Author’s response to reviews

Title: Sustained serological and complete responses in HBeAg-Positive Patients treated with Peginterferon alfa-2b: a 6-Year Long-Term Follow-Up of a Multicenter, Randomized, Controlled Trial in China

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Pros: 
- There are only limited number of studies on long-term peginterferon in HBV infection

Cons: 
- Statements should be corrected, to reflect statistical results: "The six year cumulative response rate was significantly higher in the 48-week treatment group as compared to the two other treatment groups"

- Since almost half of patients also received additional antivirals during followup, the the six-year cumulative response rates cannot be fully attributed to the respective treatment group

Author response: We have addressed the topic of additional antivirals in our study subjects at follow-up in our response to the “major comment” below.

Editor comment: The number of patients with sustained virological response/complete response should be given for patients WITHOUT additional antivirals during follow up. Please provide the exact numbers for patients with/without additional NUC treatment in followup in the flow chart.
Author response: Many thanks for reminding the study team of this important point. To better capture information on additional antivirals for HBV in the follow-up study, we assessed all possible medical records of subjects, including insurance documents and medical history since the end of our previous RCT. Use of additional antivirals was confirmed if any regular treatment of antivirals was prescribed and administered against HBV CHB, regardless of the subject’s disease status. The exact numbers for patients with/without additional antivirals were added into the follow-up section in the flow chart.

Editor comment: Rates of HBe seroconversion should be shown for the timepoint of end of long-term follow-up.

Author response: We agree and the rates of HBe seroconversion have been added into Table 1 accordingly.

Editor comment: Please confirm whether you have obtained an approval of your local ethics committee for your long-term follow study, and if so, did all patients give written and informed consent for both the original (48 weeks) as well as the 6 year follow up study?

Author response: We have included both approval of the ethics committees and obtaining signed informed consent from all patients undergoing a follow-up visit as well as in the original RCT. In China, all clinical trials and follow-up procedures are ICH GCP-compliant. Local ethics approvals are accessible upon the journal’s request. Please see the revised content describing ethics approval and the patients’ informed consent in the ‘Study Design and Patients’ paragraph in the Methods section.

Editor comment: The authors should not duplicate/include previously published data (including the tables and the figure) that were included in the publication of the previous study: Cheng J, Wang Y, Hou J, Luo D, Xie Q, Ning Q, et al. Peginterferon alfa-2b in the treatment of Chinese patients with HBeAg-positive chronic hepatitis B: a randomized trial. J Clin Virol. 2014;
Author response: We apologize for causing any confusion. We aimed to display response rates at different time points from the original study through long-term follow-up visits among all 322 subjects who were included in the long-term follow-up. We accomplished this by re-counting and analyzing numbers of clinical responses for the 322 patients based on laboratory measurements from the original study (baseline) through the LTFU. Therefore, no results were duplicated from the original study. To make this clearer to readers, we have modified the statistical analysis paragraph and the corresponding table. Please see the revised Table 1 Title and footnote to explain the data results.

Editor comment: Complications of decompensation/during followup: i.e. hepatocellular carcinoma, ascites, variceal bleeding, encephalopathy, jaundice, or liver transplant), and administration of (other) antiviral therapy after the end of the initial study.

Please provide the numbers of the clinical events that happened - so the reader can learn about the complication rate after PEG - IGN treatment after a mean follow up of up to six years.

Author response: In the single long-term follow-up visit, the investigators assessed each patient’s clinical complications. However, as confirmed repeatedly among the patients, only 1 patient had liver complications at the long-term follow-up visit. The data were added into the new Table 3 (Clinical Responses in the Long-term Follow-up Study Grouped by Additional NA Treatment). The possible explanation for the lack of complications may be that patients were younger (<30 years of age at the time of P05170 study treatment) and relatively healthier in the original study. Also, patients who had liver complications may not have been able to make the follow-up visit. These points were added into the Limitations paragraph of the Discussion section to explain possible study limitations and bias.

