Infect Dis Ther (2021) 10:2259–2270
https://doi.org/10.1007/s40121-021-00497-5

ORIGINAL RESEARCH

Efficacy of Peginterferon alfa-2b in Nucleoside Analogue Experienced Patients with Negative HBeAg and Low HBsAg: A Non-Randomized Clinical Trial

Jun Chen · Min Qi · Xue-Gong Fan · Xing-Wang Hu · Cheng-Jin Liao · Li-Yuan Long · Xiao-Ting Zhao · Min Tan · Hai-Fu Li · Ruo-Chan Chen · Ze-Bing Huang · Yan Huang

Received: April 7, 2021 / Accepted: June 30, 2021 / Published online: July 25, 2021

ABSTRACT

Introduction: Hepatitis B surface antigen (HBsAg) clearance is the treatment goal for hepatitis B e antigen (HBeAg)-negative patients with chronic hepatitis B (CHB). However, its rate is extremely low with nucleoside (acid) analogues (NAs) monotherapy. Peginterferon could enhance HBsAg clearance. This study aimed to evaluate the efficacy of peginterferon alfa-2b (PegIFNα-2b) in NAs-experienced patients with CHB with negative HBeAg and low HBsAg level.

Methods: HBeAg-negative patients with CHB who had received NAs therapy over 24 weeks with HBsAg < 1500 IU/mL and HBV DNA < 100 IU/mL were enrolled. Patients received either PegIFNα-2b add-on therapy (n = 108) or continuous NAs monotherapy (n = 75). The primary endpoint was HBsAg clearance rate at week 48.

Results: At week 48, serum HBV DNA was undetectable among all PegIFNα-2b add-on therapy patients. Almost all patients maintained HBV DNA suppression in the PegIFNα-2b add-on group (100%, 108/108) and NAs monotherapy group (97.33%, 73/75). Only patients with PegIFNα-2b add-on therapy achieved HBsAg clearance (50.93%, 55/108) and HBsAg seroconversion (48.15%, 52/108) at week 48. Patients with baseline HBsAg < 100 IU/mL achieved the highest HBsAg clearance rate and HBsAg seroconversion rate at week 48 (60.87%, 28/46 and 58.70%, 27/46 respectively). HBsAg clearance and HBsAg seroconversion at week 72 had no significant difference with continuing or discontinuing PegIFNα-2b therapy after 48 weeks of treatment. PegIFNα-2b add-on therapy was well tolerated.

Conclusions: PegIFNα-2b add-on therapy increases HBsAg clearance rate and seroconversion rate for HBeAg-negative patients with CHB, particularly for those with lower HBsAg level. It would be unnecessary to prolong PegIFNα-2b duration after 48 weeks of PegIFNα-2b treatment.

Keywords: Add-on therapy; Chronic hepatitis B; PegIFNα-2b; Nucleoside (acid) analogues

J. Chen · M. Qi · X.-G. Fan · X.-W. Hu · C.-J. Liao · L.-Y. Long · X.-T. Zhao · M. Tan · H.-F. Li · R.-C. Chen · Z.-B. Huang (✉) · Y. Huang (✉)
Department of Infectious Diseases, Xiangya Hospital, Central South University, Xiangya Road 87, Kaifu District, Changsha 410008, China
e-mail: 36165934@qq.com

Y. Huang
e-mail: drhyan@126.com

J. Chen · X.-G. Fan · X.-W. Hu · R.-C. Chen · Z.-B. Huang · Y. Huang
Key Laboratory of Viral Hepatitis, Hunan, China

Key Summary Points

Why carry out this study?

HBsAg clearance rate is extremely low with nucleoside (acid) analogues (NAs) monotherapy and most patients with CHB need lifelong medication.

The efficacy and optimization of PegIFNα-2b add-on therapy for HBeAg-negative patients with CHB were analyzed in this study.

What was learned from the study?

PegIFNα-2b add-on therapy could enhance HBsAg clearance for HBeAg-negative patients with CHB.

Low HBsAg level is a potential predictor of HBsAg clearance by PegIFNα-2b add-on therapy.

Prolonging PegIFN treatment duration after 48 weeks of PegIFN treatment may not improve the HBsAg clearance.

INTRODUCTION

Hepatitis B virus (HBV, a hepatotropic virus) can establish a persistent and chronic infection in humans through immune anergy. Currently, about 2 billion people worldwide suffer from HBV infection, and 3.5% of the global population (about 257 million individuals) is chronically infected [1, 2]. Chronic HBV infection is a major public health problem, leading to cirrhosis, liver failure, or hepatocellular carcinoma [3]. Active HBV infection includes hepatitis B e antigen (HBeAg)-positive and HBeAg-negative patients with chronic hepatitis B (CHB). Most HBeAg-negative patients with CHB have a long course, low spontaneous hepatitis B surface antigen (HBsAg) clearance rate, and low sustained response rate [4–6].

