Peginterferon and Entecavir combination therapy improves outcome of non-early response Hepatitis B e antigen-positive patients

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Key points.

HBeAg+ patients without early response from pegylated interferon treatment, continued with entecavir and interferon combination therapy could benefit more, including HBsAg decline/loss and HBeAg clearance.
Abstraction

**Background.** It is still controversial that the efficacy of nucleot(s)ide analogs (NAs) and pegylated interferon (PegIFN) combination therapy for hepatitis B e antigen positive (HBeAg⁺) patients. It was assessed whether PegIFN and entecavir (ETV) combination therapy could bring more benefit for HBeAg⁺ patients.

**Methods.** The treatment naïve HBeAg⁺ patients initiated with PegIFN alfa-2a (PegIFNα-2a) for 24 weeks without early response (early response: HBsAg < 1500 IU/mL and HBV DNA < 10⁵ copies/mL) were recruited in the current study. Among total of 94 patients, 51 were continued with PegIFNα-2a monotherapy, 43 were offered PegIFNα-2a and ETV combined therapy.

**Results.** It was demonstrated that better outcomes in response to the combined therapy compared to that of the monotherapy, including more HBsAg decline and loss, HBV DNA decline and HBeAg clearance. Importantly, the patients with HBsAg levels between 1500 - 20,000 IU/mL initially or between 5000 - 20000 IU/mL after 24 weeks of PegIFNα-2a were more benefit from the combined therapy, compared to that of monotherapy.

**Conclusions.** The combined therapy of PegIFNα-2a and ETV is more efficacy for HBeAg⁺ patients without early response to PegIFN monotherapy, and the HBsAg levels are a good predictor of the treatment outcomes.

**Keywords.** chronic hepatitis B, Interferon, nucleos(t)ide analogues, antiviral treatment
Introduction

Chronic hepatitis B (CHB) patients often progress further to liver fibrosis, cirrhosis and hepatocarcinoma. The covalently closed circular HBV DNA (cccDNA), persistently replicates within the nucleus of hepatocytes, is the most important contributing factor for the chronic infection of hepatitis B virus. HBsAg level could surrogate cccDNA level in the nucleus, thus the ultimate goal for antiviral therapy of CHB is HBsAg loss and sustained remission afterwards. Pegylated interferon (PegIFN) and nucleot(s)ide analogs (NAs) are two first-line treatments recommended by AASLD, APASL, EASL, and Chinese guidelines for the treatment of CHB patients. NAs effectively suppress viral replication, and PegIFN enhances the efficacy of outcomes via burst host immunity, but it remained to be explored the effect of NAs and PegIFN on the reduction and/or elimination of HBsAg.

HBeAg+ patients can reach up to 40% response to PegIFN treatment, including HBeAg seroconversion and HBV DNA decline, but only with ~5% HBsAg loss. However, no response or incomplete response is still observed in ~60% of HBeAg+ patients. More recently, it has been reported that a multi-center and prospective study of HBeAg+ patients in response to the extension of PegIFNα-2a and/or adding adefovir in China. It has been demonstrated that a response-guided-therapy (RGT) strategy for the first time is involved, i.e. these patients continued to treated with different therapy strategies based on their 24-week response to PegIFNα-2a. Interestingly, there is no additional benefit in extending PegIFNα-2a nor combining with adefovir for these non-early responders. Controversial data has been also reported from others to optimize the efficacy of these none or partial response patients.

However, the international guidelines do not recommend the use of combination therapy with NAs and PegIFN, due to controversial in the real world or lack of sufficient evidence to support the combination therapy. Thus, it is ideal to verify the efficacy of the combined therapy and to optimize the benefit of the combination approach, including who and when to start the combination, which NAs should be added, and how long the combination time should last.
In the current study, we prospectively analyzed HBeAg+ patients, who initiated with PegIFNα-2a for 24 weeks without early response, either extended with PegIFNα-2a along or combined with entecavir (ETV) for 72 weeks. We aimed to clarify if a longer time combination with ETV could bring more efficacy in these difficult-to-treat patients.

