

# CONSORT: Effects of adding adefovirdipivoxil to peginterferon alfa-2a at different time points on HBeAg-positive patients

## A prospective, randomized study

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### Abstract

**Background:** The aims of this study were to compare the efficacy and safety of the addition of adefovir dipivoxil (ADV) (started at different time points) to pegylated interferon alpha-2a (PEG-INF- $\alpha$ 2a) and PEG-INF- $\alpha$ 2a monotherapy. This prospective, randomized study sought to evaluate the safety and efficacy of the combination of PEG-INF- $\alpha$ 2a and ADV at different time points. 120 patients were randomized into groups that received PEG-INF- $\alpha$ 2a as monotherapy (group A) or in combination with ADV started at week 0 (group B), 12 (group C), or 24 (group D). All patients were followed for 48 weeks. Efficacy and safety analyses were performed.

**Methods:** Patients in group A received 135  $\mu$ g of PEG-INF- $\alpha$ 2a by subcutaneous injection once weekly for 48 weeks. Patients in the ADV add-on group received 135  $\mu$ g of PEG-INF- $\alpha$ 2a subcutaneously once weekly and received 10 mg of ADV administered once daily for 48 weeks. HBV DNA, HBsAg, HBeAg, and hepatitis B e antibody levels were determined. Responses were determined at week 12 (ADV add-on), the end of treatment for PEG-INF- $\alpha$ 2a (48 weeks) and ADV (EOT) and at the end of 96 weeks of follow-up (EOF).

**Results:** The rate of HBV DNA loss were higher in the combination groups than group A at the week 12, week 48, the EOT and EOF ( $P < 0.05$ ). The rates of HBeAg seroconversion and HBsAg loss were similar among the treatment groups ( $P > 0.05$ ). The alanine aminotransferase (ALT) normalization rate was higher in the combination group than group A only at the EOT ( $P = 0.007$ ). By the EOF, the patients with ADV added at week 12 achieved higher rates of HBV DNA loss (71.9%), HBeAg seroconversion (50.0%), HBsAg loss (15.6%), and ALT normalization (78.1%).

**Conclusions:** PEG-INF- $\alpha$ 2a plus ADV combination therapy is safe and superior to PEG-INF- $\alpha$ 2a monotherapy for decreasing serum HBV DNA and normalizing the ALT level but has no significant impact on the rate of HBeAg seroconversion and HBsAg loss. Adding ADV at week 12 may be an optimal combination strategy.

**Abbreviations:** ADV = adefovir dipivoxil, ALT = alanine aminotransferase, CHB = chronic hepatitis B, EOF = the end of follow-up, EOT = the end of treatment, ETV = entecavir, HBeAg = hepatitis B virus e antigen, HBsAg = hepatitis B virus surface antigen, HBV = hepatitis B virus, HBV = hepatitis B virus, PEG-IFN = pegylated interferon, TDF = tenofovir disoproxil fumarate.

**Keywords:** adefovir dipivoxil, chronic hepatitis B, combination therapy, pegylated interferon alfa-2a

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## 1. Introduction

Hepatitis B virus (HBV) infection is a major public health problem. More than 350 million people are chronically infected with HBV throughout the world.<sup>[1]</sup> Patients with HBV infection are at risk of progressing to long-term complications, including cirrhosis of the liver, decompensated liver disease, and/or hepatocellular carcinoma (HCC). Approximately 93 million people are chronic HBV (CHB) carriers in China, and 300,000 individuals die from chronic disease every year.<sup>[2]</sup>

