenofovir (TDF) is the major choice of treatment for HIV-HBV co-infected patients due to is dual anti-HBV and anti-HIV activity and virtually null risk of developing resistant HBV mutations [9]. Unfortunately for HBeAg-positive co-infected patients, almost half maintain HBeAg-positive serology while undergoing this treatment regimen [10–12].

In HBV mono-infection, treatment with pegylated-interferon (PegIFN) has demonstrated higher rates of HBeAg-seroconversion compared to nucleoside/nucleotide analogues (NA) with lower antiviral potency [13]. Combining PegIFN with a potent NA has shown a favorable impact on HBeAg- and HBsAg-seroclearance [14,15]. While more recent clinical trials in HBeAg-positive patients have concluded that adding or switching to PegIFN from an already existing potent NA-based regimen could increase rates of HBeAg-seroclearance and HBeAg-seroconversion, even after completing PegIFN [16,17]. Despite these encouraging data, it remains unknown whether such a treatment strategy would be beneficial to HIV-HBV co-infected patients, for whom interferon-based treatments are generally less successful [18].

The EMVIPEG study was recently conducted to examine safety and tolerability of PegIFN intensification therapy in HBeAg-positive co-infected patients with extensive TDF-containing ART [19]. This study demonstrated modest rates of HBeAg and hepatitis B surface antigen (HBsAg) seroclearance, yet the lack of comparison group with only TDF makes it difficult to establish the specific effect of treatment. The objective of the study herein was to compare seroclearance rates of HBeAg and HBsAg, including their quantifiable markers, of patients enrolled in the EMVIPEG trial to those without PegIFN intensification with similar characteristics and prospective follow-up.

MATERIALS AND METHODS

Study participants

Patients were included from two multi-center, prospective studies: ANRS HB01 EMVIPEG study (NCT00391638), a Phase II open-label trial aimed at evaluating the efficacy and safety of Peg-IFN α2a intensification (180 µg subcutaneously once a week) in HIV-HBV co-infected patients with HBeAg-positive serology; and the French HIV-HBV Cohort, a cohort study aimed at evaluating the evolution and determinants of liver fibrosis in HIV-HBV co-infected patients. Study procedures are detailed elsewhere [19,20]. For both studies, patients provided written informed consent and protocols were approved by the appropriate ethics committee (EMVIPEG, Lyon Hospital, CPP Sud-Est III and ANRS; French HIV-HBV Cohort, Pitié-Salpêtrière and Saint-Antoine Hospitals).

We included patients in the present study based on the following criteria: age >18 years, HIV-1 infection, Karnovsky index >80, stable TDF-containing ART for ≥6 months, CD4+ >200/mm^3 or

>15%, HIV viral load <10,000 copies/mL, and creatinine clearance >60 mL/min. Patients also needed to have HBsAg-positive serology, HBeAg-positive serology, no anti-HBe antibodies (anti-HBeAb), serum HBV-DNA >10,000 copies/mL (>2,000 IU/mL) at the time TDF had been initiated and <10,000 copies/mL (<2,000 IU/mL) when considered for inclusion. Patients enrolled in the EMVIPEG study were also required to have ART including lamivudine (LAM) and/or emtricitabine (FTC) along with TDF.

Exclusion criteria were as follows: hepatitis C virus RNA-positive [determined by a sensitive polymerase chain reaction (PCR)-assay]; anti-hepatitis D virus IgG antibody-positive; concomitant AIDS-defining event; alcohol consumption >50 g/day; decompensated cirrhosis; concomitant cancer or immunosuppressive drugs; pregnancy or breastfeeding; anti-HBV therapy other than TDF, LAM or FTC in <6 months and previous use of PegIFN; neutrophil count <1000/mm^3; hemoglobin <10 g/dL; platelet count <80,000/mm^3; and abnormal thyroid-stimulating hormone. Patients with no available data on qHBsAg and qHBeAg at baseline and at least once during follow-up were also excluded.

Treatment exposure groups and matching procedure

We constituted two exposure groups: (1) patients adding PegIFN therapy for 48 weeks to their pre-existing TDF-containing ART regimen (herein referred to as the “TDF+PegIFN” group) and (2) patients undergoing continuous TDF-containing ART (herein referred to as the “TDF” group). Individuals from the TDF+PegIFN intensification group were defined as index patients, who were matched 1:1 to control patients from the TDF group.

