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Switching from entecavir to PegIFN alfa-2a in patients with HBeAg-positive chronic hepatitis B: A randomised open-label trial (OSST trial)

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Background & Aims: Durable post-treatment response is uncommon in chronic hepatitis B (CHB) patients on nucleos(t)ide analogue therapy. Response, response predictors and safety were assessed in patients who switched from long-term entecavir (ETV) to peginterferon alfa-2a.

Methods: Hepatitis B e antigen (HBeAg)-positive CHB patients who had received ETV for 9–36 months, with HBeAg <100 PEIU/ml and HBV DNA ⩽1000 copies/ml, were randomised 1:1 to receive peginterferon alfa-2a 180 μg/week or ETV 0.5 mg/day for 48 weeks. The primary endpoint was HBeAg seroconversion at week 48 (ClinicalTrials.gov: NCT00940485).

Results: 200 patients were randomised; 197 received ⩾1 study drug dose. Five patients who were anti-HBe-positive at baseline were excluded from the modified intention-to-treat population (peginterferon alfa-2a, n = 94; ETV, n = 98). Patients who switched to peginterferon alfa-2a achieved higher week 48 HBeAg seroconversion rates *vs.* those who continued ETV (14.9% *vs.* 6.1%; $p = 0.0467$). Only patients receiving peginterferon alfa-2a achieved HBsAg loss (8.5%). Among peginterferon alfa-2a-treated patients with HBeAg loss and HBsAg <1500 IU/ml at randomisation, 33.3% and 22.2% achieved HBeAg seroconversion and HBsAg loss, respectively. Early on-treatment HBsAg decline predicted response at week 48; highest rates were observed in patients with week 12 HBsAg <200 IU/ml (HBeAg seroconversion, 66.7%; HBsAg loss, 77.8%). Alanine aminotransferase elevations were not associated with viral rebound (n = 38). Peginterferon alfa-2a was well-tolerated.

Conclusions: For patients who achieve virological suppression with ETV, switching to a finite course of peginterferon alfa-2a significantly increases rates of HBeAg seroconversion and HBsAg loss. A response-guided approach may identify patients with the greatest chance of success.

Introduction

In patients with chronic hepatitis B (CHB), hepatitis B surface antigen (HBsAg) loss is associated with a reduction in the progression to cirrhosis or hepatocellular carcinoma [1] and is considered the ultimate endpoint of therapy [2–4]. Profound suppression of hepatitis B virus (HBV) DNA replication can be achieved and sustained with potent nucleos(t)ide analogues (NAs) such as entecavir (ETV) but the rate of HBsAg loss during NA therapy is low (0–3% after 1 year) [5–7] and virological relapse typically occurs if therapy is withdrawn [7,8]. Indeed, it has been suggested that patients would need to be treated with an NA for a median of 52.2 years to clear HBsAg [9]. In contrast, HBsAg loss and seroconversion rates are higher with peginterferon (3–7% after 1 year) [2,10] and increase after discontinuation of therapy [7,8].

Treatment guidelines suggest that therapy may be discontinued after 6–12 months of consolidation therapy in non-cirrhotic patients who achieve hepatitis B e antigen (HBeAg) seroconversion during NA therapy [2,4]. However, over half of patients do not achieve a durable off-treatment response after consolidation therapy [11] and indefinite treatment is often required, which is associated with increased risks of viral resistance and poor adherence [2,12]. In contrast, HBeAg seroconversion rates are durable after discontinuation of peginterferon alfa-2a (PegIFN alfa-2a) therapy, increasing to up to 36% after 6 months [13,14], and are

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Abbreviations: ALT, alanine aminotransferase; CHB, chronic hepatitis B; CI, confidence interval; ETV, entecavir; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; ITT, intention-to-treat; mITT, modified intention-to-treat; NA, nucleos(t)ide analogue; NPV, negative predictive value; PegIFN alfa-2a, peginterferon alfa-2a; PPV, positive predictive value; ROC, receiver-operating characteristic; ULN, upper limit of normal.

Research Article

sustained in 83% of patients 12 months post-treatment [15]. Furthermore, the incidence of HBsAg loss is increased in patients who experience HBeAg seroconversion during PegIFN alfa-2a therapy [16,17]. Despite this, more patients are treated with NAs than with PegIFN alfa-2a [18]. Patients who do not experience HBeAg seroconversion after more than 1 year of treatment with a potent NA may be considered "difficult to treat" despite having achieved HBV DNA suppression, because of the failure to seroconvert and the high likelihood that they will require lifelong therapy [19].

Patients who switched from adefovir or lamivudine to PegIFN alfa-2a have been reported to achieve higher rates of sustained response than those continuing NA monotherapy in small studies [20–22]. The objective of this study was to determine whether switching to PegIFN alfa-2a increases rates of HBeAg seroconversion and HBsAg loss in patients with well-controlled HBV DNA replication on long-term ETV therapy.