Editor comment: Provide information on the patients who were HBe Ag negative and had ALT normalization, specifically for patients with versus without additional NA therapy at LTFU.
Author response: We have provided more information on patients who were HBeAg negative and had ALT normalization. We created a new table (Table 3) to display rates of patients who were HBeAg negative combined with ALT normalization vs. those without additional antiviral therapy at LTFU. This table shows the laboratory viral profiles and biochemical remission in patients who were sub-grouped according to additional antiviral therapy. In the follow-up study, all patients who had used documented regular antivirals since the end of the original RCT were considered ‘failed’ for endpoint analysis. Results are described in the revised ‘Results’ section.

Editor comment: Was there any HBs Ag decline in the patients treated with NA’s

Author response: Yes. HBsAg decline in NA-treated patients. In the new Table 3, any decrease in HBsAg as a categorical variable was analyzed and presented. The proportion of patients with HBsAg decline was higher in the patient subgroup treated with additional NAs at LTFU. Results are described in the revised ‘Results’ section.

Editor comment: Discuss potential predictors of response sustainability

Author response: We have added information on potential predictors of sustainable response as advised. Please see the modified Discussion section for predictors of response sustainability.

Reviewer #1 (Mayer) comments

Reviewer comment: The "long-term follow up" should be explained in more detail. You are mixing the methods of the original study ("Peginterferon alfa-2b in the treatment of Chinese patients with HBeAg-positive chronic hepatitis B: a randomized trial") with the methods of your follow up study. The authors need to clarify and outline which of the study investigations were solely carried out for the “long-term follow up” and those which belong to the original study.
Author response: As advised, we have restructured the relevant section (‘Clinical and Laboratory measurements’) to separate procedures in the long-term follow-up from those in the original study.

Reviewer comment: Did you obtain an approval of your local ethics committee for your long-term follow study, and if so, did all patients give written and informed consent for both the original (48 weeks) as well as the 6 year follow up study? Please clarify and state in the method section whether you obtained the above mentioned agreements.

Author response: In China, all clinical trials and follow-up procedures are ICH GCP-compliant. All patients provided signed informed consent for both the original RCT and the long-term follow-up study. Local ethics approvals are available upon the journal’s request. The relevant section (‘Study Design and Patients’) has been revised to better describe the ethics committees’ approval and the patients’ informed consent.


Author response: We apologize for causing confusion by presenting information from the two studies, but it was necessary to show baseline and follow-up results. We aimed to display response rates at different time points from the original study through long-term follow-up visits among all 322 subjects who were recalled for the long-term follow-up. This analysis was simple but was accomplished by re-counting and analyzing numbers of clinical responses for these 322 patients. Therefore, no results were duplicated from the original study. To make this clear for readers, the statistical analysis paragraph in the Methods section has been revised. We also have modified Table 1 Title and footnote to better explain the data results.

Reviewer comment: This belongs to the method section: 3.3; Line 1-10 : "We used univariate and multivariate Cox regression analysis to analyze factors associated with SR and CR (Table 3). Factors with p<0.05 in univariate analysis were included in the multivariate analysis" AND 3.1, Line 6.: and were requested to participate in long-term follow-up by returning for an onsite visit.
Author response: We have revised the text of the ‘Statistical Analysis’ paragraph in the Methods section of the report accordingly.

Reviewer comment: Your endpoint is the sustained combined response and the sustained serological response after 6 years. You do not provide any data regarding the median follow-up (I highly doubt that all patients were investigated EXACTLY after 6 years).

Author response: True, not all patients had an exact 6-year long-term follow-up. Some patients had the follow-up visit closer to the end of the original RCT, while others had a later follow-up visit. This was due to different timing of ethics approval at the different sites, site initiation and individual patient’s response to telephone calls made to arrange for follow-up. Median follow-up duration was displayed in Table 1 but we have modified the footnotes to make the follow-up process clearer.

Reviewer comment: Table 2. What kind of statistical test did you use for the determination of the p-value?