HBsAg clearance is the drug withdrawal indication and the goal of HBeAg-negative patients with CHB treatment [7]. However, HBsAg clearance rate is very low after long-term NAs therapy in this population and most of them need lifelong medication [8, 9]. Although nucleoside (acid) analogues (NAs) can suppress viral replication, reactivation is common when treatment is discontinued. For HBeAg-positive patients who achieved HBeAg seroconversion by NAs treatment, virological relapse was up to 71% within 1 year post NAs discontinuation. In addition, 90% of HBeAg-negative patients who maintained virological suppression on NAs therapy had a risk of relapse after consolidation therapy for at least a year or decreases [10–14]. Moreover, HBeAg-negative patients with CHB would suffer virological recurrence, liver function deterioration, severe hepatitis, and high incidence of liver cancer if they had drug withdrawal without obtaining HBsAg clearance [6, 15, 16]. Therefore, new treatment strategies are required to increase HBsAg clearance rate in HBeAg-negative patients with CHB.

Interferon alfa was the first antiviral drug for CHB treatment. Studies have reported that adding or switching to peginterferon could increase HBsAg clearance rate from 8.5% to 37.4% in NAs-treated patients with CHB [17–20]. The OSST trial and New Switch Study showed that patients with low HBsAg (< 1500 IU/mL) at baseline were more likely to achieve HBsAg clearance compared to those with HBsAg > 1500 IU/mL [19, 21]. Beyond previous studies, this study aimed to evaluate the efficacy and safety of peginterferon alfa-2b (PegIFNα-2b) treatment for HBeAg-negative patients with CHB who achieved HBsAg < 1500 IU/mL and HBV DNA < 100 IU/mL by NAs treatment.

METHODS

Patients

NAs-treated patients with CHB in this study were enrolled from outpatient departments of Xiangya Hospital, Central South University during April 2018–October 2019. Patients paid for the drug by themselves in this real-world study and all enrolled patients chose to receive

PegIFNα-2b treatment voluntarily with full knowledge and signed an informed consent. This study was in compliance with the Declaration of Helsinki and was approved by Xiangya Hospital Ethics Committee, Central South University.

Inclusion criteria:

1. Patients with CHB diagnosed according to the Guidelines for the Prevention and Treatment of Chronic Hepatitis B (2019 Edition) in China [22].
2. NAs therapy $\geq$ 24 weeks and no interferon therapy within 6 months.
3. HBeAg-negative patients with HBsAg level $<$ 1500 IU/mL and HBV DNA level $<$ 100 IU/mL.
4. Aged 18–65 years old.

Exclusion criteria:

1. Patients with other viral hepatitis (hepatitis A, C, D, and E) or other liver diseases (such as autoimmune liver disease, Wilson's disease, alcoholic liver disease, or drug hepatitis).
2. Decompensated cirrhosis or liver cancer.
3. HIV infection, lesions of important organs (such as lesions of the heart, brain, lung, kidney, or fundus), severe metabolic diseases, malignant tumors, severe mental diseases, etc.
4. Hyperthyroidism or hypothyroidism, combined with other autoimmune diseases.
5. Patients with interferon allergy, alcoholism, or drug addiction.
6. Patients receiving chemotherapy or immunosuppressive therapy.

Treatment Plan

In this non-randomized study, patients in the control group received continued NAs monotherapy. Patients in the PegIFNα-2b add-on treatment group received combination therapy of PegIFNα-2b (180 μg/week, subcutaneous injection) and NAs [entecavir (ETV), tenofovir (TDF), or tenofovir alafenamide (TAF)]. If patients took lamivudine (LAM), telbivudine (LdT), or adefovir (ADV) previously, they took ETV instead. If patients took ETV, TDF, or TAF previously, they continued previous NAs therapy.

All patients received PegIFNα-2b + NA or NA for at least 48 weeks. After finishing 48 weeks of treatment, patients chose to continue peginterferon therapy up to 72 weeks or discontinue peginterferon therapy voluntarily. If patients obtained HBsAg clearance at week 48, NA was stopped; otherwise, NA was continued.

Data Collection

1. Baseline information: gender, age, history of alcohol consumption, family history of hepatitis B, history of antiviral therapy, and history of interferon therapy.
2. Laboratory test: white blood cell (WBC), peripheral blood neutrophil (NEUT), platelet (PLT), hemoglobin (Hb), total bilirubin (TBIL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), HBV DNA quantification, HBsAg and HBeAg at baseline and at week 12, 24, 48, 72 during treatment.
3. Thyroid function, autoantibodies, alpha-fetoprotein (AFP), and abdominal ultrasound at baseline and during treatment.

The quantitative detection of HBV DNA was measured by high-sensitivity fluorescence quantitative real-time PCR with lower detection limit of 10 IU/mL. The quantitative detection of HBV markers (HBsAg, HBsAb, and HBeAg) were measured by Abbott Chemilescent Automatic Immunoanalyzer (ARCHTIECT i2000sr). The lower limit of HBsAg detection was 0.05 IU/mL, and HBsAb level $>$ 10 IU/mL was defined as positive.