**Materials and Methods**

*Study patients*

In this prospective cohort study, all of the patients were admitted to the Infectious Disease Department of Ruijin Hospital between 2016 December to 2019 July. Inclusion criteria: (1) age ≥ 18 years old, (2) confirmed diagnosis of CHB with HBeAg+, Hepatitis B e antibody (HbeAb), serum HBV DNA >10^5 copies/mL, (3) initiated with PegIFNα-2a (180 μg/week) for 24 weeks without early response (early response: HBsAg <1500 IU/mL and HBV DNA <10^5 copies/mL). The exclusion criteria were as follows: (1) received any antiviral treatment within the prior 6 months, (2) coincident with other hepatitis, including viral (hepatitis A, hepatitis C, hepatitis E), autoimmune, drug induced and alcoholic reasons, (3) decompensated liver disease (Child-Pugh score > 5), (4) hepatocellular carcinoma, (5) unnormal blood cell count (a neutrophil count < 1500 cells/mm^3 or platelet count < 90,000 cells/mm^3, hemoglobin < 11.0 g/dl for females and <12 g/dl for males).

*Patient Consent Statement*

All patient’s clinical data and serum were obtained from the patients with written consent including diagnostic and research purpose in an unidentified manner. All of the patients were adults who were older than 18 years. Our current study was approved by The Clinical Trial Ethics Committee of Ruijin Hospital, Shanghai Jiaotong University School of Medicine (reference number 2016-111).
**Treatment protocol**

The current study wasn’t a registered clinical trial. The CHB patients were recruited based on their basal levels of receiving 24 weeks of PegIFNα-2a, but without early response (early response: HBsAg < 1500 IU/mL and HBV DNA < 10^5 copies/mL). These patients were divided into groups A (continued with PegIFNα-2a along for another 72 weeks) and B (continued with ETV add-on to PegIFNα-2a for another 72 weeks of combination) depended on the consents of the patients, which was not included the attributes or confounding variables (Fig 1). The definition of early response for the current study is based on the previous study.7

The patients from both groups were followed up with examinations at 0 (the first PegIFNα-2a shot for the patients), 12, 24, 48, 72 and 96 weeks, as well as, 24 weeks post the end of treatment (EOT) (group B patients were continued with ETV), including clinical characteristics, quantitative HBsAg, HBeAg, HBeAb, HBV DNA (COBAS TagMan, Roche Molecular Diagnosis, smaller than limitation eques to negative, limitation of detection: 20 IU/mL, 1 IU/mL = 5.82 copies/mL), alanine aminotransferase (ALT), aspartate aminotransferase (AST). In this study, we used the fully automated chemiluminescent microparticle immunoassay (Architect HBsAg QT) to detect the quantitative HBsAg.

**Assessment of efficacy**

Per protocol analysis included patients who finished the predefined treatment which was totally 96 weeks and 24 weeks post-EOT, follow-up without major protocol deviation. The patients dropped out during the study (4 in group A and 7 in group B) were not included in the analysis. The efficacies of the treatment were assessed with HBsAg decline and/or loss, HBeAg serum conversion, HBV DNA decline and/or undetectable during the treatment, at the end of the treatment (EOT) and 24 weeks post-EOT. The primary endpoint was the change of quantitative HBsAg from 0-week (the time initiated with the first PegIFNα-2a shot) to EOT. There is no adjustment for multiple comparisons was made.
Statistical analysis

Graphpad 8.0 software was used for part of the statistical analysis. Quantitative variables were expressed as mean ± standard error of mean. Differences between two groups were tested using t-tests, chi-square or Fisher’s exact tests. The logistic regression was performed with SPSS software (19.0). P ≤ 0.05 (two-tailed) was considered statistically significant.

Results

Patient Characteristics

The flowchart of the study is presented in figure 2 (Fig 2). A total of 145 HBeAg+ patients eligible for PegIFNα-2a treatment were initiated with PegIFNα-2a monotherapy (the inclusion and exclusion criteria were provided in the supplemental materials). Only 105 patients without early response at 24-week were recruited, including 55 or 50 in group A or B, respectively. There were 4 or 7 patients dropped out from group A or B. In group A, two patients dropped out due to adverse effects (one was because of thyroid problems the other one was because of low level of white blood cells), one withdraws consent and one lost to follow-up. In group B, three patients dropped out due to adverse effects (two were because of thyroid problems, one was because of unacceptable itching), two patients withdraw their consents and two patients lost to follow-up (Fig 2). Finally total 94/105 (89.5%) patients finished the treatment were analyzed in this study. During the study, there were 5 and 3 patients reduced the amount of PegIFNα-2a from 180 μg/week to 135 μg/week for different time in group A and B. In group A, three patients were reduced for 3 weeks and two patients were reduced for 4 weeks. In group B, four patients were reduced for 4 weeks and one patient was for 3 weeks.