Author response: We used the log-rank test for Kaplan-Meier survival analysis to compare cumulative rates between groups. We also have modified the footnotes for the table and statistical paragraph in the Methods section to indicate the method used.

Reviewer comment: Table 3. This table doesn’t make any sense. What are you trying to present here? Do you want to show which variables you included in your stepwise model? If so, simply show which variables you included in the final model. Or do you want to show the difference between the PEG-IFN dosing schemes after 6 years?

Author response: We appreciate the advice and admit that the table is unnecessarily long. As advised, we have revised Table 3 to simply show the most clinically relevant variables that were included in the models.
Reviewer #2 (Maieron) comments

Reviewer comment: I read with interest the report of Jian Sun et al. I believe the paper can give some important insight in our current understanding of who will profit from Peg IFN treatment on the long run and therefore this paper represents an important work. However, there are some major criticism: I believe there is a great bias on the cohort as stated by the authors - up to one third refused to participate in the study - this might be due to fact that they obtained medical treatment elsewhere.

Author response: We appreciate the reviewer’s kind comment. We also agree that the follow-up study has a significant limitation in terms of subjects recalled for long-term follow-up. We have collaborated with external clinical research organisations to endeavour to use all possible procedures including telephone calls and personal visits to recall as many as possible of the subjects included in the original RCT. Some patients simply refused to participate where others could not be contacted due to lack of updated information. We agree that results should be interpreted with caution, as bias in such follow-up studies is inevitable. Limitations paragraph in the Discussion section has been revised to reflect these points.

Reviewer comment: Page 11 lines 25 - 26: For long-term follow-up (LTFU), all subjects were contacted by telephone and their continued eligibility was assessed onsite at their local participating centers. The subject was included in the LTFU group if he or she was treated in the original study and gave informed consent, and had no significant medical conditions precluding the study inclusion. The authors must state the exactly criteria for inclusion and exclusion for this study. E.g. how they dealt with patients who where on NA treatment - I guess they have been excluded for LTFU as stated on page 21 line 9 - 15. Please provide the exact numbers on how many patients entered the LTFU analyses in the flow chart.

Author response: We aimed to include as many as possible of the patients from the original RCT. We have revised the inclusion/exclusion criteria because no exclusion criterion had been set for those who were treated by additional antivirals since the end of the original RCT. The exact numbers of patients included in long-term follow-up study analysis are shown versus those without additional antivirals, who were added into the flowchart. We have modified the subsection ‘Study Design and Patients’ to reflect the exact numbers as advised.
Reviewer comment: Page 11 line 39 - 47: The investigator assessed clinical signs and symptoms, and complications of liver disease (i.e. hepatocellular carcinoma, ascites, variceal bleeding, encephalopathy, jaundice, or liver transplant), and administration of (other) antiviral therapy after the end of the initial study.

Please provide the numbers of the clinical events that happened - so the reader can learn about the complication rate after PEG - IGN treatment after a mean follow up of up to six years

Author response: In each long-term follow-up visit, the investigators assessed the patient’s clinical complications. However, as confirmed repeatedly, the majority had no complications and only 1 patient had liver complications at the long-term follow-up visits. The possible explanation for this may be that patients were younger and relatively healthier in the original study. Also, patients who had liver complications may not have been able to make the follow-up visit. We have added these points into the Discussion section accordingly. Results for liver events are displayed in the new Table 3. Results are summarized in the subsection ‘Clinical responses at LTFU grouped by additional NAs.’

Reviewer comment: At the end of the long-term follow-up the percentage of patients across treatment groups with ALT normalization and who were negative for HBe were 40.7% and 41.3%, respectively, and 44.4% of patients were receiving HBV antiviral therapy

Please provide information on the patients who were HBe Ag negative and had ALT normalisation: how many did receive NA therapy at LTFU and even more important how many did not - where there any cases who developed HBe Ag negativity or even seroconversion that did not receive NA treatment or had a SR or CR at EOS. Was there any HBs Ag decline in the patients treated with NA´s
Author response: A new table (Table 3. Clinical Responses in the Long-term Follow-up Study Grouped by Additional NA Treatment) was created to display rates of patients who were HBeAg negative combined with ALT normalization vs. those without additional antiviral therapy. Also, HBsAg decline was analyzed and results shown in this table, which also was used to show laboratory viral profiles and biochemical remission in patients sub-grouped according to additional antiviral therapy or not. In the follow-up study, all patients who used documented regular antivirals since the end of the original RCT were considered ‘failed’ for endpoint analysis. Results are summarized in the subsection ‘Clinical responses at LTFU grouped by additional NAs.’