Since patients had various durations of TDF when PegIFN was added, the goal was to find a time-point during TDF-containing ART at which index patients initiating PegIFN most closely

resembled their controls. In order to accomplish this, we decided to use a time-dependent propensity score [21] based on the time-varying covariates age, CD4+ cell count (>350 cells/mm^3), and liver cirrhosis status (F4 METAVIR equivalent). To summarize, a risk set was constructed in which the hazards of receiving PegIFN treatment were modeled for all patients using a proportional hazards model with the matching criteria as independent variables. Predicted hazards were estimated at the initiation of treatment intensification for index patients and at each time-point during TDF-containing ART for control patients. While sequentially selecting index patients from the longest to shortest cumulative TDF-duration, matched pairs were chosen by the smallest total distance in predicted hazards within matched sets. After verifying that inclusion and non-inclusion criteria still held for the selected control, matched patients were removed from the risk set and the process was repeated.

Study visits

Baseline was defined at PegIFN-initiation for index patients or the corresponding matched time-point during TDF-containing ART for control patients. Follow-up visits were performed every 6-12 months after baseline for all patients. Follow-up continued until the date of last follow-up, at the month-36 visit (to maintain consistency with the EMVIPEG study in which follow-up only extended to this time-point), permanent treatment discontinuation, treatment switch, or death; whichever occurred first.

Assessing HBV-related parameters

Plasma HBV-DNA viral load (VL) was quantified using a commercial PCR-based assay [20]. Due to varying detection thresholds, a harmonized definition of undetectable HBV-VL was

established at <60 IU/mL. Qualitative HBsAg, HBeAg, anti-HBs antibody (Ab), and anti-HBeAb were detected using a commercially-available enzyme immunoassay [20]. *HBeAg-seroclearance* was defined as any patient with HBeAg-loss during follow-up. *HBsAg-seroclearance* was similarly defined as HBsAg-loss during follow-up.

Serum qHBsAg was performed using either the Architect HBsAg or Elecsys HBsAg II assay (detection limit=0.05 IU/mL for both assays). High correlation between these assays has been observed in specifically HIV-HBV co-infected patients [22]. Serum qHBeAg was determined using either the Architect (with Architect i2000 analyzer) or Elecsys HBeAg assay (with Modular E170 analyzer). Using a previously designed protocol [23], assay-corrected signals were converted to Paul Ehrlich Institute Units (PEIU)/mL.

Alanine aminotransferase (ALT) levels were quantified using standard methods. Liver fibrosis was assessed at baseline and at least once during follow-up by a non-invasive method: Fibroscan® (Echosens, Paris, France) conducted by a trained clinical research associate and/or Fibrotest® calculated from a standard battery of biochemical levels. METAVIR equivalents of these measures were used to grade liver fibrosis [24,25].

Statistical analysis

Baseline characteristics were compared between treatment groups using Kruskal-Wallis test for continuous variables and Pearson χ^2 test or Fisher's exact test for categorical variables.

The kinetics of antigen quantification were compared between treatment groups during two follow-up periods: *intensification* (during PegIFN treatment in patients from the TDF+PegIFN group and the matched time-period in patients from the TDF group) and *post-intensification* (all

follow-up thereafter). Mixed-effect linear regression was used to estimate the average slope of antigen decline, while including a random-intercept in order to account for between-patient variation at baseline. Slopes were stratified on treatment group and follow-up period and were directly calculated via a three-way interaction model. Differences in slopes between treatment groups were compared within each follow-up period. Antigen kinetics were modeled unadjusted; adjusted *a priori* for matching criteria, i.e. age, CD4+ cell count <350/mm^3, F4 liver fibrosis (Model 1); and additionally adjusted for gender, baseline qHBeAg or qHBsAg level, and concomitant treatment with LAM and/or FTC (Model 2).

Determinants associated with time to HBeAg-seroclearance were modeled using Cox proportional hazards regression. Based on previous data suggesting low rates of HBsAg-seroclearance [10,11,26], we did not conduct analysis on this end-point. A multivariable model was constructed by forcing treatment group in the model and placing all covariables with a *P*-value <0.1 in univariable analysis.

All statistical analyses were performed using STATA software (v12.1, College Station, TX, USA) and significance was determined using a *P*-value <0.05.

RESULTS

Description of the study population

Patient flow from the source studies before and after matching is described in Supplementary Figure 1. In total, 46 patients in the TDF+PegIFN group were matched to 46 patients of the TDF control group.

Baseline characteristics per treatment group are described in Table 1. As expected, there were no significant differences in age (median=47 years, IQR=39-50), CD4+ cell count (median=500/mm^3, IQR=370-697), and percent with F4 fibrosis (9.8%). Baseline HIV-RNA VL was significantly higher among patients in the TDF group, yet no difference was observed in the proportion with undetectable HIV-RNA VL (*P*=0.8). The proportion of patients with previous LAM-exposure and concomitant treatment with LAM and/or FTC was significantly higher in the TDF+PegIFN versus TDF group.