The clinical characteristics at 0-week (the time initiated with the first PegIFNα-2a shot) were similar between two groups without significance (Table 1), even though they were not randomized. Most of these patients were male with the age around 37 years, and HBsAg levels at 0-week were both 4 Log_{10} IU/mL of two groups. HBV DNA and ALT levels were marginally higher in group A than B, while HBeAg level was lower without significance. The patients’ clinical characteristics at 24-week were also similar between two groups, including the FIB-4 index (Table 2).
The efficacy of monotherapy or combination therapy for HBsAg decline.

It was observed that a 2.8-fold increase of HBsAg decline in group B than A (group A vs. group B, -0.605 Log_{10} IU/mL vs. -1.684 Log_{10} IU/mL, P = 0.0025) at EOT, with the effect continued, group B obtained more HBsAg decline than group A at 24 weeks post-EOT as the off-treatment response (group A vs. group B, -0.855 Log_{10} IU/mL vs. -2.001 Log_{10} IU/mL, P = 0.0004) (Fig 3A). A more profound difference in HBsAg level was detected with prolonged treatment (48 weeks: group A vs. group B, -0.433 Log_{10} IU/mL vs. -0.961 Log_{10} IU/mL, P = 0.0124; 96 weeks: group A vs. group B, -0.656 Log_{10} IU/mL vs. -1.734 Log_{10} IU/mL, P = 0.0012) (Fig 3B).

There was no significant difference of HBsAg decline between 48 to 96 weeks in group A (48 weeks vs. 96 weeks, 0.409 Log_{10} IU/mL vs. 0.605 Log_{10} IU/mL, P = 0.1841), but striking difference was detected in group B (48 weeks vs. 96 weeks, 0.912 Log_{10} IU/mL vs. 1.685 Log_{10} IU/mL, P=0.0492) (Fig 3C). Interestingly, it was observed a better HBsAg decline in the group B at 48-week than that of 96-week in group A without significance (group A 96 weeks 0.605 Log_{10} IU/mL, group B 48 weeks 0.912 Log_{10} IU/mL, P=0.174).

The efficacy on HBeAg clearance and HBV DNA decline/undetectable

The clearance of HBeAg was also greater in group B than in group A at EOT even with a higher baseline (groups A vs. B, 33.3% vs. 55.8%, P = 0.0373) (Fig 4A).

Similarly, group B also achieved more HBV DNA decline at EOT (group A vs. group B, -6.674 Log_{10} copies/mL vs. -9.498 Log_{10} copies/mL, P = 0.0064) (Fig 4B), making 57% or 86% of patients achieved HBV DNA undetectable in group A or B, respectively (P = 0.01) (Fig 4C).
The HBeAg clearance and HBsAg loss at different time points

The first patient got HBsAg loss at week 48 in group B, and one more in every 24 weeks. At EOT, three patients (7%) had HBsAg loss in group B but none in group A. The off-treatment response was valued at 24 weeks post-EOT, one (2%) patient and four (9.3%) patients had HBsAg loss in group A and B at that time (Fig 5A). The percentage of HBeAg loss was increasing as the treatment time prolonged. Group A was still with 33.3% at 24 weeks post-EOT, while group B was slightly dropped to 48.4% (Fig 5B).

Prediction of response

The binary logistic regression analysis was applied to explore the values of the 0-week characteristics for the response at EOT. According to the previous study\(^7\), the HBeAg loss and HBV DNA < 2000 log\(_{10}\) copies/mL at EOT is defined as “Response”. It was detected that the HBsAg level at 0-week (continuous variable, Log\(_{10}\) IU/mL, P = 0.04) and ETV combination (with ETV = 1, without ETV = 0, P = 0.01) had influences on the response, but not age, gender, HBeAg, HBV DNA and ALT. The odds ratio (OR) for HBsAg and ETV combination was 0.461 (95% CI: 0.22 to 0.966) and 3.172 (95% CI: 1.316 to 7.647), respectively (Table 3).