Interferon (IFN) and several nucleoside/nucleotide analogs (NAs) are currently approved for the treatment of CHB in several countries. Moreover, pegylated interferon (peg-IFN) alfa-2a (40 kD) and NAs are recommended by treatment guidelines as a first-line therapy for CHB. However, a 48-week course of peg-IFN alfa-2a treatment only results in sustained immune control in 36% of HBeAg-positive patients.<sup>[3,4]</sup> After the completion of peg-IFN treatment, the response rates among HBeAg-positive patients were 13%, 25%, 14%, 21%, and 21% at 1, 2, 3, 4, and 5 years, respectively.<sup>[5]</sup> Thus, the HBeAg seroconversion rate and the HBsAg loss rate of peg-IFN remain low. In contrast, NAs have high rates of suppression of viral replication in HBeAg-positive CHB patients but rarely lead to sustained HBeAg or HBsAg loss or seroconversion.<sup>[6,7]</sup> Although ADV was not

recommended as a first-line treatment, ADV is widely used in most Asian countries for nucleos (t)ide-naïve Asian CHB patients owing to low cost and easy availability. Entecavir (ETV) is superior to adefovir (ADV) for decreasing serum HBV-DNA and normalizing the alanine aminotransferase (ALT) level but is similar to ADV for clearing HBeAg and favoring HBeAg seroconversion for HBeAg-positive NA-naïve Asian patients with CHB.<sup>[8]</sup> Tenofovir disoproxil fumarate (TDF) demonstrated superiority over ADV with respect to the primary endpoint of viral suppression in both HBeAg-positive and HBeAg-negative patients. However, the proportions of HBeAg loss and HBeAg seroconversion at week 48 were similar. In a word, combination therapy of PEG-INF- $\alpha$ 2a and ADV may be a good plan for the CHB patients in the countries with limited health resources, which can improve overall response rates.

In our previous peg-IFN  $\alpha$ -2a and ADV combination studies, the addition of ADV to PEG-INF- $\alpha$ 2a at week 24 improved the inhibition of HBV DNA in CHB patients who were HBeAg-positive, and the level of HBsAg at 12 and 24 weeks was a predictor used to evaluate the efficacy of combination therapy. However, the rates of HBeAg loss and HBeAg seroconversion were not improved compared to PEG-INF- $\alpha$ 2a monotherapy.<sup>[9,10]</sup> Takkenberg et al<sup>[11]</sup> reported the results of combination therapy with peg-IFN and ADV for 48 weeks and showed a high rate of HBeAg loss (41%) and HBsAg loss (11%) in HBeAg-positive patients after the end of treatment (EOT). However, in these studies, combination therapy was started at week 0 and compared to peg-IFN $\alpha$ -2a monotherapy. To date, no reports have described the efficacy and safety of PEG-INF- $\alpha$ 2a therapy with ADV started at different time points (week 0, 12, and 24).

To explore the optimal time to begin combination treatment with PEG-INF- $\alpha$ 2a and ADV in HBeAg-positive, treatment-naïve CHB patients, we compared the efficacy and safety of 48 weeks of treatment with PEG-INF- $\alpha$ 2a monotherapy to treatment with PEG-INF- $\alpha$ 2a combined with ADV therapy added at weeks 0, 12, and 24.

## 2. Methods

### 2.1. Study population

This was an open-labeled pilot study performed in accordance with the Declaration of Helsinki. The Ethics Committee of the Third Affiliated Hospital of Sun Yat-Sen University approved this study.

Informed consent was obtained from all patients. The inclusion criteria consisted of patients with a documented HBV infection lasting at least 6 months who were 18 to 60 years of age, were HBeAg-positive, had serum HBV DNA levels  $\geq 100,000$  copies/mL, had experienced 2 episodes of elevated serum ALT levels ( $>2$  but  $\leq 10$  times the upper limit of normal), had no history of antiviral treatment for HBV in the previous 6 months, and did not have cirrhosis (confirmed by liver biopsy or ultrasound). The exclusion criteria consisted of antiviral, antineoplastic, or immunomodulatory treatment within the previous 6 months; co-infection with hepatitis A, C, or D virus or human immune deficiency virus; evidence of nonviral chronic liver disease or liver decompensation; or a history of severe psychiatric disease, particularly depression.<sup>[12,13]</sup>

### 2.2. Study design

This investigator-initiated, prospective, randomized, open-label study (ClinicalTrials.gov registration no: 09000612) was conducted in the Department of Infectious Diseases of the Third

