PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Prospective, multicentre, randomized controlled trial comparing the seroclearance of HBsAg between combination therapy of peg-interferon alpha and tenofovir with tenofovir monotherapy in nucleos(t)ide analogue-experienced patients with HBV-related liver fibrosis: a study protocol
AUTHORS	Zhu, Shu; Wu, Lina; Mei, Yongyu; Liu, Zhihua; Lin, Luping; Yuan, Jing; Li, Jianguo; Li, Xuejun; Peng, Liang

VERSION 1 – REVIEW

REVIEWER	Kao, JiaHorng National Taiwan University Hospital, Hepatitis Research Center
REVIEW RETURNED	06-Mar-2021

GENERAL COMMENTS	General comments: In this paper, Zhu et al. proposed a protocol of a prospective, multicenter, open, randomized controlled trial to investigate the efficacy of peg-IFN in addition to NUC in NA-experienced HBV patients with liver fibrosis by evaluating the serological clearance/conversion rate of HBsAg. Nowadays, achieve functional HBV cure a clinically important issue needs to be addressed. Specific comments: 1. In page 8 “Study process”, the authors described the hepatitis B virus serological markers will be assessed every 3 months. It may be more informative to specify which serological markers will be explored. 2. Similarly, the liver biopsy will be performed before randomization and at 96 and 144 weeks. In addition to the degree of fibrosis, the authors may clarify any other index or virological profiles will be evaluated in the precious histological samples. 3. The type and duration of previous NA should be specified. 4. IFN-experience patients and patients with contraindications to IFN should be excluded. 5. Since Peg-IFN will be out of market and TDF will be replaced by TAF, the clinical value of this trial is a concern. 6. Minor point: Page 8 Line 36, “progenomic RNA” should read “pregenomic RNA”.

REVIEWER	Kothakota, Sunil KIMS Hospital, Trivandrum, Kerala, India
REVIEW RETURNED	18-May-2021

GENERAL COMMENTS	This study has been designed very well and addressing much needed concern in hepatology i.e. therapeutic cure of HBV. It

	would be much more beneficial if authors can address following issues: 1. Include the HBsAg quantitative analysis at the start and end of analysis. 2. Make it clear whether both HBeAg positive and negative or only HBeAg negative subjects are recruiting for the study.

REVIEWER	Boyd, Anders Hôpital Saint-Antoine, Service de Maladies Infectieuses et Tropicales
REVIEW RETURNED	24-May-2021

GENERAL COMMENTS	In this protocol paper, the authors present a randomized clinical trial of treatment-experienced individuals with chronic HBV infection who will be randomized 1:1 to receive either pegylated interferon with TDF or only TDF. RCTs with the same objectives as in this study have been done multiple times, hence the novelty of this study should be more convincingly highlighted. I have several other recommendations that I hope could help direct the authors to a more concise research question. Major issues: - The authors emphasize that this is a study designed for individuals with liver disease/liver fibrosis (i.e. this is assumedly the target population), yet they recruit specifically individuals with F1-F3 fibrosis. F1 is not much or a concern. F2 is where one would consider treatment more seriously. F3 is definitely indication for treatment. F4 was excluded (for toxicity reasons? It was never explained). The heterogeneity of fibrosis levels questions which populations are being referred to. - Previous studies differentiate between HBeAg-positive and HBeAg-negative status because there is a massive difference in HBsAg-seroclearance rates between these populations. Naturally, the randomization should make HBeAg-status balanced, but once again, there is a question of which population this treatment is intended to be used for. - There is no control arm with peg-interferon alone. This is imperative because if TDF has no effect when combined with peg-interferon, then there is no need to use it in combination. This has been a very important limitation of the previous RCTs, which could be resolved with this study. Minor comments: - Endpoint: "…serological clearance/conversion rate of HBsAg." Which one is it? Since seroconversion requires seroclearance, it would be simpler to leave as "seroclearance." - ref 11. Does this ref include treated patients? - combination therapy of peg-IFN-alpha … in a specific population of chronic hepatitis B patients…" which ones? - "Long-term cirrhosis, liver cancer, and other important indicators…" would the authors have enough power to conclude anything about this study? - Please state clearly which antiviral therapy recommendations and functional cure definitions are being used, rather than just cite them. - Sample size. Why is only a one-sided test being used for a superiority trial? - Primary efficacy endpoint is a cumulative proportion, not a rate, I assume. The statistical test for the sample size calculation was not