Reviewer comment: The authors should include analyse of those receiving NA treatment vs those who don't at LTFU at least giving us the numbers and indicating the rate of liver related events.

Author response: As we described in our response above, we created a new table (Table 3) to display the rates of patients who were HBeAg negative combined with ALT normalization vs. those without additional antiviral therapy. We also analyzed HBsAg decline and results are also shown in new Table 3. Only 1 subject had a liver complication event in the follow-up study. This finding is shown in the new Table 3.

Reviewer comment: How many of the pat. who experienced relapse either in the SR or the CR group would qualify for treatment

Author response: Long-term follow-up included only one follow-up visit. We judged patients who relapsed and/or used additional antivirals as failed response to long-term sustainability. We have added results for the proportions of patients who had relapse based on laboratory tests into Table 2. Results are described in the subsection ‘Sustained combined response and sustained serological response.’

Reviewer comment: Please indicate how many of those not getting NA treatment should have gotten one according to current e.g. APASL guidelines at LTFU
Author response: Relevant data of those patients not receiving NA treatment are indicated in Table 2 and results are summarized in the subsection ‘Sustained combined response and sustained serological response’.

Reviewer comment: Can the authors mention early on treatment predictors of sustainability; please include a analysis. So, we could learn whom to get through a PEG IFN treatment

Author response: We used univariate and multivariate Cox regression analyses to explore the associations between subjects’ clinical characteristics and sustainability. However, we did not find many predictors of sustainable response other than genotype and Peginterferon alfa-2b dosing scheme. We have revised the Discussion section to reflect these findings.

Reviewer #3 (Gschwantler) comments

Reviewer comment: At present only a limited number of studies assessing the long-term outcome of treatment of chronic hepatitis B with peginterferon have been published and their results have been conflicting. Therefore the authors have to be congratulated for presenting there date from a large Chinese cohort. In general, the paper is well written.

As presented in Table 2, the rates of sustainability of serological response and combined response were not significantly different between the three treatment regimens, although the rates were numerically higher in the 48-week treatment group. Therefore, the final sentence of the paper, stating “Patients treated with 1,5 mikrogram/kg/wk for 48 weeks had significantly higher rates of sustained SR and CR” is not correct or could be misunderstood. It would be correct to state that “The six-year cumulative response rate was significantly higher in the 48-week treatment group as compared to the two other treatment groups”.

Author response: As advised, we have revised our statement about rates of SR and CR accordingly throughout the manuscript wherever applicable.
Reviewer comment: It is difficult to interpret the six-year cumulative response rates. For example, the six-year cumulative combined response rate was 70\% in the 48-week treatment group (see table 2). However, 47.7\% of patients in this group had received additional antiviral treatment during long-term follow-up (see Table 1). Therefore the 70\% six-year cumulative combined response rate is not only an effect of the original peginterferon-therapy but also of the additional antiviral treatment during long-term follow-up. I would like to know, how many patients, who had NOT achieved serological or combined response at the end of the original study, had achieved these endpoints WITHOUT further antiviral therapy at the end of long-term follow-up. (It has been reported that response rates increase even after six months after end of interferon treatment).

Author response: We have calculated the rates and they are displayed in Table 2. We also have summarized the results in the subsection ‘Sustained combined response and sustained serological response’.

Reviewer comment: In Table 1 the rates of HBe seroconversion are given for the time points EOT and EOS, but not for the time point end of long-term follow-up.

Author response: We have added the rates of HBe seroconversion into Table 1 as advised.