**Title: Prediction model for HBeAg seroconversion to peginterferon-alfa in treatment-experienced patients with HBeAg-positive chronic hepatitis B based on a response-guided therapy strategy**(Manuscript NO: 89628)

Dear editors and expert reviewers,

We sincerely thank you for your reply and the reviewers' constructive comments on our manuscript entitled "Prediction model for HBeAg seroconversion to peginterferon-alfa in treatment-experienced patients with HBeAg-positive chronic hepatitis B based on a response-guided therapy strategy" (Manuscript NO: 89628). These comments are very valuable and important to improve the quality of the manuscript. I have made some changes according to the suggestions of expert reviewers. Changes/additions to the manuscript are highlighted with yellow color. Point-by-point replys to reviewers are listed as follows. Comments of reviewers are shown in orange color and our responses are given in black color.

Thank you for your consideration. I look forward to hearing from you.

Sincerely,

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**Comment 1**: The author should state more clearly the selection criteria, whether the patient was being treated with NAs, or had been treated and then stopped. If they stopped, how long had they stopped the treatments?

**Reply**: Thank you for your valuable advice.The aim of this study was to establish a predictive model for the efficacy of PEG-IFN therapy in HBeAg-

positive CHB patients after NAs treatment. Based on the data collected, a total of 75 patients did not experience HBeAg seroconversion after a median duration of 2 years (at least half of one year) of NAs treatment. Among them, 13 patients discontinued NAs treatment and commenced monotherapy with PEG-IFN, while 62 patients continued NAs treatment in combination with PEG-IFN therapy. These patients did not interrupt the treatment because HBeAg seroconversion did not occur after NAs therapy. They either immediately started PEG-IFN therapy after stopping NAs treatment or received combination therapy with PEG-IFN. This criterion has been revised and incorporated into the inclusion criteria. (Changes can be found in the first paragraph, 6th to 7th lines, of the manuscript's methods section. )

**Comment 2**: This is a medical research. To evaluate the results accurately, the author should use the same drug and the same manufacturing company. In this study, the author used many different types: Pegasys; Roche, Shanghai, China or Peginterferonα-2b; Amoytop Biotach, Xiamen, China.

**Reply**: Your comments are pertinent and I acknowledge that this retrospective study has small sample size and spans a duration of 13 years. And the literature search revealed a limited number of patients who did not undergo HBeAg seroconversion after NAs treatment. Due to the lengthy duration of the study and the availability of the drug at the beginning of the research, we needed to use the same category of PEG-IFN to ensure a sufficient sample size. Therefore, we included patients who received two types of PEG-IFN therapy. These two types of PEG-IFN are known as Pegasys (also known as Peginterferonα-2a) and Peginterferonα-2b. We ensured that these two types of PEG-IFN were sourced from their respective manufacturers: Pegasys from Roche, produced in Shanghai, China; and Peginterferonα-2b from Amoytop Biotach, produced in Xiamen, China. On the other hand, several large-scale studies[1,2] have indicated that there is minimal difference in efficacy between

these two forms of PEG-IFN. Patients can choose between them based on their economic capacity and adverse treatment reactions. At last, we conducted an efficacy analysis on patients treated separately with the two aforementioned PEG-IFN (peginterferonα-2a, peginterferonα-2b) and found no statistically significant difference in their effectiveness.

References:


1. Scotto G, Fazio V, Fornabaio C, Tartaglia A, Tullio RD, Saracino A, Angarano G. Early and sustained virological response in non-responders with chronic hepatitis C: a randomized open-label study of pegylated interferon-alpha-2a versus pegylated interferon-alpha-2b. *Drugs*. 2008;68 (6):791-801. [PMID: 18416586 DOI: 10.2165/00003495-200868060-00005]
2. Laguno M, Cifuentes C, Murillas J, Veloso S, Larrousse M, Payeras A, Bonet L, Vidal F, Milinkovic A, Bassa A, Villalonga C, Pérez I, Tural C, Rebollar M, Calvo M, Blanco JL, Martínez E, Tapias JM, Gatell JM, Mallolas J. Randomized trial comparing pegylated interferon alpha-2b versus pegylated interferon alpha-2a, both plus ribavirin, to treat chronic hepatitis C in human immunodeficiency virus patients. *Hepatology*. 2009;49 (1):22-31. [PMID: 19085908 DOI: 10.1002/hep.22598]


**Comment 3**: Evaluation of treatment response should include additional factors such as normal ALT enzyme, negative HBVDNA. In addition, liver fibrosis should be assessed.