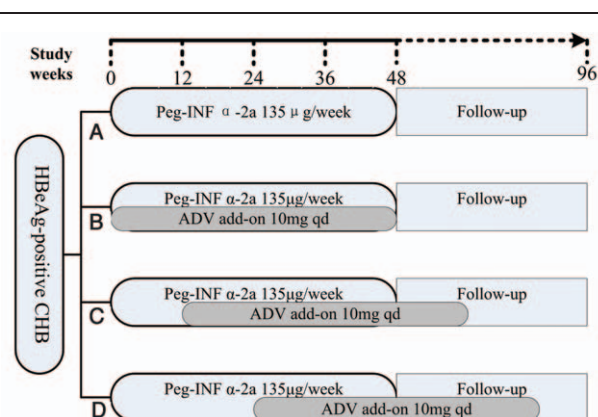
Affiliated Hospital of Sun Yat-Sen University between October 2009 and December 2014. Eligible patients were randomized into 4 treatment groups. Randomization was centralized, and a patient identification number assigned by the randomization system was used to allocate the patients to treatment groups. Patients in the PEG-INF- $\alpha$ 2a monotherapy group received 135  $\mu$ g of peg-IFN  $\alpha$ -2a (Pegasys, F. Hoffmann-La Roche Ltd., Basel, Switzerland) by subcutaneous injection once weekly for 48 weeks (group A). Patients in the ADV add-on group received 135  $\mu$ g of PEG-INF- $\alpha$ 2a subcutaneously once weekly for 48 weeks and were divided into 3 groups according to the ADV add-on time (group B, ADV add-on at week 0; group C, ADV add-on at week 12; and group D, ADV add-on at week 24). These patients received 10 mg of ADV (GelanSushike [Tianjin] Co., Ltd, China) administered once daily (Fig. 1). All patients were followed for 48 weeks. Routine blood tests were performed each month. HBV DNA, HBsAg, HBeAg, and hepatitis B e antibody levels were determined every 4 to 8 weeks. The efficacy parameters were the EOT viral and biochemical responses. Responses were determined at week 12 (ADV add-on), the EOT for PEG-INF- $\alpha$ 2a (48 weeks) and ADV, and at the end of 96 weeks of follow-up (EOF). The biochemical response was defined as the normalization of ALT levels. Viral responses included the HBV DNA loss rate, HBeAg seroconversion rate, and HBsAg loss rate. Response definitions were previously described in detail.<sup>[11]</sup>

### 2.3. Laboratory assessments

HBeAg, HBsAg, and hepatitis B e antibody level testing were performed using Roche chemiluminescent assays (Roche Elecsys 2010 analyzer; Roche Diagnostics, Indianapolis, IN). Serum HBV DNA levels were measured using in-house real-time PCR with a sensitivity limit of 500 copies/mL. Testing was performed at baseline and at weeks 4, 8, 12, 24, 36, 48, 72, and 96 at a central laboratory of the Third Affiliated Hospital of Sun Yat-Sen University. All patients were assessed for thyroid function, autoimmune antibodies, blood sugar, and routine blood tests before and during treatment.

### 2.4. Sample size calculation

A previous study demonstrated that about 27 subjects in each group can reveal the different effects of peg-IFN  $\alpha$  on HBeAg-positive patients. Therefore, we decided to include 30 patients in each group, which allowed for an expected 15% dropout rate.



**Figure 1.** Study design. ADV=adefovirdipivoxil, CHB=chronic hepatitis B, HBeAg=hepatitis B e antigen, Peg-IFN- $\alpha$ 2a=pegylated interferon  $\alpha$ -2a.

## 2.5. Statistical analysis

Continuous variables were expressed as mean  $\pm$  standard deviation (SD) or median (interquartile) depending on the result of normality test. The Shapiro–Wilk test of normality was performed. Normally distributed variables were analyzed with ANOVA test, otherwise with nonparametric test. Categorical variables were expressed as the number of patients (percentage) and tested with the  $\chi^2$  test or Fisher exact test, as appropriate. All analyses were performed as 2-sided tests with a 0.05 level of significance. The Bonferroni correction was used for multiple testing. In addition, the logistic regression analysis techniques were also used to access the difference of efficacy for 4 different treatment schemes, adjusting for the possibly influencing covariates. Patients with missing data were counted as nonresponders. SPSS software (version 22.0; SPSS Inc, Chicago, IL) was used to perform the statistical analyses.