Evaluation of Therapeutic Efficacy

The primary endpoint was HBsAg clearance at week 48. The secondary endpoint was HBsAg serological conversion rate and HBV DNA levels at week 48, HBsAg clearance, and HBsAg serological conversion at week 12, 24 and 72. Complete response was defined as

HBsAg < 0.05 IU/mL and non-complete response was defined as HBsAg ≥ 0.05 IU/mL.

Statistical Analysis

Data analysis of primary and secondary endpoints in this study was performed in the per-protocol (PP) population. Response rates were summarized by calculating percentage at week 48 and week 72. Data analysis for safety was performed in the safety population. Measurement data with normal distribution were expressed as mean ± standard deviation (SD). Measurement data with non-normal distribution were expressed by median [25th, 75th] (M[P25, P75]). Counting data were expressed in frequency (%). *T* test or non-parametric test was used for comparison of continuous variables between groups. Categorical variables were compared by chi-square test or Fisher exact test. All data were analyzed by SPSS 20.0 and graphpad prism 7.0. $P < 0.05$ was statistically significant.

RESULTS

Patients and General Information

A total of 196 NAs-treated patients with CHB met the inclusion criteria, including three patients with loss to follow-up in the PegIFNα-2b add-on group (one because of hyperthyroidism, one because of continuous aminotransferase elevation, and one because of severe flu-like symptoms during the treatment), and 10 lost to follow-up in the NAs monotherapy group (Fig. 1). Therefore, 183 NAs-treated patients with CHB (108 in the PegIFNα-2b add-on group and 75 in the NAs monotherapy group) were enrolled in the study eventually. Baseline characteristics of gender, HBsAg level, HBV DNA, WBC, NEUT, Hb, TBIL, ALT, and AST were not statistically different between the two groups, whereas age and PLT level were (Table 1).

Virological Response

Ten patients were HBV DNA positive at baseline and all turned HBV DNA negative (HBV DNA < 10 IU/mL) after 48 weeks of PegIFNα-2b treatment. At the end of 48 weeks of treatment, 100% (108/108) and 97.33% (73/75) patients maintained HBV DNA suppression in the PegIFNα-2b add-on group and NAs monotherapy group, respectively. Three patients (4.0%, 3/75) had increased HBV DNA at week 48 by NAs monotherapy, but no significant difference compared with PegIFNα-2b add-on therapy ($P = 0.067$) (Table 2).

Serological Response

At week 48, HBsAg clearance (50.93%, 55/108) and HBsAg seroconversion (48.15%, 52/108) were achieved only in patients with PegIFNα-2b add-on therapy. Significantly more patients with PegIFNα-2b add-on therapy had low HBsAg level (0.05 ≤ HBsAg < 10 IU/mL) than those with NAs monotherapy (23.15% vs 4.0%, $P = 0.000$) (Table 3). In addition, HBsAg level showed a downward trend during PegIFNα-2b treatment. The percentage of patients with HBsAg < 0.05 IU/mL increased gradually, while those with HBsAg > 10 IU/mL decreased gradually during PegIFNα-2b add-on therapy (Fig. 2).

For 108 patients with PegIFNα-2b add-on therapy, HBsAg clearance and HBsAg seroconversion were increased over 48 weeks of treatment (Table 4). Patients with baseline HBsAg level < 100 IU/mL and HBsAg level 100–500 IU/mL achieved significantly higher HBsAg clearance than those with baseline HBsAg level 500–1500 at week 12, 24, and 48 ($P = 0.001$ vs $P = 0.000$ vs $P = 0.042$ respectively). Similarly, baseline HBsAg level 500–1500 IU/mL was associated with the lowest HBsAg seroconversion. In addition, at week 48, the HBsAg seroconversion was significantly different among patients with HBsAg level < 100 IU/mL, 100–500 IU/mL, and 500–1500 IU/mL (58.70% vs 54.84% vs 25.81%, $P = 0.012$) (Table 4).

There were 95 patients with available data at week 72, including 47 HBsAg-positive patients and 48 HBsAg-negative patients at week 48.