Based on their HBsAg level at 0-week to predicting the effect, the CHB patients were further divided into three groups according to previous study\(^7\). It was detected 60% of the patients achieved response at EOT with HBsAg < 1500 IU/mL at 0-week (Fig 6A), irrespective to either treatment used (Fig 6B, group A vs. group B, 66.7% vs. 57.1%, P>0.05). However the lowest response was observed in these patients with HBsAg > 20,000 IU/mL at 0-week (Fig 6A, 27.6%), despite that the combination therapy increased the response rate for more than 2 folds without significance (group A vs. group B, 15.4% vs. 37.5%, P>0.05) (Fig 6B). Interestingly, for these patients with HBsAg between 1500 to 20,000 IU/mL at 0-week, the combination therapy improved significantly more the response rate in group B than group A (group A vs. group B, 34.3% vs. 70.0%, P = 0.0408) (Fig 6B).
The HBsAg level at the enrollment (24 weeks after PegIFNα-2a) could further predict the outcomes of the treatment. Previous study showed that the HBsAg cut off of 1500 IU/ml at 24-week is used to predict the outcome. In our study, the CHB patients included were all with HBsAg > 1500 IU/ml. The cut off of 5000 IU/ml was selected, instead of 1500 IU/ml. It was detected that the patients with HBsAg levels between 5000 and 20000 IU/mL at 24-week had better response in combine therapy than that from the monotherapy group (group A vs. group B, 12.5% vs. 50%, P = 0.0484) (Fig 6C). The patients with HBsAg < 5000 IU/mL displayed higher response rate with combine therapy than monotherapy, but without statistical difference (Fig 6C). However, there was no patient had response at EOT with HBsAg > 20000 IU/mL at 24-week in neither group.

Discussion

“APASL clinical practice guidelines on the management of hepatitis B: a 2015 update” recommend the ideal endpoint of hepatitis B is sustained off-therapy HBsAg loss (A1 recommendation). PegIFN and NAs with potent virus suppression are recommended to be the first choice of antiviral therapy. NAs should be taken long time long with rare HBsAg loss. Currently, PegIFN could have more chance to achieve HBeAg seroconversion and HBsAg clearance. However less CHB patients’ responder is detected with PegIFN monotherapy. Thus it is critically important to optimize the combination of NAs and PegIFN therapy at this stage. Response-Guided-Treatment (RGT) is a promising approach which has been successfully applied for treatment of CHC, while its usage in CHB patients still remains to be clarified. The RGT strategy was also applied in the current study, but more focusing on these non-early responders.

The combination of PegIFNα and NAs are not recommended currently by the guidelines because of unproven superior efficacy and lack of well-designed randomized clinical trial. The data from classic global registration study doesn’t show the impressive from combination of PegIFNα and lamivudine, which is consistent with studies in China. A more recent multi-center clinical study in China also demonstrates that extending and/or combining PegIFNα with adefovir doesn’t provide superiority for non-early responders. However, NAs with stronger HBV DNA suppression and less resistance are not included, and NAs are only offered for a short duration (< 24 weeks), which might lead to the unsatisfied results. It has been reported that PegIFNα combined with tenofovir improves the reduction of HBsAg, even
in HBeAg negative patients in 2016. Interestingly, our study demonstrated that regardless of HBsAg decline/loss, HBeAg clearance or HBV DNA declined in the combination therapy was better than that from monotherapy for non-early responders based on the RGT strategy. Our data further demonstrated that the reduction of HBsAg in the combined therapy group B was 2.8 times more than that of monotherapy at EOT, which is consistent to the decrease in HBsAg value of group B from 4 to 2.66 Log_{10} IU/mL, while only 4 to 3.344 Log_{10} IU/mL in group A. In addition, 13 out of 43 (30.2%) patients in group B had 2 Logs of HBsAg reduction, compares with 2 out 51 (3.9%) patients in group A at EOT. Even more, 3 out of 43 (7%) patients in group B achieve HBsAg loss, while none in group A was cleared. Similarly, the combination therapy also provides more HBeAg clearance and HBV DNA decline/loss. The explanation for the discrepancy between ours and others might be as such: (1) ETV is chosen as the added on NAs because ETV is able to suppress virus stronger in short time than adefovir or lamivudine; (2) We initiated in advanced and further extended ETV added on time, which started at 24 week and combined for total 72 weeks, which is the longest combination therapy in the literature.