Kinetics of HBeAg quantification during follow-up

Median follow-up was 33.4 months (IQR=19.0-36.3), with no significant difference in follow-up time between treatment groups (*P*=0.5). From baseline to month-12 and end of follow-up visits, respectively, median qHBeAg levels decreased from 6.1, 4.1, and 1.7 PEIU/mL in the TDF and 7.6, 1.0, and 0.8 PEIU/mL in the TDF+PegIFN group (Figure 1A). Average decline of qHBeAg was significantly faster among patients undergoing TDF+PegIFN compared to TDF alone during the intensification period, yet no differences in qHBeAg slope were observed thereafter (Table 2). These results held after adjusting for baseline matching criteria or when also including sex, baseline qHBeAg level and concomitant treatment with LAM and/or FTC.

Declines of >1.0 $\log_{10}$ PEIU/mL were observed in 19 patients, resulting in a cumulative percent of 13.6% at month-12 and 23.2% at the end of follow-up. One-$\log_{10}$ declines or greater occurred more frequently in those undergoing TDF+PegIFN (*n*=17, cumulative 34.1%) versus TDF (*n*=2, cumulative 6.9%) during follow-up (*P* log-rank test=0.001).

HBeAg seroclearance and treatment strategy

HBeAg-seroclearance occurred in 22 patients, of whom 13 (28.3%) and 9 (19.6%) were in the TDF+PegIFN and TDF groups, respectively. In the TDF+PegIFN group, the incidence rate (IR) of HBeAg-seroclearance was faster during the intensification period (IR=24.4/ person•years) compared to post-intensification (IR=9.0/100 person•years), averaging 13.2/100 person•years (cumulative 50.9%) during overall follow-up. In the TDF group, IR were similar between matched intensification period (IR=13.6/100 person•years) and post-intensification (IR=10.3/100 person•years), averaging 12.6/100 person•years (cumulative 46.1%) during overall follow-up. As shown in Figure 1C, no significant difference in time to HBeAg-seroclearance was observed between treatment groups during the intensification period, post-intensification, or overall (*P* for log-rank test=0.19, 0.5, 0.5, respectively).

Of those who seroconverted, 3 (13.6%) acquired anti-HBe antibodies (one treated with TDF and two treated with TDF+PegIFN) and 6 (27.3%) reverted back to HBeAg-positive serology (all six treated with TDF+PegIFN). Assuming patients with HBeAg-seroclerance reverting HBeAg-positive never had HBeAg-loss during follow-up, IR would have been 6.5/100 person•years in the TDF+PegIFN group, still with no significant difference between treatment groups (*P*=0.4).

In multivariable analysis (Table 2), PegIFN intensification did not emerge as a significant determinant of HBeAg-seroclearance (*P*=0.5). Significantly lower rates of HBeAg-seroclearance were observed in patients with longer duration of TDF or ART prior to baseline and those with higher baseline qHBsAg levels. Of note, no specific level of baseline qHBeAg was predictive of HBeAg-seroclearance when added to the multivariable model (≤10 PEIU/mL, adjusted-HR=2.70, 95%CI=0.37-19.71). When using specific cut-offs of baseline qHBsAg, patients with ≤1000 IU/mL and ≤100 IU/mL had a 7.06-times (95%CI=2.93-17.02) and 3.02-times (95%CI=0.27-33.78) higher adjusted hazards of HBeAg-seroclearance, respectively.

Kinetics of HBsAg quantification during follow-up

From baseline to month-12 and end of follow-up visits, respectively, median qHBsAg levels were 3.71, 3.67, and 3.61 $\log_{10}$ IU/mL in the TDF group versus 3.72, 3.39, and 3.14 $\log_{10}$ IU/mL in the TDF+PegIFN group (Figure 1B). The rate of qHBsAg decline tended to be faster in the TDF+PegIFN versus TDF group, with no significant difference between treatment groups during post-intensification follow-up (Table 2). Similar observations were found after adjusting for baseline matching criteria or in the fully-adjusted model.

Declines of >1.0 $\log_{10}$ IU/mL were observed in 12 patients, resulting in a cumulative percent of 10.3% at month-12 and 18.1% at the end of follow-up. One-$\log_{10}$ declines or greater occurred more frequently in those undergoing TDF+PegIFN (*n*=10, cumulative 24.3%) versus TDF (*n*=2, cumulative 14.5%) during follow-up (*P* log-rank test=0.04).

HBsAg seroclearance and treatment strategy

Only three patients achieved HBsAg-seroclearance (IR=1.6/100 person•years), none of whom acquired anti-HBs antibodies. The IR of HBsAg-seroclearance was 1.3/100 person•years (cumulative 4.0%) in patients with TDF and 1.8/100 person•years (cumulative 4.4%) with TDF+PegIFN. Accordingly, time to HBsAg-seroclearance was not significantly different between treatment groups (Figure 1D, *P* for log-rank test=0.7).

