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ETV add-on Peg-interferon therapy plays a positive role in reversing hepatic fibrosis in treatment-naïve chronic hepatitis B patients: a prospective and randomized controlled trial

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Abstract

Background and Aim The efficacy of entecavir (ETV) add-on peg-interferon therapy compared with ETV monotherapy in treatment-naïve hepatitis B virus (HBV) patients remains controversial. We investigated whether adding Peg-interferon to ongoing ETV treatment leads to a better curative effect or not. Methods Eligible HBV patients (n=144) were randomly divided (1:1) to receive either ETV monotherapy (n=70) or peg-interferon add-on therapy from weeks 26 to 52 (n=74). Patients were followed-up for 2 years. We evaluated hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg) seroconversion rate, sustained virologic response (SVR), transient elastography value, and histological scores. Results At week 26, no patient achieved HBsAg seroconversion in either group. At week 52, one patient in the monotherapy group was HBsAg-negative but there was none in the combination therapy group. The monotherapy group showed significantly better liver function recovery results than the combination therapy group. At week 78, one patient in combination group had HBsAg seroconverted. At week 104, only three patients in the combination therapy group were HBsAg-negative compared with one patient in monotherapy. The mean alanine aminotransferase (ALT)and aspartate aminotransferase (AST) levels and transient elastography values decreased significantly compared with baseline. Both group showed a favorable decrease in alpha fetoprotein(AFP) (monotherapy:23.4±77.3 vs 2.4±0.91, P=0.149; combination therapy: 33.3±96.9 vs 4.3±5.5,P=0.085) and an improved result of liver biopsy examination scores. The combination group showed a better improvement in histology compared with the monotherapy group(mean transient elastography value 7.5±3.4 kPa [SD 1.9] vs. 12.8±13.9 kPa [SD 1.9], P=0.037). But this research didn't show significant difference in HBsAg conversion rate (1.79% (1/56) vs 4.11% (3/73), P=0.632) as well as HBV-DNA sustained virologic response(93.2% vs 98.5%,P=0.15) between two groups. Conclusions Both therapies supported liver function recovery and histology improvement. Combination therapy did not show better antiviral efficacy in HBsAg or HBeAg seroconversion compared with monotherapy. However, combination therapy played a more positive role in reversing hepatic fibrosis compared with monotherapy.

Background

There are approximately 240 million patients infected with Hepatitis B virus（HBV）worldwide, which represents a challenge to public health despite clinical application of new drugs and efficacious vaccines[1]. Nucleos(t)ide analogues (NAs) are used more commonly in HBV treatment protocols because they can suppress HBV-DNA replication, help to recover liver function, improve histology, and obtain a functional cure which defined as hepatitis B surface antigen(HBsAg)-negative regardless of the presence or absence of (hepatitis B surface antibody) HBsAb. Peg-IFNα-2a is an immune-modulating drug that can induce cytotoxic T-cells, which clears the HBV virus from infected cells through the immunomodulatory pathway and reduces the covalently closed circularDNA(cccDNA)levels[2, 3]. Peg-IFNα-2a was shown to achieve a higher rate of HBeAg seroconversion and HBsAg seroclearance compared with entecavir (ETV) treatment[4, 5]. Combination therapy of lamivudine (LAM) and peg-IFNα-2a showed a higher virologic response rate but there was no improvement in the post-therapy response compared with monotherapy[6]. However, peg-IFNα-2a has several disadvantages including a moderate antiviral effect, sustained risk of adverse events, inferior tolerability, and subcutaneous injections. What's more，it requires regular clinical follow-up visits and a strict administration schedule. Therefore, NAs and peg-IFNα-2a combination therapy has been carried out to find an optimal efficacy, but the results of these trials are controversial because of the varying designs and evaluation criteria[7-9]. Some studies considered that combination therapy was better than monotherapy, but antiviral therapeutic options are largely influenced by the cost of drugs in China[10]. Most treatment-naïve patients generally start on NAs as an initial treatment because they are inexpensive and have a confirmed curative effect despite drug resistance.