It is reported that extending PegIFNα treatment brings advantage of virological response or HBsAg decline in HBeAg− patients. We also demonstrated that HBsAg decline wasn’t improvement significantly with extension of PegIFNα monotherapy from 48 to 96 weeks (group A 48 weeks vs. 96 weeks, -0.433 Log_{10} IU/mL vs. 0.656 Log_{10} IU/mL, P > 0.05), which was the same as the HBeAg clearance (group A 48 weeks vs. 96 weeks, 31.37% vs. 33.3%, P > 0.05). Interestingly, the HBV DNA loss was improved significantly (group A 48 weeks vs. 96 weeks, 19.6% vs. 56.8%, P = 0.0002). Our data suggests that monotherapy extension maybe not necessary for non-early responders, 48 weeks might provide the maximum effect, and 96 weeks may not be unnecessary without additional benefit, but possible of more adverse effects of PegIFNα. Although HBV DNA loss is benefit, NAs monotherapy can achieve HBV DNA lose without less adverse effects. We also acknowledge that there was no powered to detect a difference in these two groups between weeks 48 and 96.

Our current finding showed that 0-week HBsAg level may influence on the EOT response, which is consistent with the previous study. For the patients with 0-week HBsAg < 1500
IU/mL, 60% were the responders at EOT, whereas for the patients with baseline HBsAg > 20,000 IU/mL, only 27.6% were the responders, and either monotherapy or combination therapy. Thus our data further suggests that the patients with lower 0-week HBsAg have better chance to response to PegIFNα-2a, and no combination with NAs is necessary. On the other hand, it is not recommended the patient with high HBsAg to initial PegIFNα therapy, because long-term NAs probably is more appropriate to achieve lower HBsAg level prior to PegIFNα therapy.

More importantly, the HBsAg levels at 24-week could predict the treatment outcomes in advance. The patients with HBsAg levels at 24-week between < 5000 IU/mL, could lead to optimize response rate with either monotherapy or combination therapy (group A vs. group B, 42.4% vs. 66.7%, P > 0.05). Furthermore, the combination therapy seems to be more benefit for the patients with HBsAg levels between 5000 and 20000 IU/mL. In addition, it is recommended that the patients with HBsAg levels between 5000 and 20000 IU/mL should be added with first-line NAs as soon as possible, and extended the combination therapy for at least 72 weeks. It remains to be explored the cutoff value of HBsAg for combination therapy with the same HBsAg range. Unfortunately, the patients with HBsAg level still higher than 200000 IU/mL at 24-week should stop the IFN therapy switch to NAs because of the poor outcomes at EOT.

Although this was a prospective cohort study in single-center with < 150 patients, our data offers some useful information in utilizing RGT strategy in HBeAg+ patients, as well as, shows the optimistic results of the combination therapy of PegIFNα-2a and ETV. We realize that this isn’t the best selection. Nevertheless, it was the best option we had at the time. We will, of course, extend our study with randomized and multi-center investigation in future.
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Author contributions: Lu Chen and Lanyi Lin designed the experiments and wrote the manuscript draft. Huijuan Zhou, Weiliang Tang, Hui Wang and Wei Cai were involved in patient recruitment and data collection. Shisan Bao and Simin Guo conceived the study and critically reviewed the manuscript. Qing Xie had full access to all the data in the study and takes responsibility for the integrity of the data.