One patient treated with TDF had fairly high qHBeAg and qHBsAg levels at baseline (4.88 $\log_{10}$ IU/mL and 517.6 PEIU/mL). Large drops in qHBeAg (-517.16 PEIU/mL) and qHBsAg (-6.18 $\log_{10}$ IU/mL) were observed during the first six months of TDF-containing ART, followed by

HBeAg- and HBsAg-negative serology. Two patients treated with TDF+PegIFN had low baseline levels of qHBeAg (range=0.13-5.77 PEIU/mL) and qHBsAg (range=0.32-2.57 log_{10} IU/mL), proceeding to drop until HBeAg-seroclearance (after 5.6-7 months) and then HBsAg-seroclerance (after 10.5-11.2 months). At the end of follow-up, one patient reconverted to HBsAg-positive serology, while the other reconverted back to HBeAg- and HBsAg-positive serology.

Evolution of transaminases and liver fibrosis during follow-up

In the TDF group, median ALT/AST levels slowly declined from 36 (IQR=23-47)/29 (IQR=25-36) IU/mL at baseline to 32 (IQR=25-47)/29 (IQR=24-33) IU/mL at month-12 and 27 (IQR=19-43)/28 (IQR=23-33) IU/mL at the end of follow-up. Patients in the TDF+PegIFN group had a slight peak of transaminase levels after PegIFN intensification (ALT/AST): 33 (IQR=27-39)/30 (IQR=24-34) IU/mL at baseline, 37 (IQR=28-57)/35 (IQR=25-47) IU/mL at month-12, and 27 (IQR=21-38)/25 (IQR=21-32) IU/mL at the end of follow-up; with no significant difference over time when compared to TDF alone (*P* for interaction=0.10/0.4).

Percentage of patients with ≥F3 liver fibrosis, as measured using non-invasive markers, remained stable for both TDF and TDF+PegIFN groups, respectively: baseline, 12.8% and 14.3% and end of follow-up 15.2% and 21.7%. There were no significant differences in liver fibrosis levels over time between treatment groups (*P* for interaction=0.4).

Serious adverse events and liver-related mortality

During follow-up, grade 3/4 laboratory abnormalities (Table 4) occurred in 16 patients overall and were significantly more frequent in patients from the TDF+PegIFN versus TDF groups

(32.6% and 2.2%, respectively, $p<0.001$). No patient permanently discontinued TDF due to a serious adverse event. While no patient in the TDF+PegIFN group experienced a severe event, one patient in the TDF group with baseline cirrhosis was diagnosed with HCC after 34 months of TDF.

DISCUSSION

Seroclearance is rare for HIV-HBV co-infected patients undergoing highly potent anti-HBV agents [20]. Further evaluation of therapeutic combinations that could increase the rates of these outcomes is therefore strongly needed. In our study, we evaluated the strategy of adding PegIFN to an already existing TDF-containing antiretroviral regimen in carefully matched co-infected patients with similar characteristics during the course of TDF-containing ART. Although faster declines in qHBeAg and, to a lesser extent, qHBsAg were observed during intensification, there was no advantage in qHBeAg or qHBsAg decline thereafter and no difference in HBeAg- or HBsAg-seroclearance rates in patients with PegIFN intensification compared to TDF alone. More importantly, PegIFN intensification was associated with a significantly higher rate of grade 3/4 laboratory abnormalities [15].

Indeed, the two principal agents used in this study have different mechanisms of action [27]– PegIFN has an immunomodulatory effect allowing some patients to produce host responses and clear viral infection whereas TDF directly inhibits viral polymerase activity. These treatments would then be ideal candidates for combination therapy. For HBeAg-positive HBV mono-infected patients, previous research has shown higher rates of HBeAg-seroclearance with PegIFN versus NA-based regimens, while no synergistic benefit has been observed when initiating combined therapy [13,15,28,29]. Preliminary evidence has suggested that higher

seroconversion rates could be achieved when adding PegIFN to potent NA-based therapy [30]. In several randomized-control trials, adding PegIFN after 12 or 24 weeks of treatment with the potent NA entecavir (ETV) appears to provide a marginal increase in HBeAg-seroclearance rates, which increases slightly further when treatment is discontinued [17,31].