Therefore, we designed this prospective trial to evaluate the curative effect of adding peg-interferon to ongoing ETV therapy in treatment-naïve patients. Given the side effects of peg-IFNα-2a monotherapy and drug-resistance of ETV long-term therapy, we evaluated whether shortening the add-on course (26 weeks) of peg-IFNα-2a improve outcomes such as enhancing virologic response and sustained suppression of HBV DNA or not.

Methods

Study design

All patients have been recruited from Shanghai Public Health Clinical Center and Zhongshan Hospital. Eligible participants were randomly divided into two groups at the start of the study by using a computerized randomization program. 144 eligible patients were randomly divided, in a 1:1 ratio, to receive either ETV monotherapy (n=70) or Peg-interferon add-on therapy (n=74). Both groups were given ETV for 2 years, but after 26 weeks, the combination therapy group received additional peg-IFN once weekly from week 26 to week 52. The efficacy of NAs monotherapy and combination therapy was compared every 3 months until the end of the study. Liver histology was also evaluated when participants were enrolled into the trial and after 2 years of therapy. They underwent a liver biopsy at the beginning and end of study.

Inclusion and exclusion criteria

This randomized controlled trial was approved by legally instituted ethics committees at Shanghai Public Health Clinical Center and Zhongshan Hospital. All chronic HBV patients were positive for HBsAg and had an HBV-DNA viral load >500 IU/mL with two consecutive tests at least 6

months apart, and the patients were treatment-naïve. Patients who were either HBeAg-positive or negative were included. Exclusion criteria were as follows: 1) co-infected with hepatitis A virus, hepatitis C virus, hepatitis D virus, or human immunodeficiency virus ; 2) decompensated liver disease(such as hepatocyte dysfunction and portal hypertension) or a history of esophageal varices; 3) hepatocellular carcinoma; 4) pregnancy or lactation, or liver transplantation; 5) alcoholic hepatitis, drug hepatitis, autoimmune disease, or metabolic liver disease; 6) comorbidities such as diabetes mellitus, arterial hypertension, dyslipidemia, coronary artery diseases, thyropathy. All patients signed an informed consent for indicating that their participation was voluntary and that their samples could be used for research. They were also informed of adverse events. The trial design is shown in Fig. 1.

Measurements

Before participants were enrolled in the trial, laboratory tests and routine examinations were performed at 3-month intervals for 6 months. Serum HBsAg and HBeAg levels were tested by Architect i2000 analyzer (Abbott Diagnostics, Abbott Park, IL, USA; HBsAg range of 0.05 to 52,000 IU/mL and HBeAg cut-off index <10 m IU/mL), HBV DNA were amplified using the iCycler device (Bio-Rad, USA; lower limit of quantification: 500 IU/mL).Liver biopsies were assessed at Zong Shan Hospital and Shanghai Public Health Clinical Center, Fudan University, Shanghai, China.

Evaluation criteria

HBsAg/HBeAg, serum HBV-DNA viral load, and liver function were assessed at the beginning of treatment. The rate of patients with HBsAg loss was defined as the primary endpoint.. The proportion of patients with sustained suppression of HBV DNA below 500 IU/mL, HBeAg seroconversion loss or seroconversion, histology improvement, and liver function were defined as secondary endpoints. HBsAg loss refers to undetectable serum HBsAg <0.05 IU/mL, and HBsAg/HBeAg seroconversion means the detectable HBeAb /HBsAb antibodies. HBV-DNA< 500 IU/mL was defined as an undetectable level. Ishak score was evaluated by professional pathologist. A reduction in the Ishak score or liver stiffness measurement (LSM)between the first day of treatment and the end of therapy was defined as the histology improvement criterion. The liver cirrhosis diagnosis were based on pathological stages and grades of liver histology and transient elastography standard (LSM >12.4 kPa in CHB patients with normal bilirubin and 1×ULN<ALT<2×ULN considered the progression of liver fibrosis)[11]. We Analyzed HBsAg/HBeAg loss or seroconversion, viral load, and liver function every 3 months and the end of treatment between the two groups. Adverse events were also recorded in both groups.

Statistical analysis

All statistical analyses were processed using the SPSS version 19 (SPSS Inc. Chicago, IL, USA). Continuous variables were compared using the Student's *t*-test and categorical data were presented as n (%). The HBsAg/HBeAg seroconversion rate between the two groups was compared using the Chi-square test. *P* value of <0.05 was defined as statistically significant.