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Reference

1. Trepo C, Chan HL, Lok A. Hepatitis B virus infection. *Lancet* 2014;384(9959):2053-63. doi: 10.1016/S0140-6736(14)60220-8 [published Online First: 2014/06/24]

2. European Association For The Study Of The L. EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. *J Hepatol* 2012;57(1):167-85. doi: 10.1016/j.jhep.2012.02.010 [published Online First: 2012/03/23]

3. 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):1-98. doi: 10.1007/s12072-015-9675-4 [published Online First: 2015/11/14]

4. Liaw YF, Kao JH, Piratvisuth T, et al. Erratum to: Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2012 update. *Hepatol Int* 2012;6(4):809-10. doi: 10.1007/s12072-012-9386-z [published Online First: 2012/10/01]

5. Terrault NA, Lok ASF, McMahon BJ, et al. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. *Hepatology* 2018;67(4):1560-99. doi: 10.1002/hep.29800 [published Online First: 2018/02/07]

6. Buster EH, Flink HJ, Cakaloglu Y, et al. Sustained HBeAg and HBsAg loss after long-term follow-up of HBeAg-positive patients treated with peginterferon alpha-2b. *Gastroenterology* 2008;135(2):459-67. doi: 10.1053/j.gastro.2008.05.031 [published Online First: 2008/07/01]

7. Sun J, Ma H, Xie Q, et al. Response-guided peginterferon therapy in patients with HBeAg-positive
chronic hepatitis B: A randomized controlled study. *J Hepatol* 2016;65(4):674-82. doi: 10.1016/j.jhep.2016.05.024 [published Online First: 2016/05/31]

8. Marcellin P, Lau GK, Bonino F, et al. Peginterferon alfa-2a alone, lamivudine alone, and the two in combination in patients with HBeAg-negative chronic hepatitis B. *N Engl J Med* 2004;351(12):1206-17. doi: 10.1056/NEJMoa040431 [published Online First: 2004/09/17]

9. Lau GK, Piratvisuth T, Luo KX, et al. Peginterferon Alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. *N Engl J Med* 2005;352(26):2682-95. doi: 10.1056/NEJMoa043470 [published Online First: 2005/07/01]

10. Xie Q, Zhou H, Bai X, et al. A randomized, open-label clinical study of combined pegylated interferon Alfa-2a (40KD) and entecavir treatment for hepatitis B "e" antigen-positive chronic hepatitis B. *Clin Infect Dis* 2014;59(12):1714-23. doi: 10.1093/cid/ciu702 [published Online First: 2014/09/06]

11. A SPECIAL MEETING REVIEW EDITION: Advances in the Treatment of Hepatitis C Virus Infection From EASL 2014. *Gastroenterol Hepatol (N Y)* 2014;10(6 Suppl 2):1-19. [published Online First: 2015/07/17]

12. Lampertico P. The royal wedding in chronic hepatitis B: The haves and the have-nots for the combination of pegylated interferon and nucleos(t)ide therapy. *Hepatology* 2015;61(5):1459-61. doi: 10.1002/hep.27731 [published Online First: 2015/02/04]

13. Hu P, Zhao GD, Li H, et al. Effects of oral antiviral agents on long-term outcomes of treatment-naive patients with HBV-related decompensated cirrhosis: a retrospective cohort study. *Zhonghua Gan Zang Bing Za Zhi* 2014;22(11):806-11. doi: 10.3760/cma.j.issn.1007-3418.2014.11.002 [published Online First: 2014/12/23]

14. Marcellin P, Ahn SH, Ma X, et al. Combination of Tenofovir Disoproxil Fumarate and Peginterferon alpha-2a Increases Loss of Hepatitis B Surface Antigen in Patients With Chronic Hepatitis B. *Gastroenterology* 2016;150(1):134-44 e10. doi: 10.1053/j.gastro.2015.09.043 [published Online First: 2015/10/11]

15. Lampertico P, Vigano M, Colombo M. Why do I treat HBeAg-negative chronic hepatitis B patients
with pegylated interferon? *Liver Int* 2013;33 Suppl 1:157-63. doi: 10.1111/liv.12064 [published Online First: 2013/01/11]

16. Gish RG, Lau DT, Schmid P, et al. A pilot study of extended duration peginterferon alfa-2a for patients with hepatitis B e antigen-negative chronic hepatitis B. *Am J Gastroenterol* 2007;102(12):2718-23. doi: 10.1111/j.1572-0241.2007.01449.x [published Online First: 2007/07/31]