	given (nor was it found in the statistical analysis section), so it is difficult to determine. - Secondary efficacy endpoint on fibrosis: so the authors plan on taking liver biopsies? FibroScans? Or both? The criteria for evaluation need to be given, along with how the biopsies will be read to ensure high reliability. - Statistical analysis. Are the authors attempting to differentiate PP and ITT analyses sets? The ITT analysis set needs to be more thoroughly described. - The manuscript needs to be read by a native speaker with medical knowledge. There is a lot of inappropriate or unclear phrasing: “is a hot issue”, “who progress to fibrosis already”, “treatment plan”, “liver fibrosis takes place after liver damage”, etc.

VERSION 1 – AUTHOR RESPONSE

Reviewers' comments
Reviewer: 1
Prof. JiaHorng Kao, National Taiwan University Hospital
Comments to the Author:
General comments:
In this paper, Zhu et al. proposed a protocol of a prospective, multicenter, open, randomized controlled trial to investigate the efficacy of peg-IFN in addition to NUC in NA-experienced HBV patients with liver fibrosis by evaluating the serological clearance/conversion rate of HBsAg. Nowadays, achieve functional HBV cure a clinically important issue needs to be addressed.
Response from authors:
We are very grateful for your recognition of the significance of our study.

Specific comments:
1. In page 8 “Study process”, the authors described the hepatitis B virus serological markers will be assessed every 3 months. It may be more informative to specify which serological markers will be explored.
Response from authors:
Thanks for your kind suggestion, and we specify the serological markers of
HBV as HBsAg, HBeAg, HBeAb and HBV DNA in Study process, page 7.
2. Similarly, the liver biopsy will be performed before randomization and at 96 and 144 weeks. In addition to the degree of fibrosis, the authors may clarify any other index or virological profiles will be evaluated in the precious histological samples.
Response from authors:
Once the biopsy performs, the expression of viral markers (HBsAg, HBeAg, HBcAg) of hepatic tissue, the level of intrahepatic covalently closed circular DNA (cccDNA) and histological scores for staging (fibrosis) and grading (inflammation) will be evaluated.
3. The type and duration of previous NA should be specified.
Response from authors:
Data of the type and duration of previous NA will be collected in screening (medical and medication history, in Study process, page7). No matter the type (nucleotide or nucleoside) but the duration should be longer than 1 year according to inclusion criteria (Inclusion criteria, page5).
4. IFN-experience patients and patients with contraindications to IFN should be excluded.
Response from authors:
Thanks for your kind suggestion, and the exclusion criteria of IFN-experienced patients and patients with contraindications to IFN is added in Exclusion criteria part, page 5.

5. Since Peg-IFN will be out of market and TDF will be replaced by TAF, the clinical value of this trial is a concern.
Response from authors:
Thank you for your concern, however, we hold the other opinions. Studies have confirmed that peg-IFN is an indispensable therapeutic drug for functional cure at present. Therefore, peg-IFN will be in demand before curative drugs for HBV are available for a relatively long time in our opinions. Compared with TAF, TDF has more data on efficacy and safety, so we think it is a better option for a clinical trial to illuminate other factors influencing the HBsAg seroclearance. And taking pharmacoeconomics into account, TDF is the most cost-effective treatment for Chinese CHB patients to achieve satisfactory and optimal goals. (Cost-effectiveness analysis of first-line treatment for chronic hepatitis B in China. Clinical Microbiology and Infection. https://doi.org/10.1016/j.cmi.2021.06.024)
6. Minor point: Page 8 Line 36, “progenomic RNA” should read “pregenomic RNA”.
Response from authors:
We are very sorry for our incorrect spelling about “pregenomic” which we have corrected already.