## 3. Results

### 3.1. Patient characteristics

A total of 147 patients signed informed consent forms. Six patients did not meet the inclusion criteria because of a viral load  $<100,000$  copies/mL. One patient developed cirrhosis, which was confirmed by an ultrasound evaluation. Ultimately, 140 patients were randomized into the 4 treatment groups (group A,  $n=35$ ; group B,  $n=35$ ; group C,  $n=35$ ; and group D,  $n=35$ ). Of these patients, 11 patients discontinued treatment because of loss to follow-up (1 in group A, 1 in group B, 2 in group C, and 3 in group D), patient request (1 in group A and 1 in group B), and side effects (1 in group A and 1 in group C). In total, 129 patients completed at least 96 weeks of treatment follow-up.

### 3.2. Patient baseline clinical characteristics

Table 1 shows the age, sex, and baseline clinical and viral characteristics. These were generally similar among the 4 groups, and no significant differences were observed in the baseline characteristics between the groups.

### 3.3. Response to treatment at week 12 (ADV add-on)

At week 12 (ADV add-on for 12 weeks), patients in the ADV add-on groups (groups B, C, and D; ADV add-on at week 0, 12, 24) achieved higher rates of HBV DNA loss than those in the PEG-INF- $\alpha$ 2a monotherapy group (group A) ( $P=0.000$ ; Table 2).

However, similar rates of combined response (HBeAg seroconversion, HBsAg loss, and ALT normalization) were achieved in all treatment groups at week 12 (Table 2).

### 3.4. Response to treatment at week 48

In total, 13 of 32 (40.6%) patients in the PEG-INF- $\alpha$ 2a monotherapy group (group A), 28 of 33 (84.8%) patients in group B (ADV add-on at week 0), 25 of 32 (78.1%) patients in group C (ADV add-on at week 12), and 20 of 32 (62.5%) patients in group D (ADV add-on at week 24) achieved HBV DNA loss, and the difference across treatment groups was statistically significant ( $P=0.01$ ; Table 2). A greater proportion of patients in group B and group C achieved HBV DNA loss at week 48 (end of peg-INF) than in group A (84.8% and 78.1% vs. 40.6%). However, no difference was found in the HBV DNA loss rate at week 48 between group A and group D (40.6% vs. 62.5%). In the ADV add-on groups, patients who received ADV add-on at week 0 (group B) demonstrated a higher rate of HBV DNA loss at week 48 than group D ( $P=0.04$ ; Table 2). Similar rates of combined response (HBeAg seroconversion, HBsAg loss, and ALT normalization) were achieved in all treatment groups at week 48 (Table 2).

### 3.5. Response to treatment at the EOT

At the EOT, patients in the ADV add-on groups (groups B, C, and D) achieved higher rates of HBV DNA loss than those in group A ( $P=0.000$ ; Table 3). The rates of HBV DNA loss increased from week 48 to the EOT in group C and group D (78.1%–87.5% and 62.5%–78.1%, respectively). Similarly, the rates of ALT normalization achieved in all ADV add-on groups (groups B, C, and D) at the EOT were higher than those of group A ( $P=0.007$ ; Table 3). However, the rates of HBsAg loss and HBeAg seroconversion were similar in all groups at the EOT (Table 3).

### 3.6. Response to treatment at the EOF (week 96)

By the EOF (week 96), viral rebound occurred in all groups. However, the groups showed comparable levels at the EOF (Table 3). Patients in the ADV add-on groups (groups B, C, and D) continued to have higher rates of HBV DNA loss than patients in group A ( $P=0.004$ ; Table 3). No difference was observed for the HBV DNA loss rate at the EOF for the 3 ADV add-on groups. Patients in group C had a higher rate of HBeAg seroconversion at the EOF than the other 3 groups ( $P=0.164$ ; Table 3). Comparable rates of HBsAg loss and ALT normalization were achieved in all groups by the EOF ( $P=0.647$  and  $0.169$ ; Table 3).