In HIV-HBV co-infected patients, interferon-based regimens have been historically unsuccessful in increasing HBeAg-seroclearance rates [18], even in combination with more potent nucleoside/nucleotide analogues such as adefovir [32]. It was then assumed that long-term treatment with TDF, presenting high anti-HBV potency and a high genetic barrier to resistance [9], would efficiently reduce HBV replication, allowing ideal conditions for PegIFN intensification to evoke progression towards serological clearance [10,33]. Nevertheless, our results are disappointing with no significant difference in HBeAg- and HBsAg-seroclearance rates between treatment groups, even after three years of follow-up. Of note, HBeAg-serocleance rates in our co-infected group undergoing PegIFN intensification, at 13.2/100 person•years, were substantially lower than those reported in HBV mono-infected patients undergoing ETV-treatment with PegIFN intensification (30-34/100 person•years) or switching to PegIFN (38/100 person•years) [16,17,31].

Naturally, immunosuppression imposed by HIV-infection could explain the fairly low seroclearance rates observed in our study [18]. In HBV mono-infection, viral clearance during treatment with PegIFN, with or without lamivudine, is strongly associated with intrahepatic CD8+ T cell response [34]. PegIFN also induces secretion of specific cytokines and chemokines via IFN signaling pathways, resulting in natural killer (NK) cell proliferation and activation of CD8+ T cells [35]. These responses appear to be enhanced with HBV suppression during concomitant TDF. Nonetheless, HIV-HBV co-infected patients have much lower frequencies of intrahepatic NK and CD8+ T cells, while only marginally increasing after initiating ART with an anti-HBV

agent [36]. HIV-infection also alters the quality of cellular immunity and decreases T cell capacity to respond to HBV antigens [37]. The level of past immunosuppression, as deemed by nadir CD4+ T cell count, was fairly low and homogeneous in our study population and perhaps the lack of effect with PegIFN intensification could be due to the inability to mount appropriate responses required for HBeAg-seroclearance.

Some insight as to why seroclearance rates were so similar between treatment groups can be inferred from qHBeAg and qHBsAg. Previous studies in HBV mono-infected patients have observed significantly faster short-term declines in both qHBeAg and qHBsAg during PegIFN alone, PegIFN added to ETV treatment, and ETV added to PegIFN treatment when compared to ETV alone [17,31,38]. Likewise, we observed that changes in both qHBeAg and qHBsAg were much faster during the PegIFN intensification period compared to TDF alone, even after adjusting on several factors. After intensification, declines were no different between treatment groups and were slow. The faster decreases in antigen levels during PegIFN intensification, albeit significant, were therefore not sufficient enough to increase HBeAg- or HBsAg-seroclearance rates. However, it should be mentioned that much lower levels of qHBsAg at the moment of switching from ETV to PegIFN, around 200 IU/mL, are highly predictive of HBeAg-seroconversion and HBsAg-loss in HBV mono-infection [16]. Roughly 90% of patients in our study had levels >1000 IU/mL, possibly making them non-ideal candidates for successful PegIFN therapy. In addition, these results lend to question if prolonged PegIFN could increase seroclearance rates, although the major drawback would be potentially increasing the rate of PegIFN-associated adverse events.

Flares in transaminases were rare and although a slight increase in ALT/AST levels was observed during PegIFN intensification, they were not significantly different compared to TDF alone. In addition, there were no major changes in the proportion of patients with liver fibrosis or

cirrhosis over time, mostly owing to the extensive period of TDF-containing ART prior to inclusion [39]. The lack of treatment effect on fibrosis levels, coupled with transaminase levels, would suggest no particular clinical benefit to adding PegIFN.

Certain limitations need to be addressed. First, since inclusion was based on a convenience sample of all available patients in the EMVIPEG and French HIV-HBV Cohort studies, we did not perform a sample size calculation prior to conducting analysis. Assuming that TDF-alone would have the same HBeAg-seroclearance rates as presented herein, HBeAg-seroclearance would have to occur in 34.6% of TDF+PegIFN-treated patients per year in order to achieve 80% power in detecting a significant difference. Nevertheless, data from qHBeAg and qHBsAg help reinforce conclusions regarding the observed inefficacy of PegIFN intensification. Second, some bias could have resulted from differential loss-to follow-up. However, lost to follow-up was mostly due to ending participation in the French HIV-HBV cohort and not to causes that would have influenced seroclearance events. Third, qHBsAg and qHBeAg were measured using different assays, particularly for patients enrolled in the French HIV-HBV cohort. Despite the almost perfect correlation between assays in co-infected patients [22,23], we cannot rule out potential measurement error.

In conclusion, adding PegIFN to pre-existing TDF-containing ART does not increase HBeAg or HBsAg seroclearance rates in HIV-HBV co-infected patients with HBeAg-positive serology. Due to its high cost, lower tolerance, and low efficacy, PegIFN intensification is not recommended for this specific patient group. Whether this treatment strategy is efficacious in patients without previously severe immunosuppression and/or lower levels of qHBsAg remains unresolved. Still, our findings underscore the need for other therapeutic agents to increase seroclearance and seroconversion rates in co-infected individuals.