Results

All baseline characteristics of the two groups are shown in Table 1. Almost all clinical data from the two groups were comparable. All patients were HBsAg positive, with an HBV viral load >500 IU/ml, no participant had decompensated liver cirrhosis.

Table 1. Baseline clinical data of the two study groups

Variables	NAs monotherapy(n=56)	Combination Therapy(n=73)	*P* value
Age(Year)	45.5±12.7	42.9±12.2	0.489
Male（%）	35（62.5%）	48（65.75%）	0.367
BMI	23.1±3.1	23.4±2.9	0.536
ALT（U/L）	79.5±64.9	92.4±104.2	0.447
ALT>40U/L	15(26.78%)	23(31.51%)	0.560
AST（U/L）	60.8±38.9	69.1±66.7	0.435
AST>40U/L	14(25%)	21(28.77%)	0.633
TBil（umol/L）	14.5±6.9	14.8±8.8	0.791
AFP（ng/ml）	23.4±77.3	33.3±96.9	0.839
LSM(KPa)	20.0±13.4	17.2±13.2	0.323
PLT（10^9/L）	127.3±50.8	122.8±53.1	0.643
HBV DNA（$\log_{10}$IU/ml）	5.68±1.23	5.56±1.44	0.636
HBeAg positive	28（50%）	32（43.8%）	0.612

Data are shown as the mean± standard deviation (SD) or n (%) and they were compared using the independent-sample test or a chi-square test.

Abbreviations: BMI, Body Mass Index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBil, total bilirubin; AFP, alpha-fetoprotein; LSM, liver stiffness measurement ;PLT, platelet; HBeAg, hepatitis B e antigen

Clinical efficacy of two groups

The clinical efficacy was different between the two groups at different time points, as shown in Table 2. Baseline and endpoint treatment variables were also compared in each group (Table 3). These results indicated that at week 52, the monotherapy group had better liver function recovery compared with the add-on treatment group (ALT: 26.0±12.1 U/L vs. 37.4±34.4, *P*=0.029; AST: 26.8±7.0 U/L vs. 36.4±23.6 U/L, *P*=0.007), but there was no significant difference in ALT and AST levels between the two groups at week 104. The results also showed that the monotherapy group might have favorable AFP decrease compared with the combination therapy group (2.4±0.84 ng/mL vs. 4.7±5.7 ng/mL, *P*=0.011). At the end of 2 years, the mean ALT and AST levels and the transient elastography value decreased significantly in each group compared with baseline data.

Table 2. On-treatment laboratory examination comparative results at different time points between the two groups

Variable	group	Baseline	26 weeks	*P*	52 weeks	*P*	78 weeks	*P*	104 Weeks	*P*
ALT（U/L）	monotherapy	79.5±64.9	30.5±16.1	0.927	26.0±12.1	**0.029***	24.4±9.5	0.158	24.8±11.5	0.2
	Add on	92.4±104.2	30.8±17.6		37.4±34.4		29.9±24.6		40.1±62.7	
ALT>40U/L	monotherapy	15(26.8%)	8(14.3%)	0.738	6(10.7%)	0.469	1(1.8%)	0.228	4(7.1%)	0.948
	Add on	23(31.5%)	12(16.4%)		11(15.1%)		6(8.2%)		5(6.8%)	
AST（U/L）	monotherapy	60.8±38.9	31.2±16.1	0.956	26.8±7.0	**0.007***	25.8±6.2	0.108	25.4±7.5	0.403
	Add on	69.1±66.7	31.3±13.1		36.4±23.6		29.3±13.7		28.5±18.2	
ALB (g/L)	monotherapy	46.8±5.5	45.3±2.9	0.799	45.3±2.1	0.208	46.5±4.3	0.91	48.2±4.7	0.774
	Add on	42.5±4.9	45.4±3.8		44.4±4.3		45.1±4.1		47.8±6.3	
TBil(umol/L)	monotherapy	14.5±6.9	14.3±5.4	0.781	15.9±7.0	0.649	14.3±5.5	0.292	20.6±32.7	0.143
	Add on	14.8±8.8	14.0±6.2		18.4±38.6		13.2±5.8		12.4±5.0	
AFP(ng/ml)	monotherapy	23.4±77.3	3.5±1.7	0.221	3.3±2.8	0.058	2.4±0.84	**0.011***	2.4±0.9	**0.07**
	Add on	33.3±96.9	6.8±19.1		4.4±3.2		4.7±5.7		4.3±5.5	
LSM measurement	monotherapy	20.0±13.4	12.9±7.9	0.853	12.3±8.3	0.195	11.1±10.7	0.357	12.8±13.9	**0.037***
	Add on	17.2±13.2	13.3±12.4		10.5±5.9		9.5±5.9		7.5±3.4	
HBV DNA (log_{10}IU/ml)	monotherapy	5.68±1.23	3.07±0.94	0.759	3.11±0.41	0.552			/	/
	Add on	5.56±1.44	3.01±0.48		2.95±0.04				/	