Reviewer: 2
Dr. Sunil Kothakota, KIMS Hospital, Trivandrum, Kerala, India
Comments to the Author:
This study has been designed very well and addressing much needed concern in hepatology i.e., therapeutic cure of HBV. It would be much more beneficial if authors can address following issues:
1. Include the HBsAg quantitative analysis at the start and end of analysis.
Response from authors:
We are very appreciated for your valuable advice. HBsAg quantitative will be measured in screening and every 3 months, and we will analyze it at the start, 48weeks, 96weeks, and the end of study for that the reduction of HBsAg quantitative is an important efficacy index.
2. Make it clear whether both HBeAg positive and negative or only HBeAg negative subjects are recruiting for the study.
Response from authors:
I am sorry that this part was not clear in the original manuscript. Our study will enroll both HBeAg-positive and HBeAg-negative patients and we clarified that in Inclusion criterion in page4 in marked copy.
We noticed that there is a difference in HBsAg-seroclearance rates between these populations. However, we aim to figure out that the clearance of HBsAg under combination therapy among HBV-related liver fibrosis patients compared with TDF monotherapy, thus, the status of HBeAg is not the main concern. Nevertheless, we do take it into consideration. The randomization should make HBeAg-status balanced, and when necessary, we will conduct a stratified analysis for the status of HBeAg.

Reviewer: 3
Dr. Anders Boyd, Hôpital Saint-Antoine
Comments to the Author:
In this protocol paper, the authors present a randomized clinical trial of treatment-experienced individuals with chronic HBV infection who will be randomized 1:1 to receive either pegylated interferon with TDF or only TDF. RCTs with the same objectives as in this study have been done multiple times, hence the novelty of this study should be more convincingly highlighted. I have several other recommendations that I hope could help direct the authors to a more concise research question.

Major issues:
1. The authors emphasize that this is a study designed for individuals with liver disease/liver fibrosis (i.e. this is assumedly the target population), yet they recruit specifically individuals with F1-F3

fibrosis. F1 is not much or a concern. F2 is where one would consider treatment more seriously. F3 is definitely indication for treatment. F4 was excluded (for toxicity reasons? It was never explained). The heterogeneity of fibrosis levels questions which populations are being referred to.
Response from authors:
We are very appreciated your question and we have thought about it for a long time. We would like to explain to you as below.
1) The enrollment of this study is chronic HBV infected patients who have had definite antiviral treatment indications and have received NA for more than 1 year. If HBsAg is still positive, treatment should be continued no matter which stage of fibrosis.
2) The efficacy and safety of the combination therapy of interferon and NA in NA-experienced CHB patients have been confirmed. We want to know whether consistent results can be obtained in patients with liver fibrosis. At the same time, does combination therapy have a better outcome than NA monotherapy in reversing liver fibrosis. In fact, we hope that the combination therapy can further prevent the progression of liver fibrosis to cirrhosis. So, we enroll the patients with liver fibrosis.
3) F4 may cover compensated and decompensated cirrhosis, and IFN is not recommended to the latter population. And yes, the AEs are our concern among cirrhosis patients. We intend to carry out similar clinical trial in compensated cirrhosis population in the future and the frequency of follow up will be adjusted higher.
2. Previous studies differentiate between HBeAg-positive and HBeAg-negative status because there is a massive difference in HBsAg-seroclearance rates between these populations. Naturally, the randomization should make HBeAg-status balanced, but once again, there is a question of which population this treatment is intended to be used for.
Response from authors:
Our study will enroll both HBeAg-positive and HBeAg-negative patients. We aim to figure out that the clearance of HBsAg under combination therapy among HBV-related liver fibrosis patients compared with TDF monotherapy, thus, the status of HBeAg is not the main concern. However, we do take it into consideration. The randomization should make HBeAg-status balanced as you mentioned, and when necessary, we will conduct a stratified analysis for the status of HBeAg.
3. There is no control arm with peg-interferon alone. This is imperative because if TDF has no effect when combined with peg-interferon, then there is no need to use it in combination. This has been a very important limitation of the previous RCTs, which could be resolved with this study.
Response from authors:
We are very appreciated to your suggestion. It troubled us for a while in the
early phase of study designation. After comprehensive consideration, we design this two-arm trial. For NA-experienced population, cessation of NA may lead to virological relapse, even hepatitis flare. On the other hand, IFN promote the adaptive immune activities. If we get promising results in the future, we can further design a protocol to explore the way of combination, such as sequential or add-on, under frequently follow-up. And with no doubt, more closely follow-up is in need.