Patients and methods

Study participants

The Optimising HBeAg Seroconversion in HBeAg-positive CHB patients with combination and Sequential Treatment of PegIFN alfa-2a and ETV (OSST) study was a phase IV, open-label, randomised study conducted at 7 hepatology centres in China between April 2009 and December 2011. Patients aged 18–65 years who were HBeAg-positive prior to initiation of ETV treatment, had received ETV treatment for $\geqslant$12 months, had been positive for HBsAg for $\geqslant$6 months prior to enrolment and who had serum HBV DNA $\leqslant$1000 copies/ml and serum HBeAg <100 PEIU/ml were eligible. HBeAg levels above this threshold have been associated with a low likelihood of HBeAg seroconversion [23]. Loss of HBeAg can be a transient phenomenon during treatment with NA [22], thus, patients who had lost HBeAg during ETV therapy, but had not undergone HBeAg seroconversion were included. Patients who had been pre-treated with other antiviral or immunomodulatory agents were excluded, as were patients co-infected with hepatitis A, C or D, and patients with a history or evidence of chronic liver disease associated with another medical condition or decompensated liver disease (Child-Pugh score >5).

To increase the number of eligible patients, the protocol was amended such that the minimum duration of ETV treatment was 9 months and patients who had previously been treated with conventional interferon or adefovir were allowed.

Patients were randomised 1:1 to receive PegIFN alfa-2a 180 μg/week or continue ETV 0.5 mg daily, for 48 weeks (Fig. 1). Patients assigned to PegIFN alfa-2a continued ETV for the first 8 weeks of PegIFN alfa-2a therapy to reduce the risk of alanine aminotransferase (ALT) flares during the switching period. The randomisation schedule was generated by SAS PROC PLAN and stratified by centre with a block size of 4. Patient assignments were communicated to the investigators in sealed envelopes prepared by the statistician.

The study was performed in accordance with Good Clinical Practice and the ethical principles of the Declaration of Helsinki (ClinicalTrials.gov: NCT00940485). The study protocol was approved by the independent central ethics committee of Tongji Medical College at Wuhan. All patients provided written informed consent.

Laboratory measurements

Laboratory tests were performed at weeks 2, 4, 8, 12, 24, and 36. HBsAg, HBeAg, and HBV DNA levels were measured at a central laboratory with the ARCHITECT HBsAg assay (Abbott Laboratories, Lake Forest, IL, USA; dynamic range 0.05–250.0 IU/ml), the AxSYM HBe 2.0 assay (Abbott; dynamic range 0.15–200 PEIU/ml), and either a standard generic HBV DNA assay (ACON Biotech Co. Ltd, Hangzhou, China; dynamic range 1000 copies/ml–$\sim$1 $\times$ 10^8 copies/ml) or the COBAS TaqMan HBV Test (Roche Molecular Diagnostics, Pleasanton, CA, USA; dynamic range 20 IU/ml–1.7 $\times$ 10^8 IU/ml). Anti-HBs and anti-HBe were determined by the ARCHITECT qualitative assays (Abbott). Laboratory personnel were unaware of treatment assignments.

Adverse events (AEs) and laboratory test results were recorded according to the International Conference on Harmonisation Guideline *Clinical Safety Data Management: Definitions and Standards for Expedited Reporting.*

Statistical analysis

The primary endpoint was HBeAg seroconversion (HBeAg loss and detection of anti-HBe antibodies) at week 48. Secondary endpoints included rates of HBeAg loss, HBsAg loss, HBsAg seroconversion, ALT normalisation (ALT level <1 $\times$ the upper limit of normal [ULN]) and HBV DNA levels <1000 copies/ml at week 48. Other secondary endpoints included the change in quantitative serum HBeAg, HBsAg, and HBV DNA levels between baseline and the end of treatment.

All patients who received at least one dose of study drug were included in the intention-to-treat (ITT) and safety populations. In addition a modified ITT (mITT) analysis was conducted, excluding patients who were anti-HBe-positive at baseline prior to randomisation.

It was assumed that the HBeAg seroconversion rate would be 40–45% in the PegIFN alfa-2a arm based on phase III data in treatment-naive HBeAg-positive patients [13], and that the difference in HBeAg seroconversion rates between groups would be 25%. Using these assumptions, a sample size of 90 patients per treatment group would provide a statistical power of at least 85% to detect a between-group difference of 25% at a significance level of 0.05. The sample size was set at 100 per group to allow for a 15% withdrawal rate. Response rates for primary and secondary endpoints were assessed by calculating percentages and 95% confidence intervals (CIs) for the ITT and mITT populations. Patients with missing categorical data at week 48 were classified as non-responders. Categorical variables were analysed by the χ^2 test or Fisher's exact test, and continuous variables were analysed by *t* test or Wilcoxon test as appropriate. Logarithmic transformation was performed in the case of skewed data. Statistical analyses were conducted by SAS version 9.2.