Note: Data were mean ± SD and were compared by Independent-Sample Test. *: P value of <0.05 was defined as statistically significant. Monotherapy means that patients in this group only received ETV treatment. Add on means that patients in this group received add-on pegylated interferon alfa-2a to ongoing ETV treatment. There were few patients HBV DNA viral load > 500 IU/ml at week 78 and week 104，so the data was not comparable.

Table 3. Clinical efficacy at baseline and at the end of the study in each group

Variable	monotherapy		*P*	combination therapy		*P*
	Baseline	2years		Baseline	2years	
ALT（U/L）	79.5±64.9	24.8±11.5	**0.000**	92.4±104.2	40.1±62.7	**0.007**
AST（U/L）	60.8±38.9	25.4±7.5	**0.000**	69.1±66.7	28.5±18.2	**0.001**
ALB (g/L)	46.8±5.5	48.2±4.7	0.32	42.5±4.9	47.8±6.3	**0.000**
TBil（umol/L）	14.5±6.9	20.6±32.7	0.207	14.8±8.8	12.4±5.0	0.142
AFP（ng/ml）	23.4±77.3	2.4±0.91	0.149	33.3±96.9	4.3±5.5	0.085
LSM(KPa)	20.0±13.4	12.8±13.9	**0.035**	17.2±13.2	7.5±3.4	**0.000**
HBV viral load	5.68±1.23	<500	<0.05	5.56±1.44	<500	<0.05
Liver histology						
Inflammation grades	2（2,3）	1（1,1）	**0.000**	2（2,3）	1（1,1.25）	**0.001**
Fibrosis stage	3（2,4）	1.5（1,3）	**0.021**	3（2,4）	1.5（1,3.25）	**0.011**
Ishak fibrosis score	4（1,4）	2（1,3.75）	0.47	4（3,4）	1.5（1,3.25）	**0.001**

Note: All data are presented as the mean±SD, or median (IQR: 1st, 3rd quartiles) as appropriate. *P value of <0.05 was defined as statistically significant.

HBsAg/HBeAg clearance or seroconversion results

At week 26, patients in both groups only received ETV for treatment, and none of them showed HBsAg loss, whereas some patients began to lose and/or seroconvert HBeAg.

At week 52, one patient in the monotherapy group but none in the combination therapy group had lost HBsAg. In both groups, another three patients had lost and/or seroconverted HBeAg. The patient who had lost HBsAg in the monotherapy group also had an undetectable HBV viral

load, but his liver function test results remained abnormal during the treatment. Additionally, before receiving ETV monotherapy treatment, the patient had already been followed-up in our department for 2 years with slightly high ALT and AST levels and HBV viral load.

At week 78, one patient from combination group had lost and/or seroconverted HBsAg.

At the end of our study, there was altogether one patient who lost HBsAg in the monotherapy group while three patients in the combination therapy group showed HBsAg clearance (1.79% (1/56) vs 4.11% (3/73), P=0.632). All data are presented in Table 4. Additionally, Figure 2A and 2B provide supplementary information about the total number of patients who lost HBsAg, HBeAg and/or seroconverted with the respective antibody formation at different time points. Data are presented as n (%). Figure 2C shows the change of HBsAg levels. Figure 2D presents the results of sustained suppression of HBV DNA. There was no significant difference in sustained suppression of HBV DNA between the two groups.