**Table 1**  
Demographics and baseline characteristics of the patients.

Characteristics	peg-IFN-mono	ADV add-on		
	Group A	Group B	Group C	Group D
Number of patients (n)	32	33	32	32
Age, y	28.56 $\pm$ 7.95	30.15 $\pm$ 7.19	29.50 $\pm$ 8.08	29.28 $\pm$ 6.94
Male sex, No.	25 (78.1%)	26 (78.8%)	24 (75.0%)	26 (81.3%)
ALT $\times$ ULN	4.3 (2.6)	3.9 (2.4)	4.1 (2.7)	3.7 (2.4)
HBV DNA level (log <sub>10</sub> copies/mL)	7.15 $\pm$ 1.14	6.78 $\pm$ 1.35	7.03 $\pm$ 1.21	7.08 $\pm$ 1.04

ADV = adefovir dipivoxil, ALT = alanine aminotransferase, HBV = hepatitis B virus,  $P > 0.05$  for comparisons of all baseline characteristics, peg-IFN-mono = peg-IFN alpha-2a monotherapy, ULN = upper limit of normal.

**Table 2****Outcome measures at week 12 and 48.**

Response	Week 12 (ADV add-on)				Week 48 (end of Peg-IFN)			
	Group A	Group B	Group C	Group D	Group A	Group B	Group C	Group D
HBV DNA loss, n (%)	5 (15.6%)	18 (54.5%)	22 (68.8%)	18 (56.2%)	13 (40.6%)	28 (84.8%)	25 (78.1%)	20 (62.5%)
Intergroup <i>P</i> value		0.000				0.01		
Pairwise comparison <i>P</i> value		0.003*	0.000*	0.002*		0.000*	0.003*	0.04†
HBeAg seroconversion, n (%)	4 (12.5%)	7 (21.2%)	10 (31.2%)	10 (31.2%)	9 (28.1%)	11 (33.3%)	12 (37.5%)	10 (31.3%)
Intergroup <i>P</i> value		0.231				0.879		
HBsAg loss, n (%)	1 (3.1%)	2 (6.1%)	3 (9.4%)	2 (6.2%)	2 (6.3%)	4 (12.1%)	4 (12.5%)	3 (9.4%)
Intergroup <i>P</i> value		0.783				0.827		
ALT normalization, n (%)	14 (43.8%)	21 (63.6%)	21 (65.6%)	17 (53.1%)	18 (56.3%)	27 (81.8%)	24 (75.0%)	20 (62.5%)
Intergroup <i>P</i> value		0.258				0.105		

Pairwise comparisons are only reported for variables with a significant intergroup *P* value. ADV = adefovir dipivoxil, ALT = alanine aminotransferase, HBeAg = hepatitis B e antigen, HBsAg = hepatitis B surface antigen, HBV = hepatitis B virus, peg-IFN = pegylated interferon.

\* Compared to group A.

† Compared to group B.

### 3.7. Safety and tolerability

Combined peg-IFN and ADV therapy was generally well tolerated. No side effects related to the combination of peg-IFN and ADV therapy were reported. The most common side effects in the add-on arm were those known to occur with peg-IFN therapy. The most frequent treatment-related side effects consisted of abnormal laboratory results, such as decreased white blood cell and neutrophil counts, which are known to occur with PEG-INF- $\alpha$ 2a treatment. No unexpected side effects were reported. Six patients in the combination therapy group required symptomatic treatment for persistent fever before week 24, but they all completed the treatment. One patient assigned to group B experienced an ALT flare after stopping ADV. One patient in group A experienced thyroid dysfunction.