17. Milan JL, Lavenus S, Pilet P, et al. Computational model combined with in vitro experiments to analyse mechanotransduction during mesenchymal stem cell adhesion. *Eur Cell Mater* 2013;25:97-113. doi: 10.22203/ecm.v025a07 [published Online First: 2013/01/18]
### Table 1. The characteristics of the patients at 0-week initiated with the first PegIFNα-2a shot

|                        | Total (n=94) | Group A (n=51) | Group B (n=43) | P       |
|------------------------|--------------|----------------|----------------|---------|
| Male, n (%)            | 68 (72.3)    | 34 (66.7)      | 34 (79.0)      | NS      |
| Age (years)            | 37.9±8.1     | 37.8±8.6       | 38.0±7.8       | 0.8887  |
| BMI                    | 23.3±2.7     | 22.9±2.9       | 23.7±2.2       | 0.1284  |
| HBsAg (Log_{10} IU/mL)| 4.0±0.6      | 4.0±0.6        | 4.0±0.7        | 0.9573  |
| HBeAg (Log_{10} PEIU/mL)| 2.5±0.9     | 2.3±1.0        | 2.6±0.8        | 0.0815  |
| HBV DNA (Log_{10} IU/mL)| 7.3±1.4     | 7.3±1.6        | 7.1±1.0        | 0.3628  |
| ALT (IU/mL)            | 222.0±182.4  | 248.3±193.1    | 191.0±165.6    | 0.1326  |
Table 2. The characteristics of the patients at 24-week

|                         | Total (n=94) | Group A (n=51) | Group B (n=43) | P (Group A vs. B) |
|-------------------------|--------------|----------------|----------------|-------------------|
| HBsAg (Log₁₀ IU/mL)     | 3.7±0.6      | 3.7±0.6        | 3.6±0.7        | 0.2285            |
| HBeAg (Log₁₀ PEIU/mL)   | 2.0±1.0      | 1.9±1.0        | 2.1±0.9        | 0.2121            |
| HBV DNA (Log₁₀ cps/mL)  | 5.2±1.8      | 5.0±1.7        | 5.4±1.8        | 0.2507            |
| ALT (IU/mL)             | 121±96.7     | 125.8±81.6     | 115.3±112.7    | 0.6149            |
| FIB-4                   | 1.7±0.7      | 1.7±0.7        | 1.6±0.7        | 0.2976            |
Table 3 The logistic regression analysis of the characteristics

| Variable                               | Regression coefficient | Standard error | Wald $\chi^2$ value | P value OR | 95% CI of OR | Lower | Upper |
|----------------------------------------|------------------------|----------------|---------------------|------------|--------------|-------|-------|
| HBsAg, Log$_{10}$ IU/mL               | -0.774                 | 0.378          | 4.206               | 0.040      | 0.461 - 0.220 | 0.966 |
| ETV Combination, (with ETV = 1, without ETV = 0) | 1.154                  | 0.449          | 6.611               | 0.010      | 3.172 - 1.316 | 7.647 |
| Constant                               | 2.165                  | 1.497          | 2.090               | 0.148      | 8.714 -      | -    | -     |
**Figure Legend**

Fig.1 The treatment protocol of the study.

Fig.2 The flowchart of the study.

Fig.3 The effect of HBsAg in the study. A. The HBsAg decline of two groups from 0 to 96 weeks and 24 weeks post-EOT. B. The HBsAg value of two groups at 48- and 96-week. C. The HBsAg decline of two groups with 48- or 96-weeks treatment.

Fig.4 The comparison of HBeAg clearance and HBV DNA decline/loss between two groups. A. The HBeAg clearance of two groups at 96-week. B. HBV DNA decline of two groups from baseline to 96-week. C. HBV DNA clearance at 96-week of two groups.

Fig.5 Percentage of patients with HBsAg loss and HBeAg conversion in different time points. A. Percentage of patients with HBsAg loss in different time points. B. Percentage of patients with HBeAg conversion in different time points.

Fig.6 The prediction value of HBsAg at 0- and 24-week. A. The response rate of all patients at 96-week with different HBsAg levels at 0-week. B. The response rate of patients in the two groups at 96-week with different HBsAg levels at 0-week. C. The response rate of patients in the two groups at 96-week with different HBsAg levels at 24-week.
Non-early responders

Peg IFN α-2a 180 μg/week

A

B

Peg IFN α-2a 180 μg/week

ETV 0.5mg once daily

Enrollment:

0 24 48 72 96 weeks