Table 4. The number of patients who had HBsAg, HBeAg clearance, and/or seroconversion with emerging antibody during the research period

Variable	group	0 weeks	26 weeks	52 weeks	78 weeks	104 Weeks
HBsAg lost	monotherapy	0	0	1	1	1
	Add on	0	0	0	1	3
HBsAb acquire	monotherapy	0	0	1	1	1
	Add on	0	0	3	3	3
HBeAg lost	monotherapy	0	1	4	5	7
	Add on	0	2	5	6	8
HBeAb acquire	monotherapy	0	1	4	4	6
	Add on	0	1	4	5	7

Note: Data are presented as the number of patients.

Complementary liver histology results

Combination therapy showed better histology improvement than the monotherapy group (mean liver stiffness value 7.5±3.4 kPa [SD 1.9] vs. 12.8±13.9 kPa [SD 1.9], P=0.037). The liver stiffness value decreased significantly in each group compared with baseline data. Liver histology improved remarkably in both groups after 2 years. The results are shown in Figs 3 and 4.

Supplement Table: Changes of liver histological evaluations

Fibrosis stage	Monotherapy		Combination therapy	
	Baseline	2 years	Baseline	2 years
G1S1	0	9	0	12
G1S2	13	7	12	9
G2S2	6	5	6	6
G2S3	6	3	15	6
G2S4	5	2	10	5
G3S2	6	2	3	3
G3S3	6	3	3	3
G3S4	14	4	24	6

Note：Not all patients agreed second liver biopsy after 2 years of treatment.

Adverse events

During treatment, three patients showed thyroid dysfunction (one patient in monotherapy) and two patients had granulopenia (both in the combination therapy group). Six patients had a fever in the combination therapy group after they received their treatment. One patient felt fatigue in the ETV monotherapy group.

Table 5. Incidence of discontinuation of treatment and adverse events

Variable	Monotherapy(N=56)	Combination therapy(N=73)
Discontinuation		
For safety reasons	4(7.14%)	5(6.85%)
For other reasons	8(14.28%)	3(2.81%)
Adverse events		
thyroid dysfunction	1(1.79%)	0
granulopenia	0	2(2.74%)
fever	0	6(8.22%)
fatigue	1(1.79%)	0

Discussion

In present, various therapies have been proceeded for CHB, but the optimal regimen remains unclear. The clinical cure rate of combined treatment with NAs and peg-IFN is not sufficient to treat naïve chronic hepatitis B patients. peg-IFN monotherapy was also found to be efficient for HBsAg loss and seroconversion, but combination therapy was thought to cause more adverse events[12-13]. However, other studies have reported that the therapeutic efficacy of NAs combined with peg-IFN was better than monotherapy[14].NAs are thought to directly inhibit HBV DNA replication, while peg-IFNα-2a as an immunomodulator can enhance the innate and adaptive immune responses to play a synergistic antiviral role[15, 16]. In one study[10], addition of NAs to peg-IFNα-2a therapy enhanced the virologic response in chronic hepatitis B patients who did not have an early response to peg-IFNα-2a. This suggests that in patients with an early poor virologic response to Peg-IFNα-2a, addition of NAs could inhibit viral replication. An trial directed by Ning also found that patients who switched from entecavir to peg-IFNα-2a significantly had increased rates of HBeAg seroconversion and HBsAg loss[17]. Presently，a new switching study[18] showed that HBeAg-positive chronic hepatitis B patients who switched from NAs to pegylated interferon achieved 12.5% and 16.2% HBeAg seroconversion and HBsAg loss, respectively. In a recent study, Patients on long-term NA who are unlikely to meet therapeutic goals can achieve high rates of HBsAg loss by switching to Peg-IFN alfa-2a [19]. It seems add-on therapy resulted in more viral decline and appeared to prevent relapse after stopping ETV compared with monotherapy. Therefore, based on this synergistic mechanism, combination therapy may be an ideal method for chronic HBV patients. However, we did not observe these results. In our study, we did not find a significant difference in clinical efficacy between the two groups, which is similar to other studies that reported that combination therapy failed to improve clinical efficacy[20]. However, a recent meta-analysis also showed that combination therapy increased the virologic response and SVR[7]. Adding peg-IFNα-2a to ADV（adefovir dipivoxil）or ETV showed better clinical efficacy in HBeAg-negative patients[21].