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TABLES

Table 1. Description of the study population at baseline

	TDF (*n*=46)	TDF+PegIFN (*n*=46)	*P*†
Demographics			
Sex ratio, males/females (% males)	42/4 (91.3)	44/2 (95.7)	0.7
Age, years*	45 (40-51)	47 (39-50)	0.8
Originating from HBV-endemic zone**	3 (6.5)	3 (6.5)	0.9
HIV Infection			
Duration of known HIV infection, years*	15.6 (12.3-19.5)	14.9 (9.4-19.1)	0.2
AIDS-defining illness**	15 (32.6)	17 (37.0)	0.7
CD4+ cell count, /mm^3*	474 (357-686)	510 (372-701)	0.8
CD4+ cell count, /mm^3**			0.7
≥500	21 (45.7)	25 (54.4)	
≥350 and <500	14 (30.4)	11 (23.9)	
<350	11 (23.9)	10 (21.7)	
Nadir CD4+ cell count, /mm^3 [N=83]*	197 (72-321)	170 (78-295)	0.5
Undetectable HIV-RNA (<50 copies/mL)**	40 (87.0)	41 (89.1)	0.8
HIV-RNA viral load, $\log_{10}$ copies/mL*††	2.05 (1.95-2.43)	1.90 (1.85-1.91)	0.01
Duration of ART, years*	9.6 (8.1-13.7)	11.1 (8.2-13.2)	0.8
HBV characteristics			
Estimated duration of HBV infection, years*	11.1 (6.4-17.0)	9.8 (4.8-15.4)	0.3
Undetectable HBV-DNA (<60 IU/mL)**	36 (78.3)	35 (76.1)	0.9
HBV-DNA viral load, $\log_{10}$ copies/mL*††	2.58 (2.37-2.91)	2.17 (2.02-2.97)	0.7

Table 1 (con't). Description of the study population at baseline

	TDF (*n*=46)	TDF+PegIFN (*n*=46)	*P*†
Previous LAM/FTC-exposure**	46 (100)	45 (97.8)	0.9
Cumulative FTC duration, months*‡	33.5 (11.1-38.9)	16.2 (9.5-27.2)	0.03
Cumulative LAM duration, months*‡	76.8 (53.3-95.8)	93.3 (52.3-124.0)	0.2
Previous TDF-exposure**	46 (100)	46 (100)	*ntp*
Cumulative TDF duration, months*‡	38.1 (20.8-62.9)	41.4 (25.0-59.3)	0.8
Concomitant LAM/FTC-treatment**	36 (78.3)	45 (97.8)	0.007
qHBsAg, $\log_{10}$ IU/mL*	3.71 (3.46-4.06)	3.72 (3.26-4.02)	0.9
qHBeAg, PEI U/mL*	6.1 (1.3-26.7)	7.6 (2.1-26.5)	0.5
ALT, IU/mL*	36 (23-47)	33 (27-39)	0.9
AST, IU/mL*	29 (25-36)	30 (24-34)	0.6
F4 liver fibrosis**	5 (10.9)	4 (8.7)	0.9

*Median (IQR). **Number (%).

†Significance between treatment groups determined using Kruskal-Wallis test for continuous variables and Pearson χ^2 test or Fisher's exact test for categorical variables. *ntp* - no test performed

††Only among patients with detectable HIV or HBV viremia.

‡Only among patients with previous exposure to given treatment.

Table 2. Kinetics of hepatitis B "e" antigen quantification (qHBeAg) and hepatitis B surface antigen quantification (qHBsAg) during follow-up

	Intensification		Post-intensification	
	Δ (95%CI)	*P* for intx	Δ (95%CI)	*P* for intx
qHBeAg (log_{10} PEIU/mL per month)				
Unadjusted		0.001		0.7
TDF	-0.027 (-0.043, -0.011)		-0.009 (-0.019, 0.001)	
TDF+PegIFN	-0.066 (-0.081, -0.050)		-0.012 (-0.025, 0.001)	
Model 1		0.001		0.7
TDF	-0.027 (-0.043, -0.011)		-0.009 (-0.020, 0.001)	
TDF+PegIFN	-0.066 (-0.081, -0.050)		-0.012 (-0.025, 0.001)	
Model 2		0.001		0.5
TDF	-0.029 (-0.045, -0.014)		-0.009 (-0.019, 0.001)	
TDF+PegIFN	-0.066 (-0.081, 0.051)		-0.015 (-0.027, -0.002)	
qHBsAg (log_{10} IU/mL per month)				
Unadjusted		0.09		0.9
TDF	-0.026 (-0.045, -0.007)		-0.006 (-0.016, 0.004)	
TDF+PegIFN	-0.049 (-0.068, -0.031)		-0.005 (-0.020, 0.010)	
Model 1		0.08		0.9
TDF	-0.026 (-0.046, -0.007)		-0.006 (-0.017, 0.004)	
TDF+PegIFN	-0.050 (-0.069, -0.031)		-0.006 (-0.021, 0.010)	
Model 2		0.09		0.9
TDF	-0.027 (-0.046, -0.007)		-0.008 (-0.019, 0.003)	
TDF+PegIFN	-0.050 (-0.068, -0.031)		-0.008 (-0.023, 0.008)	