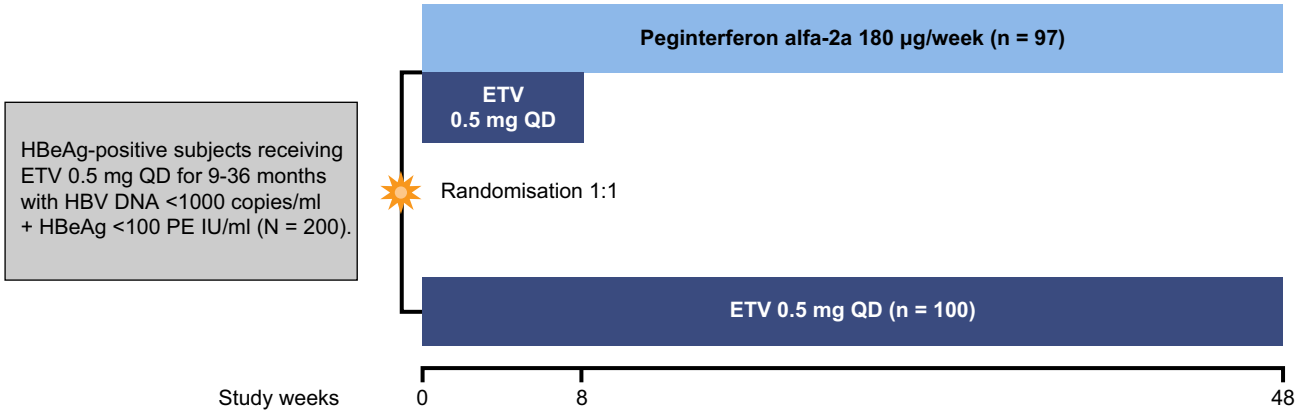

Fig. 1. Study design. Patients in the PegIFN alfa-2a arm received both PegIFN alfa-2a and entecavir (ETV) in the first 8 weeks. HBeAg, hepatitis B e antigen; QD, once daily. *All patients were HBeAg-positive when they started ETV therapy and 55.3% (52/94) and 51.0% (50/98) in the PegIFN alfa-2a and ETV arms, respectively, were HBeAg-negative at the time of randomisation.