In this randomized controlled trial, our results showed that combination therapy did not have better antiviral efficacy than ETV monotherapy for sustained virologic suppression, HBsAg/HBeAg clearance, and seroconversion, but its histologic results showed improvement in the combination group. Additionally, the rate of HBsAg/HBeAg clearance and seroconversion was not different from combination therapy compared with ETV monotherapy after adding peg-IFNα-2a. But a recent retrospective cohort study[22] showed that patients in the peg-IFN add-on therapy group were more likely to achieve HBeAg seroconversion (44% vs. 6%; $P < 0.0001$) compared with those in the ETV monotherapy group. The reason may be that the duration of the added peg-IFNα-2a administration was too short, and the different was not significant. Although some guidelines recommend 48 weeks of Peg-IFN administration for patients with chronic hepatitis B, we administered 26 weeks of peg-IFN add-on therapy because of the side effects and cost of the drug. Additionally, Brouwer WP [23] indicated that peg-IFN add-on therapy led to a higher proportion of HBeAg response compared with ETV monotherapy for HBeAg-positive chronic hepatitis B. The inclusion and exclusion criteria for patients may lead to differences in the outcomes.

In generally speaking, the sustained benefit of combination therapy requires further investigation. In our 2-year study, we compared the sustained efficacy between the two groups at different time points and we found that there was no significant difference between sustained suppression of HBV DNA in the two groups. However, this was not in consistent with the results of a recent study, which showed that combination therapy

enhanced the virologic response and sustained suppression of HBV DNA [7]. The ideal result of anti-HBV therapy is HBsAg seroconversion, which often means a viral clearance. Achieving full viral clearance still remains a challenge. During the trial, three patients (two in the monotherapy group and one in the combination group) had hepatitis relapses even though their HBsAg was cleared and their viral load was undetectable during treatment. This phenomenon requires further study.

For histology improvement, we analyzed the transient elastography value and histological fibrosis score between the first day of treatment and the end of therapy. A significant difference in the transient elastography value was seen between the monotherapy and combination groups. The transient elastography value of combination groups decreased more than the monotherapy group. However, the ALT and AST recovery results were not in agreement with the transient elastography value. The monotherapy group had a tendency to have lower ALT AST and lower AFP levels compared with combination therapy, but not the transient elastography results. Liver histology improved remarkably in each group after treatment for 2 years. Some studies showed that combination of peg-IFNα-2a may improve liver histology better than monotherapy by immunologically modulating activity of effector T-cells, which generate a robust cytotoxic T lymphocyte (CTL) response[24]. CTLs can kill infected cells, so it results in an increase in transaminase levels. Additionally, it improved histology following long-term treatment. We also found that the AFP levels in the monotherapy group were lower than that in the combination group during the treatment. Lower post-treatment AFP levels were reported to be significantly correlated with liver fibrosis regression[25]. This phenomenon may be explained by the original baseline AFP levels in the combination therapy that were higher than those in the monotherapy group, although there is no significant difference between the two groups. Liver biopsy histological scores were evaluated at the end of the study, and the combination therapy was likely to have improved histology results compared with monotherapy, but the difference was not significant.

The most frequent adverse events reported in combination group were fatigue, headache, fever, and myalgia [26-28]. These adverse events were mostly related to adding Peg-IFNα-2a. As expected, more chronic hepatitis B patients taking combination therapy had side effects compared with those taking monotherapy in our study. Three patients had thyroid dysfunction, two patients had granulopenia, and one patient had fatigue in the ETV monotherapy. Therefore, attention must be paid to side effects of combination treatment.

There are some limitations in this trial, such as the small sample size, especially because some patients were lost to follow-up during treatment resulting from pregnancy, economic conditions, or the patient was unwilling to continue in the trial. However, the advantage of this study was that it was a randomized controlled perspective study. We evaluated the changes in ALT, AST, HBsAg/HBeAg clearance and seroconversion rate, and SVR at regular intervals, and we observed liver histological examination results. We also studied the effect of reducing the use time of interferon Peg-IFNα-2a.