**Reply**: Thanks for your constructive suggestion. During the stepwise regression analysis, we included relevant factors such as ALT, HBVDNA, HBeAg, HBsAg levels at the initiation of NAs treatment (referred to as initial data) and PEG-IFN therapy (referred to as baseline data), as well as their corresponding dynamic changes. The analysis revealed that HBV DNA ≤ 4.3 log IU/ml, HBsAg ≤ 30000 IU/ml, HBeAg ≤ 1000 S/CO at the initiation of treatment, and HBsAg ≤ 1000 IU/ml, HBeAg ≤ 3 S/CO at baseline were

statistically significant ($P < 0.05$). When these factors were included in the multiple regression analysis, the two most significant independent predictive factors were HBsAg ≤ 1000 IU/ml and HBeAg ≤ 3 S/CO at baseline. Similarly, HBsAg ≤600 IU/ml and HBeAg ≤3 S/CO at week 12, and HBsAg ≤300 IU/ml and HBeAg ≤2 S/CO at week 24 as the most notable independent predictive factors. The specific statistical results can be found in Supplementary Table 1.

On the other hand, we did not assess liver fibrosis as an indicator in this study for the following reasons. Currently, there are several non-invasive methods available for assessing the degree of liver fibrosis. Firstly, there are serum markers for evaluating liver fibrosis, such as APRI and FIB-4. However, due to fluctuations in ALT and AST levels during PEG-IFN treatment, these markers can be affected, thus impacting the assessment of liver fibrosis. Secondly, liver stiffness measurement can reflect the degree of liver fibrosis. In the early stages of this study, our hospital did not have the equipment to measure liver stiffness, so some patients did not have measurements for liver stiffness. Thirdly, liver imaging examinations, such as ultrasound, CT, and MRI, can also reflect the degree of liver fibrosis. However, these imaging techniques provide a rough rather than quantitative assessment of liver fibrosis, making statistical analysis unfeasible. In brief, based on this suggestion, we will improve the experimental design and incorporate this indicator to evaluate treatment efficacy in future studies.

**Comment 4**: The author used many treatment regimens: PegINF mono, PegINF + ETV, PegINF + TDF. The author should discuss factors associated with the effectiveness of different regimens.

**Reply**: Your suggestion is helpful, and the relative discussion is as follows: Whereas, some other factors seem to be unrelated to treatment efficacy, such as PEG-IFNα monotherapy or combination therapy with NAs. Our study showed that the occurrence of response at EOF was not significantly correlated with the

treatment regimen, whether it was PEG-IFNα monotherapy, PEG-IFNα+ETV, or PEG-IFNα+TDF. However, a recent meta-analysis indicated that compared with IFN monotherapy, the combined therapy of interferon plus NAs had a higher e-antigen serological response at EOT. These influencing factors may include whether the patient has received prior treatment, viral load, HBsAg levels, HBeAg status, and the degree of liver fibrosis. ( Revisions can be found in the third paragraph, 12th to 20th lines, of the manuscript's discussion section.)

**Comment 5**: The 6-month follow-up period was a little short. The follow-up period should be longer, maybe 1 year because hepatitis B virus can recur slowly when treated with PegIFN.

**Reply**: Your comments are insightful. The 6-month follow-up period after the completion of PEG-IFN therapy is somewhat short. However, many large-scale studies have observed patient responses at 24 weeks of follow-up after completing the 52-week treatment. This appears to be the internationally accepted follow-up period. Additionally, according to the 2022 edition of the Chinese Guidelines for the Prevention and Treatment of Chronic Hepatitis B, it is recommended that effective patients receiving PEG-IFN therapy complete the standard treatment course of 52 weeks. The treatment duration can be extended based on the patient's condition, but should not exceed 96 weeks. The likelihood of hepatitis B virus relapse after discontinuation of PEG-IFN therapy is lower following completion of the standard PEG-IFN therapy course or an extended course. Finally, based on the above suggestions, we hope to carry out large-scale prospective studies in multiple centers in the future.