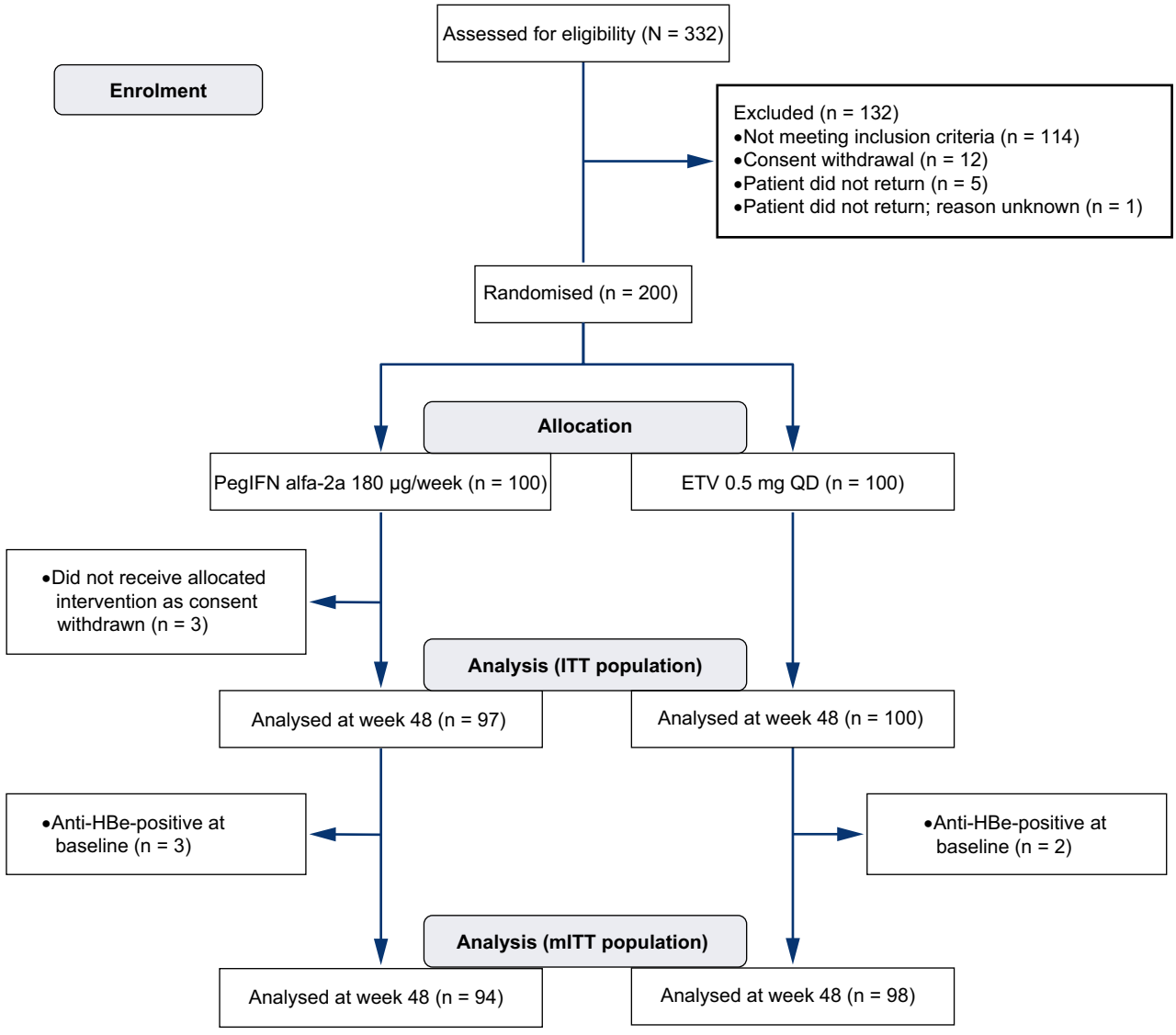

Fig. 2. Patient randomisation and flow. Anti-HBe, antibody to hepatitis B e antigen; ETV, entecavir; PegIFN alfa-2a, peginterferon alfa-2a; QD, once daily. A total of 49 of 114 individuals who did not meet inclusion criteria had HBeAg levels ⩾100 PEIU/ml.

Predictors of HBeAg seroconversion, HBsAg loss, viral rebound and viral breakthrough at week 48 were identified by post-hoc logistic regression analyses of data from patients in the PegIFN alfa-2a arm with available HBsAg, HBeAg and HBV DNA values at baseline, week 12 and week 24. Area under the receiver-operating characteristic (ROC) curves were calculated to identify cut-offs for response and non-response. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were assessed to identify optimal cut-offs for response or non-response at week 48.

Results

Baseline characteristics and patient disposition

Of the 200 patients randomised, 197 patients received ⩾1 dose of the study drug (PegIFN alfa-2a, n = 97; ETV, n = 100) (Fig. 2). Five patients were excluded from the mITT analysis (PegIFN alfa-2a, n = 94; ETV, n = 98); 4 patients achieved HBeAg seroconversion between screening and baseline and one was positive for both HBeAg and anti-HBe at baseline. Baseline demographic and disease characteristics were similar between the two arms (Table 1). Of note, the mean duration of prior ETV treatment was ~20 months. Patients in the PegIFN alfa-2a and ETV arms received a mean of 99.8% and 100% of the target dose, respectively. All patients were HBeAg-positive when they started ETV therapy and 55.3% (52/94) and 51.0% (50/98) in the PegIFN alfa-2a and ETV arms, respectively, were HBeAg-negative at the time of randomisation.

Baseline characteristics of patients were similar when stratified by baseline HBeAg status (i.e., there were no statistically significant differences between those patients who were HBeAg-negative and -positive at baseline, Supplementary Table 1).

Efficacy

The primary endpoint of HBeAg seroconversion at week 48 was achieved in a significantly greater proportion of patients treated

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Table 1. Patient demographics and baseline characteristics.

Demographic or baseline characteristic	Modified ITT	
	PegIFN alfa-2a (n = 94)	ETV (n = 98*)
Males, n (%)	75 (79.8)	85 (86.7)
Mean (SD) age, yr	33.4 (8.3)	33.3 (9.0)
Mean (SD) body mass index, kg/m^2	22.9 (2.7)	22.9 (2.9)
Mean (SD) duration of previous treatment with ETV, mo	19.9 (8.1)	20.5 (8.5)
Mean (SD) HBsAg, $\log_{10}$ IU/ml	3.3 (0.6)	3.3 (0.5)
Mean (SD) HBV DNA by PCR, $\log_{10}$ copies/ml	3.0 (0.1)	3.0 (0)
Mean (SD) HBV DNA by PCR, $\log_{10}$ IU/ml	1.6 (0.4)	1.5 (0.4)
Mean (SD) ALT, U/L	27.3 (21.4)	24.2 (13.8)
Mean (SD) HBeAg, PE IU/ml	8.5 (18.9)	5.7 (10.6)

*Two patients had received prior adefovir therapy.

with PegIFN alfa-2a than ETV (14.9% *vs.* 6.1%; difference = 8.8%; 95% CI = 0.2–17.4; $p = 0.0467$) (Table 2). The trend toward higher HBeAg seroconversion rates in patients treated with PegIFN alfa-2a remained intact when the population was stratified by HBeAg status at baseline (HBeAg positive and HBeAg-negative), although the p values for the comparison with ETV-recipients were not statistically significant because of the small sample sizes (Supplementary Table 2).

The results of the ITT analysis were consistent with the mITT analysis with significantly higher HBeAg seroconversion rates in the PegIFN alfa-2a arm than the ETV arm (15.5% *vs.* 6.0%; difference = 9.5%; 95% CI = 0.9–18.0%; $p = 0.0314$) (Supplementary Table 3).