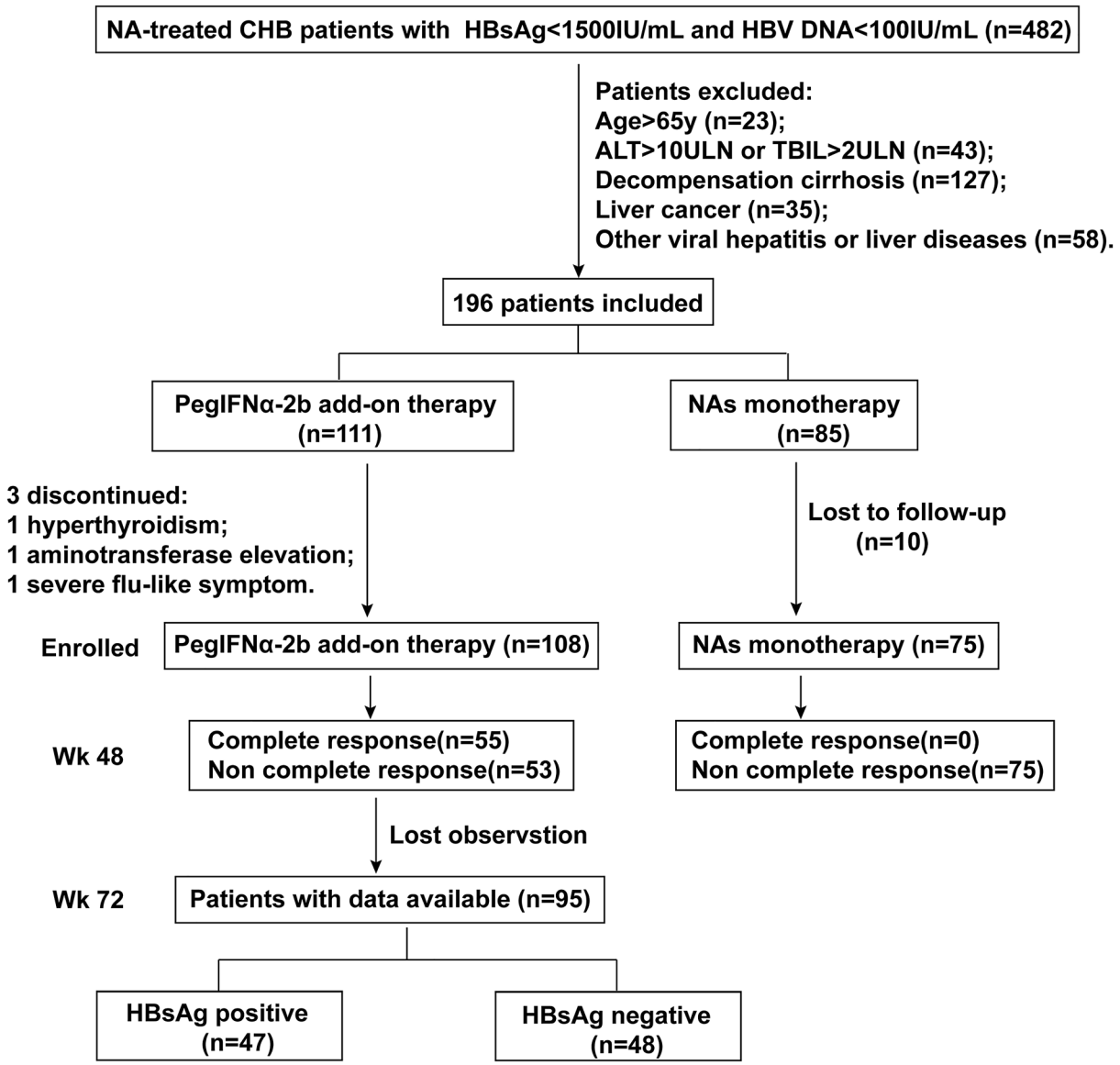

Fig. 1 Flow chart of interferon therapy of 183 NAs-treated patients with CHB

Both HBsAg clearance and HBsAg seroconversion were not significantly different between continued (18 patients) and discontinued (29 patients) PegIFNα-2b therapy at week 72 in 47 HBsAg-positive patients (16.67% vs 13.79%, $P = 0.582$ and 33.33% vs 20.69%, $P = 0.493$ respectively). In addition, 83.33% (5/6) and 95.24% (40/42) achieved HBsAg seroconversion for continued or discontinued PegIFNα-2b therapy respectively at week 72 in 48 HBsAg-negative patients ($P = 0.336$) (Table 5).

Safety

During the 48 weeks of treatment, more patients in PegIFNα-2b add-on therapy group experienced adverse events, such as transient influenza-like symptoms in the early stage (fever, fatigue, and muscle soreness),

Table 1 Baseline characteristics of 183 patients with CHB treated with NAs or PegIFNα-2b combination therapy

	PegIFNα-2b + NAs (N = 108)	NAs (N = 75)	P value
Age (years)	40.04 ± 8.42	45.15 ± 10.54	0.001
Male, *n* (%)	95 (87.96%)	61 (81.33%)	0.214
Previous NA treatment, *n* (%)			–
ETV	79 (73.15%)	62(82.67%)	
TDF	17 (15.74%)	13 (17.33%)	
Others (LAM, ADV, OR LdT)	12 (11.11%)	0	
HBsAg level (IU/mL)			0.272
< 500	77 (71.30%)	45 (60.00%)	
500–1000	24 (22.22%)	24 (32.00%)	
1000–1500	7 (6.48%)	6 (8.00%)	
HBV-DNA, *n* (%)			0.767
Undetectable (< 10 IU/mL)	98 (90.74%)	69 (92.00%)	
10–100 IU/mL	10 (9.26%)	6 (8.00%)	
WBC (10^9/L)	6.05 ± 1.31	6.08 ± 1.89	0.905
NEUT (10^9/L)	3.51 ± 1.00	3.53 ± 1.49	0.943
Hb (g/L)	160 (149, 167)	158 (146, 162)	0.075
PLT (10^9/L)	205.21 ± 52.59	174.71 ± 61.88	0.001
TBIL (μmol/L)	11.55 (9.20, 16.80)	12.10 (9.90, 17.03)	0.283
ALT (U/L)	25.00 (20.73, 37.65)	25.00 (19.10, 34.86)	0.430
AST (U/L)	25.25 (22.30, 29.93)	26.25 (22.48, 31.58)	0.392