Conclusion

For treatment-naïve patients with chronic hepatitis B, both monotherapy and combination therapy successfully improved liver function and histology. However, combination therapy did not show a better effect on HBsAg and HBeAg clearance and seroconversion or satisfactorily reduce HBV-DNA to an undetectable level. Our data also showed that combination therapy played a more positive role in reversing hepatic fibrosis than did monotherapy, but the safety of combination therapy requires further study. The curative effect of combination therapy and monotherapy also requires further study.

Abbreviations

ETV entecavir

HBsAg hepatitis B surface antigen

HBeAg hepatitis B e antigen

ALT alanine aminotransferase

AST aspartate aminotransferase

HBV hepatitis B virus

NAs Nucleos(t)ide analogues

HBsAb hepatitis B surface antibody

cccDNA covalently closed circularDNA

LAM lamivudine

TBil total bilirubin

AFP alpha-fetoprotein

PLT platelet

ADV adefovir dipivoxil

Declarations

Availability of data and material

I’m sorry the data and material were not applicable , because this study is still going on.

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Authors’ contributions

All authors participated in conceiving and planning the study, YJM，CLP, WYJ, Bei Lv, ZH, SZY , LJ，FZY collected and analyzed the data. YJM，CLP drafted the manuscript and had the same contribution to this paper. WSD,MX,LX helped to revise the manuscript. HSP，CJL did critical revision of manuscript. All authors contributed to final critical revision of the manuscript, and have read and approved the final manuscript.

Ethics approval and consent to participate

This study was conducted in compliance with the Declaration of Helsinki and approved by the Ethical Committee of Shanghai Public Health Clinical Center (2016-S026-04). All patients provided written documentation of informed consent to enter the study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

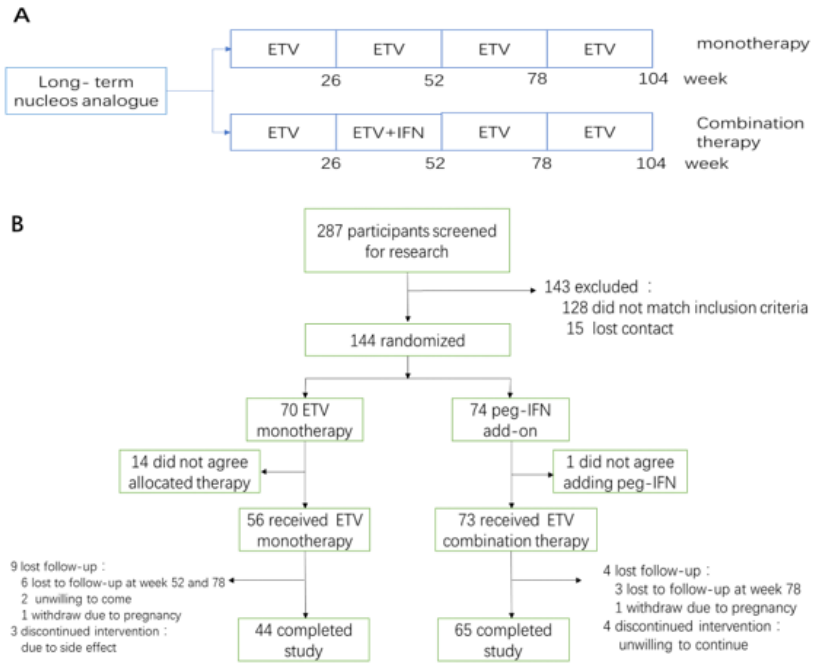

Figure 1

A: Trial design. Combination therapy adding on pre-IFN-α-2a from weeks 26 to 52. All patients were followed-up at least for 104 weeks. B: Flowchart showing the disposition of patients during the research.