HBsAg loss (8.5%) and HBsAg seroconversion (4.3%) at week 48 occurred only in patients in the PegIFN alfa-2a arm (Table 2). Among the 8 patients who lost HBsAg, 3 and 5 individuals were HBeAg-positive and -negative at baseline, respectively, and all of the patients who experienced HBsAg seroconversion were HBeAg-negative at baseline (Supplementary Table 2). HBsAg loss at week 48 occurred in 13.6% (8/59) of patients who lost HBeAg and 28.6% (4/14) of patients who had experienced HBeAg seroconversion at week 48. Significantly more patients who switched to PegIFN alfa-2a had low levels of HBsAg at the end of treatment than those who continued ETV; 52.4% and 30.4%, respectively ($p = 0.0032$) had HBsAg levels <1000 IU/ml, while 15.9% and 0% ($p < 0.0001$) had HBsAg levels <10 IU/ml (Table 2).

HBV DNA level <1000 copies/ml was maintained at week 48 in 72.0% and 97.8% of patients in the PegIFN alfa-2a and ETV arms, respectively (Table 2). Results were consistent between the generic and the Roche COBAS assays (data not shown). Similarly, 58.5% and 91.3% of patients in the PegIFN alfa-2a and ETV arms, respectively, had normal ALT levels (<1 × ULN) at week 48 (Table 2). Declines in HBsAg and HBeAg from baseline to week 48 are shown in Supplementary Fig. 1, and the declines in HBsAg and HBeAg levels in relation to HBsAg loss and HBsAg/HBeAg seroconversion are shown in Supplementary Fig. 2.

Thirty-eight patients, 27 of whom were HBeAg-positive at the start of PegIFN alfa-2a therapy, experienced virological breakthrough (HBV DNA >1 $\log_{10}$ IU/ml from nadir) or viral rebound (HBV DNA >1000 copies/ml but not meeting the criterion for virological breakthrough) during treatment with PegIFN alfa-2a (HBV DNA levels returned to <1000 copies/ml by week 48 in 15 individuals). Outcomes in these patients are shown in Supplementary Table 4.

Baseline and on-treatment predictors of response (HBsAg loss or HBeAg seroconversion by week 48) in patients who switched to PegIFN alfa-2a

ROC analyses identified a baseline HBsAg level <1500 IU/ml as the optimal cut-off to predict HBsAg loss or HBeAg seroconversion. The combination of baseline HBeAg negativity and HBsAg <1500 IU/ml (n = 18) resulted in the highest rates of HBeAg seroconversion (33.3%) and HBsAg loss (22.2%) (Table 3A). Neither baseline HBV DNA level (Supplementary Table 5) nor patient demographics, including duration of prior ETV therapy (Supplementary Table 6) predicted response, although there was a trend toward increased response in patients with longer durations of prior ETV therapy.

The week 12 HBsAg level <200 IU/ml was associated with higher rates of HBsAg loss (77.8%; PPV = 78%) and HBeAg seroconversion (66.7%; PPV = 67%) than higher HBsAg levels (Table 3B). Conversely, an HBsAg level ⩾1500 IU/ml was associated with the lowest rates of HBsAg loss (1.7%; NPV = 98%) and HBeAg seroconversion (5.2%; NPV = 95%). These findings remained consistent when patients were stratified by HBeAg status at the start of PegIFN alfa-2a therapy (Table 3B).

Association between ALT levels and viral rebound/breakthrough or response (HBsAg loss or HBeAg seroconversion by week 48)

ALT elevations did not appear to be temporally associated with viral rebound (HBV DNA ⩾1000 copies/ml) since they preceded HBV DNA level elevations by several weeks (Fig. 3). ALT elevations were observed early after switching to PegIFN alfa-2a in some patients with sustained HBV DNA suppression (mean ALT levels increased from 28.1 IU/ml at week 2 to 63.7 IU/ml at week 4 in these patients). Among patients who did not achieve a response there were no apparent associations between the maximum on-treatment ALT level (stratified as <1 × ULN, 1–3 × ULN, and 3–10 × ULN) and achievement of HBV DNA <1000 copies/ml, viral rebound or viral breakthrough. Most patients who achieved a response had maximum ALT levels in the range of 1–3 × ULN, although response rates were not significantly different when stratified by maximum ALT levels (Supplementary Table 7).

Safety

More patients in the PegIFN alfa-2a arm experienced AEs and required dose modifications that patients in the ETV arm (Table 4). Eight patients in the PegIFN alfa-2a arm discontinued treatment for safety reasons compared with none in the ETV arm. ALT flares (>5 × ULN) occurred in 10 out of 97 PegIFN alfa-2a recipients, and resolved in 9 individuals by week 48. One patient had an ALT level 7 × ULN at week 48, despite a relatively low HBV DNA level (1850 copies/ml). No ETV recipients experienced an ALT flare and no PegIFN alfa-2a recipients had an ALT level >10 × ULN. A large proportion of patients in the PegIFN alfa-2a arm experienced decreases in platelet, neutrophil and white blood cell counts during treatment; however, the majority of such episodes (⩾95%) were mild and cell counts returned to normal by week 48 in 88% of patients.