### 4. Discussion

ADV and peg-IFN are 2 antiviral drugs with different targets and pharmacokinetics. Peg-IFN produces viral suppression and immunomodulatory activity simultaneously. The favorable meta-analysis outcomes of peg-IFN include the sustained suppression of viral replication and HBeAg seroconversion.<sup>[14]</sup> However, the rate of HBeAg seroconversion remains low. Combining ADV and peg-IFN may be a logical approach because

they activate different mechanisms to control HBeAg-positive CHB. A series of previous studies with thorough viral analyses showed that the combination of ADV and peg-IFN induced a higher viral response but with differences in the rate of HBeAg seroconversion and HBsAg loss.<sup>[11,15,16]</sup> Interestingly, these studies selected the beginning of treatment as the time of combination of PEG-INF- $\alpha$ 2a and ADV. Based on current knowledge and previous studies, this study compared the efficacy and safety of 48 weeks of combination treatment with PEG-INF- $\alpha$ 2a and ADV (with ADV started at different time points) with PEG-INF- $\alpha$ 2a monotherapy.

In our study, a greater proportion of patients in the combination therapy group than in the monotherapy group achieved HBV DNA loss at week 48, the EOT, and the EOF. Despite the more pronounced on-treatment suppression of HBV DNA in the combination therapy group compared to PEG-INF- $\alpha$ 2a monotherapy, the rates of HBV DNA loss were similar in all 3 combination therapy groups at the different endpoints. This finding is consistent with previous reports of HBV DNA kinetics in combination studies of lamivudine, ADV, or ETV with PEG-INF- $\alpha$ 2a monotherapy.<sup>[13,17,18]</sup> Recently, Gu et al<sup>[19]</sup> observed that combination therapy of adefovir dipivoxil and PEG-INF- $\alpha$ 2b also achieved higher HBV DNA uncertainty rate than the group treated with adefovir dipivoxil only at the 12<sup>th</sup>, 24<sup>th</sup>, 36<sup>th</sup>,

**Table 3****Outcome measures at the EOT and EOF.**

Response	EOT				EOF			
	Group A	Group B	Group C	Group D	Group A	Group B	Group C	Group D
HBV DNA loss, n (%)	13 (40.6%)	28 (84.8%)	28 (87.5%)	25 (78.1%)	10 (31.3%)	19 (57.6%)	23 (71.9%)	22 (68.8%)
Intergroup <i>P</i> value		0.000				0.004		
Pairwise comparison <i>P</i> value		0.000*	0.000*	0.003*		0.022*	0.001*	0.003*
HBeAg seroconversion, n (%)	9 (28.1%)	11 (33.3%)	15 (46.9%)	11 (33.3%)	8 (25.0%)	10 (30.3%)	16 (50.0%)	10 (31.3%)
Intergroup <i>P</i> value		0.439				0.164		
HBsAg loss, n (%)	2 (6.3%)	4 (12.1%)	5 (15.6%)	3 (9.4%)	2 (6.3%)	3 (9.1%)	5 (15.6%)	3 (9.4%)
Intergroup <i>P</i> value		0.664				0.647		
ALT normalization, n (%)	18 (56.3%)	27 (81.8%)	29 (90.6%)	26 (81.3%)	18 (56.3%)	24 (72.7%)	25 (78.1%)	25 (78.1%)
Intergroup <i>P</i> value		0.007				0.169		
Pairwise comparison <i>P</i> value		0.033*	0.004*	0.034*				

Pairwise comparisons are only reported for variables with a significant intergroup *P* value. ALT = alanine aminotransferase, EOF = end of follow-up, EOT = end of treatment, HBeAg = hepatitis B e antigen, HBsAg = hepatitis B surface antigen, HBV = hepatitis B virus.

\* Compared to group A.

and 48th week. Because these 2 drugs have different function mechanisms and combination of them can produce coordination or superimposed effect, the higher rate of HBV DNA loss in the combination therapy group is not difficult to understand. Interestingly, we found that patients treated with peg-IFN and ADV add-on at week 12 (group C) achieved a significantly higher rate of HBV DNA loss than the other combination treatment groups (ADV add-on at week 0 or 24). At the EOT and EOF, the HBV DNA loss rates were 87.5% and 71.9%, respectively, in the patients assigned to the group with ADV add-on at week 12. Our result suggests that ADV add-on at week 12 may lead to a higher proportion of HBV DNA loss than the other ADV add-on times. Zheng et al's<sup>[20]</sup> study observed that total intrahepatic HBV-DNA and cccDNA had significantly decreased after 12 weeks of ADV treatment; meanwhile, HBV-specific IFN- $\gamma$ -producing CD4+ T cells were greatly enhanced and reached the highest level at week 12 with viral load reduction and the level was further increased between week 36 and week 48. So, patients treated with peg-IFN and ADV add-on at week 12 achieved a significantly higher rate of HBV DNA loss because of strengthening and improving of immune response.