ETV entecavir, *TDF* tenofovir, *LAM* lamivudine, *ADV* adefovir, *LdT* telbivudine, *WBC* white blood cell, *NEUT* neutrophil, *Hb* hemoglobin, *PLT* platelet, *TBIL* total bilirubin, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase

neutropenia (NEUT < 1.50 × 10^9/L), thrombocytopenia (PLT < 100 × 10^9/L), and elevated ALT or AST (Table 6). Three patients discontinued the treatment and were removed from the PegIFNα-2b add-on therapy group (one because of hyperthyroidism, one because of continuous elevation of transaminase, and one because of severe flu-like symptoms). One out of 111 PegIFNα-2b recipients developed ALT flare (ALT > 5 upper limit of normal). No NAs recipients discontinued treatment for safety reasons or experienced ALT flare. All side events were alleviated by timely treatment.

DISCUSSION

Most patients with CHB failed to withdraw the drug after achieving HBeAg clearance and HBV DNA inhibition by NAs therapy, because HBsAg clearance rate was extremely low by NAs monotherapy. PegIFN-based sequential/combination therapy is important to achieve optimal clinical cure. Previous study showed that 52.2% of HBeAg-negative patients with CHB with HBsAg < 10 IU/mL achieved clinical cure by PegIFN-based therapy [23]. This study focused on HBeAg-negative patients with CHB with low

Table 2 Virological response at 48 weeks of treatment

HBV DNA (*n*, %)	PegIFNα-2b + NAs (*N* = 108)	NAs (*N* = 75)	*P* value
Undetectable (< 10 IU/mL)	108, 100.0%	72, 96.0%	0.067
HBV DNA suppression (< 100 IU/mL)	108, 100.0%	73, 97.33%	0.167
HBV DNA increased	0, 0.0%	3, 4.0%	0.067

Table 3 Serological response at 48 weeks of treatment

Serological response (*n*, %)	PegIFNα-2b + NAs (*N* = 108)	NAs (*N* = 75)	*P* value
HBsAg clearance (< 0.05 IU/mL)	55, 50.93%	0, 0.0%	0.000
0.05 ≤ HBsAg < 10 IU/mL	25, 23.15%	3, 4.0%	0.000
HBsAg seroconversion	52, 48.19%	0, 0.0%	0.000

HBsAg level (< 1500 IU/mL) and low viral load (HBV DNA < 100 IU/mL) after NAs treatment, aiming to evaluate the efficacy and safety of PegIFNα-2b add-on therapy.

NAs and PegIFN can achieve virologic responses through different mechanisms [24–26]. After 48 weeks of add-on treatment of PegIFNα-2b, HBV DNA was undetectable in all patients. To our acknowledge, NAs are very effective in suppressing HBV DNA in patients with CHB. Previous studies showed that the virologic response of PegIFN and NAs combination therapy was as good as that of NAs monotherapy [27, 28]. Our study also showed that PegIFNα-2b add-on therapy was effective at maintaining viral suppression. No patients experienced viral rebound or HBV DNA increase during PegIFNα-2b add-on therapy.

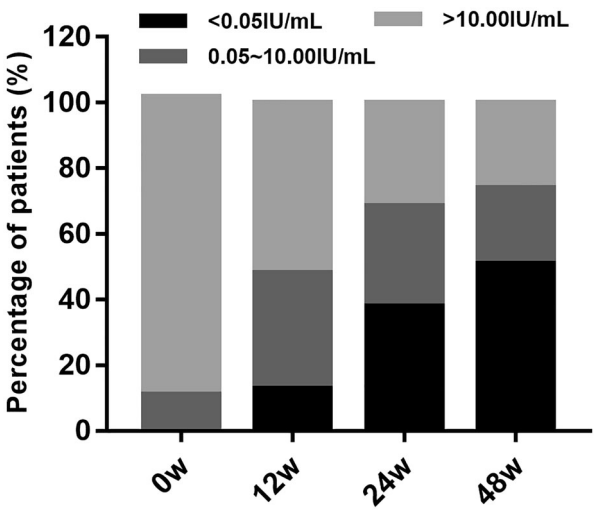

Fig. 2 Different HBsAg levels during treatment. HBsAg level showed a downward trend during PegIFNα-2b treatment