Table 2. Rates of response at week 48 (mITT population).

Outcome	PegIFN alfa-2a		ETV		Difference estimate (95% CI)†	*p* value
	n/N	% (95% CI)	n/N	% (95% CI)		
HBeAg seroconversion	14/94	14.9 (8.4, 23.7)	6/98	6.1 (2.3, 12.9)	8.8 (0.2, 17.4)	0.0467‡
HBeAg loss†	16/42	38.1 (23.6, 54.4)	16/48	33.3 (20.4, 48.4)	4.8 (-15.1, 24.6)	0.6378‡
HBV DNA <1000 copies/ml*	59/82	72.0 (60.9, 81.3)	90/92	97.8 (92.4, 99.7)	-25.9 (-36.0, -15.7)	<0.0001‡
HBsAg loss	8/94	8.5 (3.8, 16.1)	0/98	0 (0, 3.7)	8.5 (2.9, 14.2)	0.0028§
HBsAg seroconversion	4/94	4.3 (1.2, 10.5)	0/98	0 (0, 3.7)	4.3 (0.2, 8.3)	0.0556§
ALT normalisation (<1 x ULN)*	48/82	58.5 (47.1, 69.3)	84/92	91.3 (83.6, 96.2)	-32.8 (-44.9, -20.7)	<0.0001‡
HBsAg <10 IU/ml*	13/82	15.9 (8.7, 25.6)	0/92	0 (0, 3.9)	15.9 (8.0, 23.8)	<0.0001‡
HBsAg <100 IU/ml*	22/82	26.8 (17.6, 37.8)	4/92	4.4 (1.2, 10.8)	22.5 (12.0, 32.9)	<0.0001‡
HBsAg <1000 IU/ml*	43/82	52.4 (41.1, 63.6)	28/92	30.4 (21.3, 40.9)	22.0 (7.7, 36.3)	0.0032‡

*Eighteen patients with missing data were excluded.
†Difference estimate was calculated for the PegIFN alfa-2a group compared with the ETV group.
‡χ^2 test.
§Fisher's exact test.
††Only patients who were HBeAg-positive at the start of treatment with PegIFN alfa-2a are included in calculations.

Table 3. Predictors of response to PegIFN alfa-2a using (A) baseline parameters and (B) on-treatment parameters (mITT population).*

A

Baseline HBeAg status	Baseline HBsAg (IU/ml)	Wk 48 HBsAg loss		Wk 48 HBeAg seroconversion	
		n/N (%)	*p* value†	n/N (%)	*p* value†
HBeAg-negative	<1500	4/18 (22.2)	0.0133	6/18 (33.3)	0.0191
	≥1500	0/32		2/32 (6.3)	
HBeAg-positive	<1500	2/12 (16.7)	n.s.	2/12 (16.7)	n.s.
	≥1500	1/29 (3.5)		3/29 (10.3)	

B

Baseline HBeAg status	Wk 12 HBsAg (IU/ml)	Wk 48 HBsAg loss		Wk 48 HBeAg seroconversion	
		n/N (%)	*p* value‡	n/N (%)	*p* value‡
All patients	<200	7/9 (77.8)	<0.0001	6/9 (66.7)	<0.0001
	200-<1500	0/25		5/25 (20.0)	
	≥1500	1/58 (1.7)		3/58 (5.2)	
HBeAg-positive at baseline	<200	2/2 (100.0)	0.0027	1/2 (50.0)	0.0747
	200-<1500	0/11		2/11 (18.2)	
	≥1500	1/28 (3.6)		2/28 (7.1)	
HBeAg-negative at baseline	<200	5/7 (71.4)	<0.0001	5/7 (71.4)	<0.0001
	200-<1500	0/14		3/14 (21.4)	
	≥1500	0/30		1/30 (3.3)	

*Only patients with available data at baseline or week 12 were included in the baseline and on-treatment predictor analyses, respectively. Patients with missing data at week 48 were classified as non-responders.
†Fisher's exact test.
‡χ^2 test.

Discussion

In this study, significantly higher HBeAg seroconversion rates were observed in patients with an HBeAg level <100 PEIU/ml during treatment with ETV who switched to PegIFN alfa-2a than in those who continued ETV. The rate of HBeAg seroconversion with PegIFN alfa-2a (14.9%) was lower than that in treatment-naive patients in the phase III registration trial (27.0%) [13]. However, we enrolled patients who had not achieved HBeAg seroconversion despite long-term ETV therapy. HBeAg loss and seroconversion are less likely to occur in patients who have received long term NA therapy than in NA-naive patients. For example the rate of HBeAg loss after 52 weeks of treatment with ETV in phase III trials was 22% in NA-naive patients [5], and 10% in patients who had received extensive treatment with lamivudine [24]. Thus, it is not surprising that the response to PegIFN alfa-2a differed from that in treatment-naive patients. The results are consistent with previous small studies, in which patients on long-term lamivudine or adefovir therapy switched to IFN-based therapy [20–22], and in CHB patients with acute exacerbations (ALT levels >10 × ULN) who received sequential ETV and PegIFN alfa-2a [25], but, to our knowledge, this is the first report of the efficacy of switching to a finite course of PegIFN alfa-2a in patients on long-term ETV who have not experienced HBeAg

Research Article

seroconversion despite sustained HBV DNA suppression. Importantly, the trend toward higher HBeAg seroconversion rates in patients treated with PegIFN alfa-2a remained when patients were stratified by HBeAg status at baseline.