Table 2 (con't). Kinetics of hepatitis B "e" antigen quantification (qHBeAg) and hepatitis B surface antigen quantification (qHBsAg) during follow-up

Stratified estimates were provided for patients undergoing tenofovir alone (TDF) or tenofovir with pegylated-interferon intensification (TDF+PegIFN). Treatment groups were compared via interaction (itxn) as described in the Methods. Model 1 adjusts for baseline matching criteria (age, CD4+ cell count <350/mm^3, F4 liver fibrosis) and Model 2 additionally adjusts for gender, baseline qHBeAg or qHBsAg level, and concomitant treatment with lamivudine and/or emtricitabine.

Table 3. Determinants of hepatitis B "e" antigen seroclearance during follow-up

	Univariable		Multivariable**	
	HR (95%CI)	*P*	HR (95%CI)	*P*
Age at baseline (per year)	1.00 (0.93-1.06)	0.9		
AIDS-defining illness	2.01 (0.88-4.55)	0.1		
Baseline CD4+ cell count				
≥500/mm^3	1.00			
≥350/mm^3 and <500/mm^3	0.62 (0.21-1.89)	0.4		
<350/mm^3	1.07 (0.41-2.75)	0.9		
Nadir CD4+ cell count [N=83]				
≥250/mm^3	1.00			
≥100/mm^3 and <250/mm^3	0.71 (0.23-2.20)	0.6		
<100/mm^3	0.53 (0.18-1.50)	0.2		
Undetectable HIV-RNA*	1.01 (0.91-1.12)	0.8		
Baseline ART duration (per year)	0.87 (0.77-0.97)	0.02	0.89 (0.80-0.99)	0.04
Baseline LAM duration (per month)	0.99 (0.98-0.99)	0.02		
Baseline TDF duration (per month)	0.97 (0.95-0.99)	0.008	0.97 (0.95-0.99)	0.02
Concomitant LAM/FTC-treatment*	0.95 (0.83-1.10)	0.5		
TDF + pegIFN intensification	1.32 (0.54-3.21)	0.5	1.35 (0.53-3.45)	0.5
Undetectable HBV-DNA*	1.03 (0.96-1.12)	0.4		
Baseline qHBeAg (per PEIU/mL)	1.00 (0.99-1.01)	0.9		
Baseline qHBsAg (per $\log_{10}$ IU/mL)	0.53 (0.33-0.84)	0.006	0.48 (0.31-0.75)	0.001

Table 3 (con't). Determinants of hepatitis B "e" antigen seroclearance during follow-up

	Univariable		Multivariable**	
	HR (95%CI)	*P*	HR (95%CI)	*P*
Baseline ALT				
<1× ULN	1.00			
1-2× ULN	1.02 (0.40-2.56)	0.9		
>2× ULN	2.77 (0.97-7.93)	0.06		
Baseline F4 liver fibrosis	0.52 (0.06-4.26)	0.5		

Parameter estimates for female gender and zone of HBV-endemicity could not be calculated.

*Time varying covariate.

**When building the multivariable model, the following variables were excluded in forward-stepwise fashion because they no longer had *P*-values below the pre-specified threshold: AIDS-defining illness (*P*=0.113), baseline LAM duration (*P*=0.165), and baseline ALT levels (*P*=0.118).

Table 4. Serious adverse laboratory abnormalities during follow-up

	TDF+PegIFN (*n*=46)		TDF (*n*=46)	
	Per period*	Total	Total	*P***
Any grade 3/4 laboratory abnormalities	14 (30.4) / 2 (4.4)	15 (32.6)	1 (2.2)	<0.001
Liver enzyme elevation (>5 ULN)	5 (19.9) / 0	5 (10.9)	1 (1.2)	0.2
Anemia (hemoglobin <7 g/dL)	2 (4.3) / 1 (2.2)	3 (6.5)	0	0.2
Lymphopenia (leukocytes <2000/mm^3)	1 (2.2) / 1 (2.2)	2 (4.4)	0	0.5
Neutropenia (neutrophils <750/mm^3)	7 (15.2) / 0	7 (15.2)	0	0.01
Thrombocytopenia (platelet count <50,000/mm^3)	3 (6.5) / 0	3 (6.5)	0	0.2
Renal impairment (eGFR† <30 mL/min per 1.73m^2)	0 / 0	0	0	*ntp*

Numbers (%) are given. Laboratory abnormalities are based on ANRS criteria for HIV-infected adults.