HBsAg clearance represents clinical cure for HBeAg-negative patients with CHB. The OSST trial showed 22.2% HBsAg clearance among PegIFN alfa-2a-treated patients with HBeAg loss and HBsAg < 1500 IU/ml [19]. Yan et al. [25] showed 15% HBsAg clearance at week 48 among PegIFN add-on therapy patients with negative HBeAg. Wu et al. [20] showed 37.4% HBsAg clearance and 29.7% HBsAg seroconversion in PegIFNα-2a add-on therapy patients with HBsAg < 1500 IU/mL. Compared with those published studies [19, 20, 25], our study showed higher rates of HBsAg clearance (50.93%) and HBsAg seroconversion (48.15%) at week 48 following PegIFNα-2b add-on therapy. This better outcome might be attributed to the lower HBV DNA and HBsAg level at baseline in our study, since low HBsAg and HBV DNA levels were closely related to response to PegIFN add-on therapy and high HBsAg clearance rate [26, 28]. On the other hand, Chan et al. [29] reported

Table 4 Serological response for PegIFNα-2b add-on therapy during the treatment according to baseline HBsAg level

Baseline HBsAg level (IU/mL)	Week 12				Week 24				Week 48			
	HBsAg clearance		HBsAg seroconversion		HBsAg clearance		HBsAg seroconversion		HBsAg clearance		HBsAg seroconversion	
	n, %	*P* value	*n*, %	*P* value	*n*, %	*P* value	*n*, %	*P* value	*n*, %	*P* value	*n*, %	*P* value
All patients ($N = 108$)	14, 12.96	–	5, 4.63	–	41, 37.96	–	27, 25.0	–	55, 50.93	–	52, 48.15	–
< 100 ($N = 46$)	11, 29.31	0.001	3, 6.52	0.726	25, 54.35	0.000	14, 30.43	0.055	28, 60.87	0.042	27, 58.70	0.012
100–500 ($N = 31$)	3, 9.68		1, 3.23		14, 45.16		10, 32.26		17, 54.84		17, 54.84	
500–1500 ($N = 31$)	0, 0.0		1, 3.23		2, 6.45		3, 9.68		10, 32.26		8, 25.81	

that 50% of HBeAg-positive patients with CHB with baseline HBsAg < 500 IU/mL achieved HBsAg loss after 48 weeks of PegIFN treatment. In our study, we also showed that HBeAg-negative patients with CHB with low HBsAg (< 1500 IU/mL) and low HBV DNA (< 100 IU/mL) levels at enrollment had a good chance of achieving HBsAg clearance. In addition, we found that patients with HBsAg < 100 IU/mL had the highest rate of HBsAg clearance (up to 60.87% at week 48). Therefore, it would be valuable for clinical practice to identify them as the best candidates for PegIFN add-on therapy.

More interestingly, our results indicated that longer-term PegIFNα-2b add-on therapy could not improve HBsAg clearance or HBsAg seroconversion after 48 weeks of PegIFNα-2b add-on therapy. For HBeAg-negative or HBeAg-positive patients at week 48, HBsAg clearance and HBsAg seroconversion had no significant difference by continuing or discontinuing PegIFNα-2b therapy at week 72. Previous study showed that HBsAg clearance and seroconversion did not increase at week 72 compared with that at week 48 [25]. Hu et al. [21] also showed that the differences of HBsAg clearance were not statistically significant between week 48 and week 96, although HBsAg loss rates were improved from 14.4% to 20.7% by extending treatment from 48 to 96 weeks. Therefore, it would be unnecessary to prolong PegIFNα-2b treatment duration in clinical practice.

Generally, PegIFNα-2b add-on therapy was safe and well tolerated for NAs-treated patients with CHB. Most patients had transient flu-like symptoms, transaminase elevation (2–5 × ULN), and mild to moderate neutropenia, but could be mitigated by symptomatic treatment. Therefore, it is critical to regularly review and quickly treat adverse events during PegIFNα-2b application.

In summary, we analyzed the effect of PegIFNα-2b in HBeAg-negative patients with CHB with low HBsAg level through an observation study. We found that PegIFNα-2b could effectively inhibit HBV replication, reduce HBV antigen generation, and improve seroconversion. HBeAg-negative patients with CHB with low HBsAg level could achieve clinical cure and had drug withdrawal safely by PegIFNα-2b add-

Table 5 Serological response for PegIFNα-2b add-on therapy at week 72 according to week 48 HBsAg status

Week 48 HBsAg status	PegIFNα-2b therapy	Week 72 HBsAg clearance		Week 72 HBsAg seroconversion	
		n/*N* (%)	*P* value	*n*/*N* (%)	*P* value
HBsAg positive (N_0 = 47)	Continuous	3/18 (16.67)	0.582	6/18 (33.33)	0.493
	Discontinued	4/29 (13.79)		6/29 (20.69)	
HBsAg negative (N_0 = 48)	Continuous	–	–	5/6 (83.33)	0.336
	Discontinued	–		40/42 (95.24)	

on therapy. Baseline HBsAg < 100 IU/mL predicted a better chance of HBsAg clearance. In addition, it would be unnecessary to prolong PegIFNα-2b treatment duration after 48 weeks of PegIFNα-2b add-on therapy. However, further study should be carried out to verify the results in the future.