HBeAg loss and seroconversion are associated with an increased chance of sustained HBsAg loss [17], which is considered to be an ultimate therapeutic endpoint [2–4]. Importantly, HBeAg seroconversion has been associated with reduced morbidity and mortality [26,27]. Moreover, HBeAg seroconversion is associated with immune restoration and could be the impetus for HBsAg loss and seroconversion [28,29]. Indeed, the current study provides evidence to support this concept. Significantly higher rates of HBsAg loss were observed when patients on long-term ETV switched to a finite course of PegIFN alfa-2a rather than continuing ETV. Moreover, serum HBsAg quantitation is now frequently used both in long-term natural history studies in CHB patients and in studies of antiviral therapy. Such studies have shown that serum HBsAg levels correlate with intrahepatic covalently closed circular DNA (cccDNA) levels in HBeAg-positive patients, and that a decrease in serum HBsAg levels during treatment with PegIFN alfa may reflect a decrease in intrahepatic cccDNA and; thus, may be used to predict a sustained response [30–32]. Here we found significantly more PegIFN alfa-2a-treated than ETV-treated patients had an HBsAg level <1000 IU/ml at week 48 (52.4% *vs.* 30.4% [$p = 0.0032$]); HBsAg <1000 IU/ml is associated with a high probability of HBsAg clearance and good long-term prognosis [33,34]. It should be noted that the precise mechanisms involved in immune restoration during PegIFN alfa-2a treatment require further investigation.

The ability to identify ETV-treated patients who might benefit from switching to PegIFN alfa-2a would be valuable. The combination of HBeAg loss and HBsAg <1500 IU/ml at the time of switching was associated with high rates of HBeAg seroconversion (33.3%) and HBsAg loss (22.2%) following PegIFN alfa-2a therapy. Patients with HBsAg <200 IU/ml at week 12 had the highest likelihood of responding to PegIFN alfa-2a. In contrast, HBsAg ⩾1500 IU/ml was associated with the lowest rate of response. These data are consistent with previous reports that demonstrated that HBsAg quantification early on-treatment is a useful predictor of response in CHB patients [8,14,35]. However, the HBsAg cut-offs identified as optimal predictors of response and non-response in the present analysis were lower than those obtained in treatment-naive patients [35].

The decline in HBsAg to week 48 was greater in patients who experienced HBeAg seroconversion with PegIFN alfa-2a than with ETV and may be associated with the more durable response typical of PegIFN alfa-2a [8,13,14]. A 1-year post-treatment observational follow-up study is ongoing, which will help to further establish the potential benefits of switching to PegIFN alfa-2a after long-term ETV.

Viral rebound occurred in 38 patients after switching to PegIFN alfa-2a, 15 of whom had HBV DNA levels <1000 copies/ml by week 48. Efficacy was reduced in patients with viral rebound, but rebound did not preclude HBeAg seroconversion.

ALT flares (>5 × ULN) occurred only in the PegIFN alfa-2a arm, but were not temporally associated with viral rebound or breakthrough. Such host-induced flares have been reported in patients responding to peginterferon alfa [36] and are thought to reflect immune clearance of HBV. PegIFN alfa-2a was generally well tolerated in this study, with AE rates similar to those previously reported in treatment-naive patients [13,37].

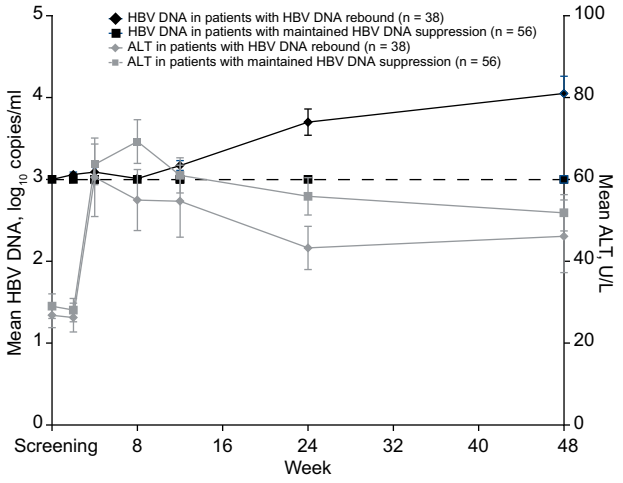

Fig. 3. HBV DNA and ALT levels in patients who switched to PegIFN alfa-2a with maintained HBV DNA suppression (HBV DNA <1000 copies/ml) through week 48 or HBV DNA rebound (HBV DNA ⩾1000 copies/ml) at any time point (mITT population). p >0.05 for a comparison of ALT levels at weeks 8, 12 or 24 for patients with maintained HBV DNA suppression compared with patients with HBV DNA rebound. ALT, alanine aminotransferase; HBV, hepatitis B virus.