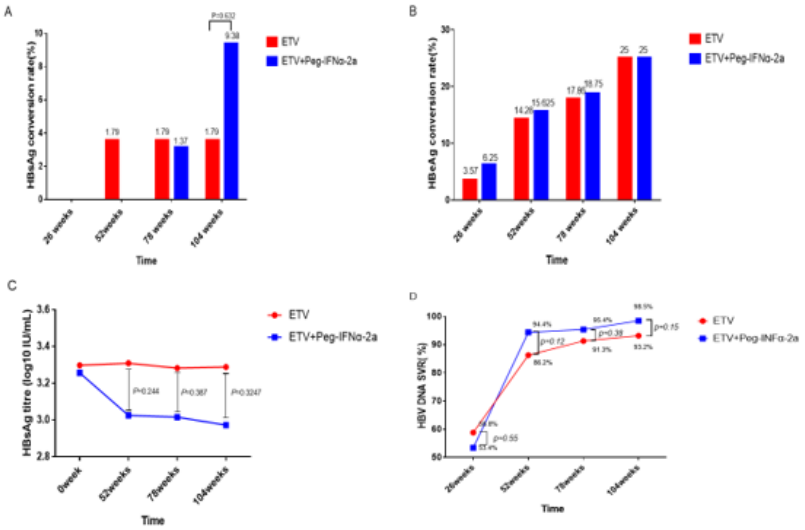

Figure 2

A/B:The rate of patients who had HBsAg, HBeAg clearance and/or seroconversion with respective antibody formation. C: The change in HBsAg levels during treatment in both groups. D: Sustained virologic response (SVR) at different time points. There was no significant difference in the

HBsAg/HBeAg conversion rate, HBsAg levels and SVR between two groups at the end of the trial. But there is a tendency that combination therapy has a lower HBsAg levels than ETV monotherapy.

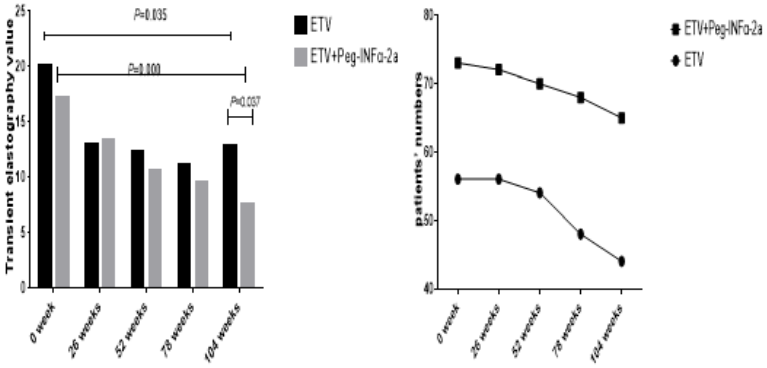

Figure 3

The transient elastography value is presented as the mean and standard deviation (SD). P < 0.05 indicates a significant difference.

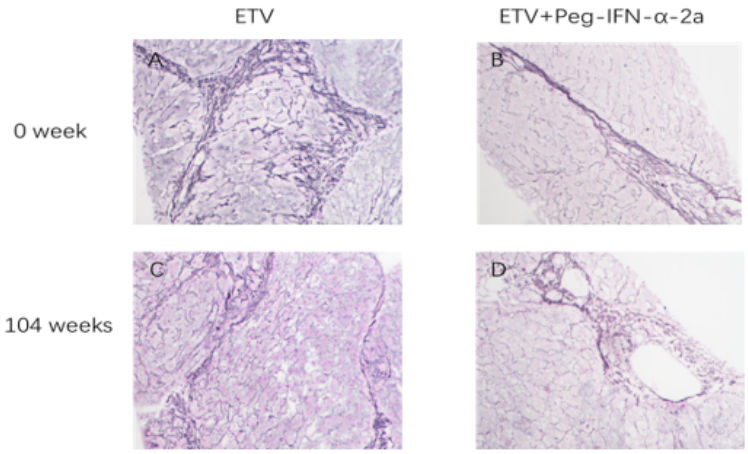

Figure 4

Liver biopsy examination results (magnification ×100) A. Histological examination results of the liver indicated inflammation grade 1 and fibrosis stage 2 in an ETV monotherapy group patient before treatment. B. Histologic examination results of the liver indicated inflammation grade 2 and fibrosis stage 2, in an ETV+ pre-IFN-α-2a group patient before treatment. C. Second liver biopsy in the same patient in the ETV monotherapy group after 2 years of treatment. The results indicate inflammation grade 1 and fibrosis stage 1. D. Second liver biopsy of the same patient in the ETV+ pre-IFN-α-2a group after 2 years of treatment. The results indicate inflammation grade 1 and fibrosis stage 1.

Supplementary Files

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- CONSORT2010Checklist.pdf
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