This study has certain limitations: (1) This is a single-center real-world study, and the sample size of HBeAg-negative CHB population is not large; (2) The current study is still under way. Some patients had limited follow-up time, which did not involve the long-term response of HBsAg clearance after drug withdrawal. (3) Baseline age and PLT level between PegIFNα-2b + NAs therapy and NAs monotherapy were statistically different. (4) The treatment response of PegIFNα-2b is affected by HBV genotype. However, as a result of the continuous HBV DNA suppression (< 100 IU/mL) in the enrolled CHB population, the impact of HBV genotype on response was not explored.

Table 6 Safety outcome of the study population

Adverse events, *n* (%)	PegIFNα-2b + NAs (*N* = 111)	NAs (*N* = 75)
Influenza-like illness	87 (78.38)	0 (0.0)
Neutropenia	81 (72.97)	3 (4.0)
Thrombocytopenia	56 (50.45)	11 (14.67)
ALT increased	81 (72.97)	8 (10.67)
AST increased	86 (77.48)	13 (17.33)
Discontinuation for safety reasons	3 (2.7)	0 (0.0)
Death	0 (0.0)	0 (0.0)
ALT flare (> 5ULN)	1 (0.9)	0 (0.0)

ALT alanine aminotransferase, *AST* aspartate aminotransferase

CONCLUSIONS

HBeAg-negative patients with CHB with low HBsAg level could achieve high HBsAg clearance and seroconversion by PegIFNα-2b add-on therapy. Patients with low baseline HBsAg levels were more likely to obtain HBsAg clearance. PegIFNα-2b add-on therapy could be a good treatment option for NAs-treated patients with CHB with negative HBeAg, particularly for those with lower HBsAg level (< 100 IU/mL). This will have important implications for PegIFNα-2b treatment screening in HBeAg-negative patients with CHB. It would be unnecessary to prolong PegIFNα-2b treatment duration after fininshing 48 weeks PegIFNα-2b treatment in clinical practice.

ACKNOWLEDGEMENTS

We thank the participants of the study.

Funding. This work was supported by the National Key R&D Program of China (No.

2017YFC0908104); the National Science and Technology Major Project of China (No. 2018ZX10732-202-001-007); the National Natural Science Foundation of China (No. 81970550); the National Natural Science Foundation of China (No. 81970523); the Natural Science Foundation of Hunan Province (2020JJ4877); the National Science and Technology Major Project (2018ZX10723203); the Natural Science Foundation of Hunan Province, China (2019JJ40496); the National Natural Science Foundation of Hunan province (No. 2019JJ30041); the National Natural Science Foundation of China (No. 82070613), and Innovation-Driven Project of Central South University (No. 2020CX044). The Rapid Service Fee was funded by the authors.

Authorship. All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Authorship Contributions. Ze-Bing Huang and Yan Huang designed the study. Min Qi collected the data. Jun Chen analyzed the data and drafted the manuscript. Ruo-Chan Chen, Xing-Wang Hu, and Xue-Gong Fan modified the manuscript. Cheng-Jin Liao, Li-Yuan Long, Xiao-Ting Zhao, Min Tan, and Hai-Fu Li checked the data. All authors contributed to the revision of the manuscript and approved the final manuscript.

Disclosures. All authors declare that they have no conflict of interest in this research.

Compliance with Ethics Guidelines. All enrolled patients chose to receive PegIFNα-2b treatment voluntarily with full knowledge and signed an informed consent. This study was in compliance with the Declaration of Helsinki and was approved by Xiangya Hospital Ethics Committee, Central South University.

Data Availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

REFERENCES

1. Yuen MF, Chen DS, Dusheiko GM, et al. Hepatitis B virus infection. Nat Rev Dis Primers. 2018;4:18035.
2. Chang MH. Natural history and clinical management of chronic hepatitis B virus infection in children. Hepatol Int. 2008;2:28–36.
3. Kao JH, Chen DS. Global control of hepatitis B virus infection. Lancet Infect Dis. 2002;2:395–403.
4. McMahon BJ. The natural history of chronic hepatitis B virus infection. Hepatology. 2009;49:S45-55.
5. Zhou KL, Contag C, Whitaker E, Terrault N. Spontaneous loss of surface antigen among adults living with chronic hepatitis B virus infection: a systematic review and pooled meta-analyses. Lancet Gastroenterol Hepatol. 2019;4:227–38.
6. Liem KS, Fung S, Wong DK, et al. Limited sustained response after stopping nucleos(t)ide analogues in patients with chronic hepatitis B: results from a randomised controlled trial (Toronto STOP study). Gut. 2019;68:2206–13.

7. Sarin SK, Kumar M, Lau GK, et al. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. Hepatol Int. 2016;10: 1–98.

8. Boglione L, D'Avolio A, Cariti G, et al. Kinetics and prediction of HBsAg loss during therapy with analogues in patients affected by chronic hepatitis B HBeAg negative and genotype D. Liver Int. 2013;33: 580–5.

9. Chevaliez S, Hezode C, Bahrami S, Grare M, Pawlotsky JM. Long-term hepatitis B surface antigen (HBsAg) kinetics during nucleoside/nucleotide analogue therapy: finite treatment duration unlikely. J Hepatol. 2013;58:676–83.