Previous study<sup>[21]</sup> confirmed that ADV was attributed not only to their direct effect on HBV suppression but also to their immune regulatory capabilities. ADV treatment can increase IFN- $\gamma$  production by CD4+T cells, which was related to the reduction of HBeAg levels.<sup>[22]</sup> Some clinical studies also suggested that combination therapy with peg-IFN and ADV for 48 weeks led to a marked decrease in both serum HBV DNA and the rate of HBeAg seroconversion (53%) compared to treatment with peg-IFN alone. Moreover, after 96 weeks of ADV monotherapy, the HBeAg loss was 80%.<sup>[15,16,23,24]</sup> However, the rates of HBeAg seroconversion and HBsAg loss at week 48, the EOT, and the EOF were not superior to the addition of ADV compared to PEG-INF- $\alpha$ 2a monotherapy in the present study. These findings support previous data, which show that combining PEG-INF- $\alpha$ 2a with lamivudine<sup>[5]</sup> or ETV<sup>[13]</sup> does not increase the post-treatment efficacy of PEG-INF- $\alpha$ 2a. In our study, 46.9% of patients in the ADV 12 week add-on combination therapy group and 28.1% of patients in the monotherapy group showed HBeAg seroconversion at the EOT. We found that patients with ADV add-on at week 12 (group C) achieved a higher rate of HBeAg seroconversion (50%), but no significant differences were found. This discrepancy may be related to the relatively small size of our studied population. In our study, the rates of HBsAg loss in the combination therapy group were 12.1%, 15.6%, and 9.4% for ADV add-on at weeks 0, 12, and 24, respectively, whereas a rate of 6.3% was observed in the monotherapy group. This result is consistent with previous reports. However, we found a significant trend for the group that received ADV add-on at week 12 at the EOF.

In our study, compared to the monotherapy group, the ALT normalization rate was similar in the combination groups at 48 weeks of peg-IFN treatment and at the EOF. Some studies<sup>[25]</sup> have reported that the ALT normalization rate after treatment was higher in the combination group. A meta-analysis also indicated that peg-IFN and ADV combination therapy is superior to IFN- $\alpha$  monotherapy for normalizing the ALT level.<sup>[24]</sup> Our results only demonstrated a difference between the combination groups and the PEG-INF- $\alpha$ 2a monotherapy group at the EOT. This discrepancy may be related to the relatively small size of our studied population or biochemical changes after the EOT.

Regarding safety, ADV add-on was generally well tolerated without unexpected adverse effects. The safety profiles of PEG-INF- $\alpha$ 2a monotherapy and combination therapy with PEG-INF- $\alpha$ 2a and ADV were similar to those reported previously in other

PEG-INF- $\alpha$ 2a and NA combination therapy studies. No unexpected adverse effects were reported.<sup>[3,13,18,26]</sup>

The limitations of this study are that the study period was only 96 weeks; a longer time could provide additional insights. In addition, the number of patients evaluated for treatment efficacy was relatively small. Furthermore, HBV genotype data were not collected. Previous studies suggest that HBsAg kinetics and rates of HBeAg seroconversion vary by HBV genotype.<sup>[27,28]</sup>

In conclusion, PEG-INF- $\alpha$ 2a and ADV combination therapy was safe and superior to PEG-INF- $\alpha$ 2a monotherapy for decreasing serum HBV DNA and normalizing the ALT level. However, patients in all treatment groups had similar rates of HBeAg seroconversion and HBsAg loss at the EOF. The addition of ADV at week 12 produced a significantly better response and may be the optimal combination time.

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