Minor comments:
1. Endpoint: "…serological clearance/conversion rate of HBsAg." Which one is it? Since seroconversion requires seroclearance, it would be simpler to leave as "seroclearance."
Response from authors:
It is really true as Reviewer suggested that serological clearance/conversion may lead to ambiguity. So we have made correction using "seroclearance" without "conversion" according to the Reviewer's comments.
2. ref 11. Does this ref include treated patients?
Response from authors:
We are very sorry for our negligence of the citation of literature, and we have canceled it in our revision.
3. combination therapy of peg-IFN-alpha … in a specific population of chronic hepatitis B patients…" which ones?

Response from authors:
In fact, we mean chronic hepatitis B patients. And we made a correction in revision.
4. “Long-term cirrhosis, liver cancer, and other important indicators…” would the authors have enough power to conclude anything about this study?
Response from authors:
Thanks for the reminder from Reviewer. we cannot draw a conclusion about the incidence of cirrhosis and liver cancer due to the limitation of sample size and follow-up duration. However, we will pay attention to the occurrence of them in different group.
5. Please state clearly which antiviral therapy recommendations and functional cure definitions are being used, rather than just cite them.
Response from authors:
We state the reason of cited recommendation of recruitment in Recruitment part, page 4.
And functional cure definition is stated clearly in line9 and 10, page3.
6. Sample size. Why is only a one-sided test being used for a superiority trial?
Response from authors:
According to results of previous studies and clinical experiences, combination therapy is no worse than NA monotherapy in terms of HBsAg clearance. So we selected one-sided test to estimate the sample size.
7. Primary efficacy endpoint is a cumulative proportion, not a rate, I assume. The statistical test for the sample size calculation was not given (nor was it found in the statistical analysis section), so it is difficult to determine.
Response from authors:
Primary efficacy endpoint is a rate at 48 weeks as mentioned in Efficacy and safety evaluation part, page6. And we use different rates of 2 therapy to calculate the sample size as mentioned in Sample size part, page 5.

8. Secondary efficacy endpoint on fibrosis: so the authors plan on taking liver biopsies? FibroScans? Or both? The criteria for evaluation need to be given, along with how the biopsies will be read to ensure high reliability.
Response from authors:
We are sorry that this part was not clear in the original manuscript. So we re-write this part according to the Reviewer’s suggestion in Efficacy and safety part from ”Each participant will undergo…” to “…reach a consensus”, page 6 in marked copy.
9. Statistical analysis. Are the authors attempting to differentiate PP and ITT analyses sets? The ITT analysis set needs to be more thoroughly described.
Response from authors:
We are sorry that this part was not clear in the original manuscript too. So, we re-write this part in Statistical analysis part, page 8 in marked copy.
10. The manuscript needs to be read by a native speaker with medical knowledge. There is a lot of inappropriate or unclear phrasing: “is a hot issue”, “who progress to fibrosis already”, “treatment plan”, “liver fibrosis takes place after liver damage”, etc.
Response from authors:
We apologize for the poor language of our manuscript. We have now worked on both language and readability and have also involved native English speakers for language corrections. We really hope that the flow and language level have been substantially improved.