HBV DNA suppression occurs rapidly after the initiation of ETV therapy. In a phase III trial, most patients had HBV DNA levels below <3 $\log_{10}$ after 24 weeks of treatment and 67% of NA-naive patients had undetectable HBV DNA after 48 weeks of treatment [5]. A total of 21% of patients experienced HBeAg seroconversion after 48 weeks of treatment with ETV in the trial [5]. In contrast, only 11% of patients experienced HBeAg seroconversion between week 48 and 96 of treatment with ETV [38]. These results suggest that patients who do not respond to NA therapy within the first 6 to 12 months of treatment might be suitable candidates for switching to a finite course of therapy with peginterferon alfa-2a.

The results of this study have practical implications for patients receiving long term ETV therapy, because they demonstrate that it is possible to enhance the chances of HBeAg seroconversion and HBsAg loss by switching to a finite course of PegIFN alfa-2a therapy. Patients who lose HBeAg and have HBsAg levels <1500 IU/ml with ETV are recommended to switch to PegIFN because they have a good chance of HBsAg loss (22.2%) and HBeAg seroconversion (33.3%). Patients with an on-treatment HBsAg level <200 IU/ml at week 12 after switching have a better chance of losing HBsAg (PPV 77.8%) and undergoing HBeAg seroconversion (PPV 67%). For those who still have HBsAg levels >1500 IU/ml at week 12 after switching have a low chance of success (NPV 98% for HBsAg loss and 95% for HBeAg seroconversion). These patients should consider stopping PegIFN. The long term follow-up data will demonstrate the durability of responses. It is unclear from the data presented in this manuscript whether the results apply to individuals with well compensated cirrhosis because these individuals were excluded from the trial.

This study has certain limitations. It was not possible to determine the HBV genotype due to the low HBV DNA levels at baseline, thus the impact of genotype on response could not be ascertained. Previous studies of PegIFN alfa-2a in treatment-naive patients have reported seroconversion rates 6 months post-treatment. The data are being collected and will be reported subsequently. Exploratory analyses into baseline

Table 4. Incidence of discontinuation of treatment, dose modification and adverse events (safety population).

Variable, n (%)	PegIFN alfa-2a (n = 97)	ETV (n = 100)
Discontinuation		
For safety reasons*	8 (8.3)	0
For other reasons	4 (4.1)	7 (7.0)
Dose modification		
Total	13 (13.4)	0
Adverse event	2 (2.1)	0
Laboratory abnormality	11 (11.3)	0
≥1 adverse event	67 (69.1)	5 (5.0)
≥1 serious adverse event	6 (6.2)	0
Deaths	0	0
ALT flare†	10 (10.3)	0
Maximum ALT level		
<1 x ULN	15 (15.5)	73 (73.0)
1—5 x ULN	72 (74.2)	27 (27.0)
5—10 x ULN	10 (10.3)	0
>10 x ULN	0	0
Adverse events‡		
AST increased	7 (7.2)	0
ALT increased	7 (7.2)	0
Platelet count decreased	26 (26.8)	0
Neutrophil count decreased	27 (27.8)	0
White blood cell count decreased	34 (35.1)	0
Pyrexia	15 (15.5)	0
Alopecia	5 (5.2)	0

*Anxiety/depression (n = 1); hyperthyroidism (n = 1); epistaxis (n = 1); platelet count decrease (n = 2); rash (n = 1); hypothyroidism (n = 1); viral myocarditis/myocardial ischaemia (n = 1).
†Defined as >5 × ULN. ULN differed across hospitals.
‡Patients may have had more than one adverse event. Listed adverse events are those reported by ⩾5% of patients in any treatment group.

and on-treatment predictors of response included small numbers of patients, and thus should be interpreted with caution.

In conclusion, this study in patients who have not seroconverted despite sustained HBV DNA suppression on long-term therapy with a potent NA (ETV) is the first to show that switching to PegIFN alfa-2a significantly increases the rate of HBeAg seroconversion and HBsAg loss and is a potential alternative to indefinite NA therapy.

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Conflict of interest

QN has been a member of Advisory Committees or Review Panels, has received consulting fees from Roche, Novartis, GlaxoSmithKline and Bristol-Myers Squibb, and has received grant/research support from Roche, Novartis and Bristol-Myers Squibb China. JH has received consulting fees from Roche, Novartis, GlaxoSmithKline and Bristol-Myers Squibb, and has received grant/research support from Roche, Novartis and GlaxoSmithKline. MZ is an employee of Shanghai Roche Pharmaceuticals Ltd. None of the other authors have any conflict of interest to declare.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhep.2014.05.044.

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