10. Seto WK, Hui AJ, Wong VW, et al. Treatment cessation of entecavir in Asian patients with hepatitis B e antigen negative chronic hepatitis B: a multicentre prospective study. Gut. 2015;64: 667–72.

11. Liu F, Wang L, Li XY, et al. Poor durability of lamivudine effectiveness despite stringent cessation criteria: a prospective clinical study in hepatitis B e antigen-negative chronic hepatitis B patients. J Gastroenterol Hepatol. 2011;26:456–60.

12. Chaung KT, Ha NB, Trinh HN, et al. High frequency of recurrent viremia after hepatitis B e antigen seroconversion and consolidation therapy. J Clin Gastroenterol. 2012;46:865–70.

13. Dai CY, Tseng TC, Wong GL, et al. Consolidation therapy for HBeAg-positive Asian chronic hepatitis B patients receiving lamivudine treatment: a multicentre study. J Antimicrob Chemother. 2013;68:2332–8.

14. Lee HW, Lee HJ, Hwang JS, et al. Lamivudine maintenance beyond one year after HBeAg seroconversion is a major factor for sustained virologic response in HBeAg-positive chronic hepatitis B. Hepatology. 2010;51:415–21.

15. Jeng WJ, Chen YC, Chien RN, Sheen IS, Liaw YF. Incidence and predictors of hepatitis B surface antigen seroclearance after cessation of nucleos(-t)ide analogue therapy in hepatitis B e antigen-negative chronic hepatitis B. Hepatology. 2018;68: 425–34.

16. Lee HW, Lee JI, Kim S, Kim S, Chang HY, Lee KS. Cumulative incidence of hepatocellular carcinoma and hepatitis B surface antigen seroclearance after nucleos(t)ide analogue-induced hepatitis B e antigen seroclearance. BMC Gastroenterol 2020;20(1): 113.

17. Boglione L, Cariti G, Di Perri G, D'Avolio A. Sequential therapy with entecavir and pegylated interferon in a cohort of young patients affected by chronic hepatitis B. J Med Virol. 2016;88:1953–9.

18. Wursthorn K, Lutgehetmann M, Dandri M, et al. Peginterferon alpha-2b plus adefovir induce strong cccDNA decline and HBsAg reduction in patients with chronic hepatitis B. Hepatology. 2006;44: 675–84.

19. Ning Q, Han M, Sun Y, et al. Switching from entecavir to PegIFN alfa-2a in patients with HBeAg-positive chronic hepatitis B: a randomised open-label trial (OSST trial). J Hepatol. 2014;61:777–84.

20. Wu FP, Yang Y, Li M, et al. Add-on pegylated interferon augments hepatitis B surface antigen clearance vs continuous nucleos(t)ide analog monotherapy in Chinese patients with chronic hepatitis B and hepatitis B surface antigen </= 1500 IU/mL: an observational study. World J Gastroenterol. 2020;26:1525–39.

21. Hu P, Shang J, Zhang W, et al. HBsAg loss with Peginterferon alfa-2a in hepatitis B patients with partial response to nucleos(t)ide analog: new switch study. J Clin Transl Hepatol. 2018;6:25–34.

22. Chinese Association of Infectious Diseases, Chinese Association of Liver Diseases. The guidelines for the prevention and treatment of chronic hepatitis B (2019 edition). Chin Hepatol. 2019;24(12): 1335–1355.

23. Brunetto MR, Moriconi F, Bonino F, et al. Hepatitis B virus surface antigen levels: a guide to sustained response to peginterferon alfa-2a in HBeAg-negative chronic hepatitis B. Hepatology. 2009;49: 1141–50.

24. Thimme R, Dandri M. Dissecting the divergent effects of interferon-alpha on immune cells: time to rethink combination therapy in chronic hepatitis B? J Hepatol. 2013;58:205–9.

25. Yan L, Zhu C, Li J, et al. Entecavir add-on or switch-to pegylated interferon improves HBsAg clearance in HBe antigen negative chronic hepatitis B patients. Infect Drug Resist. 2018;11:2001–2009.

26. Tseng TC, Kao JH. Clinical utility of quantitative HBsAg in natural history and nucleos(t)ide analogue treatment of chronic hepatitis B: new trick of old dog. J Gastroenterol. 2013;48:13–21.

27. Cao ZH, Liu YL, Ma LN, et al. A potent hepatitis B surface antigen response in subjects with inactive hepatitis B surface antigen carrier treated with pegylated-interferon alpha. Hepatology. 2017;66: 1058–66.

28. Liem KS, van Campenhout MJH, Xie Q, et al. Low hepatitis B surface antigen and HBV DNA levels

predict response to the addition of pegylated interferon to entecavir in hepatitis B e antigen positive chronic hepatitis B. Aliment Pharmacol Ther. 2019;49:448–56.

29. Chan HLY, Chan FWS, Hui AJ, et al. Switching to peginterferon for chronic hepatitis B patients with hepatitis B e antigen seroconversion on entecavir—a prospective study. J Viral Hepatitis. 2019;26:126–35.

Publisher's Note