*Provided during PegIFN intensification and post-intensification, respectively. Some events indicated in the post-intensification period could include on-going events from the intensification period.

**Comparing total numbers of serious adverse events. Significance between treatment groups determined using Pearson χ^2 test or Fisher's exact test. *ntp* - no test performed.

†Estimated glomerular filtration rate (eGFR) using the CKD-EPI equation.

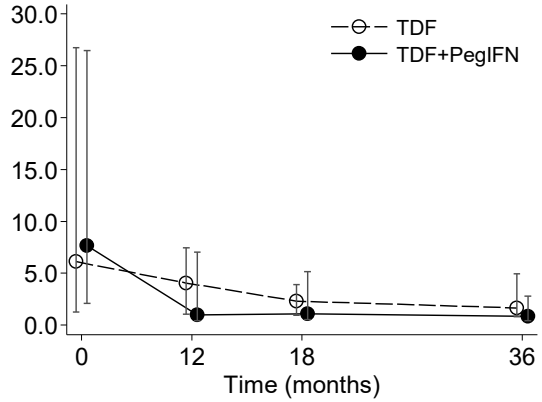
FIGURE LEGENDS

Figure 1. Hepatitis B "e" antigen (HBeAg) and hepatitis B surface antigen (HBsAg) quantification and seroclearance during follow-up

Median levels of (**A**) HBeAg quantification (qHBeAg) and (**B**) HBsAg quantification (qHBsAg) and their interquartile ranges (in bars) are given for specific time-points among patients undergoing either tenofovir alone (TDF) or tenofovir with pegylated-interferon intensification (TDF+PegIFN). qHBeAg was quantified in Paul Erhlich Institute Units (PEIU)/mL. *Visits at which antigen levels were significantly different between treatment groups (P<0.05). Cumulative proportion of patients with HBeAg-seroclearance (**C**) and HBsAg-seroclearance (**D**) are also depicted for each treatment group.

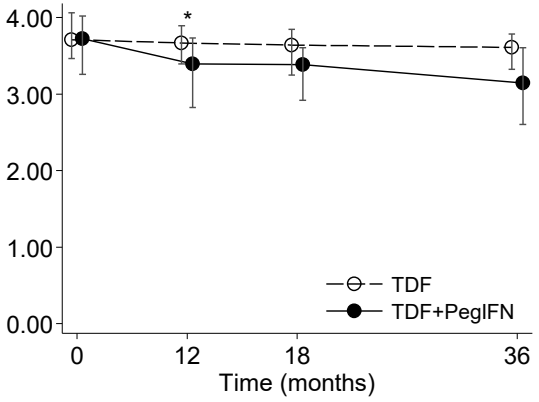
Figure 1.

A



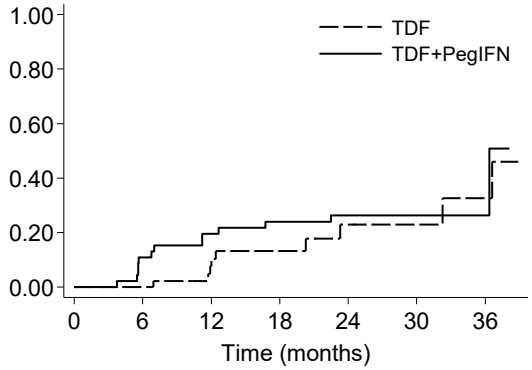
n				
TDF	46	46	16	33
TDF+PegIFN	46	46	45	33

B

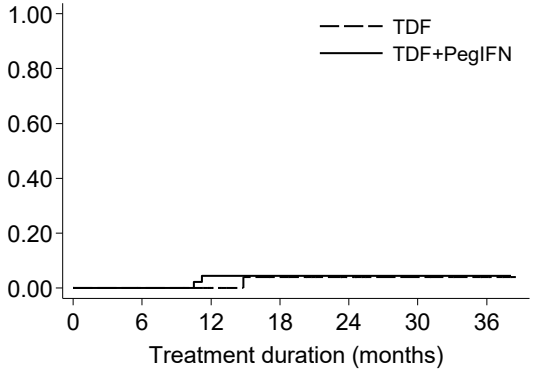


n				
TDF	46	46	20	40
TDF+PegIFN	46	46	45	33

C



D



SUPPLEMENTARY MATERIAL

Supplementary Figure 1. Patient flow