Dear editor,

We thank you for your encouragement and advice, and we would like to submit our revised manuscript “Elevated serum IL-38 level at baseline predicts virological response in telbivudine-treated patients with chronic hepatitis B ”（ESPS Manuscript NO: 23933）for your further consideration as a basic study for publication in World Journal of Gastroenterology. The major changes in the revision have been marked in red. Please see our point-by-point responses below.

If I can be of any assistance regarding the process of this manuscript, please feel free to contact me. I look forward to hearing from you soon.

Sincerely,

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Responses to the Editor,

Thank you for your review, suggestions and advice. We have clarified several issues following the guidelines for manuscript. In addition, we have carefully checked every sentence in the revision to eliminate potential syntax errors.

Please provide language certificate letter by professional English language editing companies (Classification of manuscript language quality evaluation is B).

Response: Thank you. This manuscript has been edited and proofread by two native English biologists from *Medjaden*, a professional publication service company.

Audio core tip:

In order to attract readers to read your full-text article, we request that the author make an audio file describing your final core tip, it is necessary for final acceptance. Please refer to Instruction to authors on our website or attached Format for detailed information.

Response: Thank you. We have made an audio file describing our final core tip (attached).

Peer-review:

Response: Thank you. We include the major comments provided by the peer-reviewers, which describe the characteristics, values and significance of the article. (P 15, line 421-427)

1. You need to provide the grant application form(s) or certificate of funding agency for every grant, or we will delete the "**Supported by...**".

Response: Thank you. We have deleted the "**Supported by...**".

2. Abbreviations and acronyms are often defined the first time they are used

within the main text and then used throughout the remainder of the manuscript. Please consider adhering to this convention.

Search all abbreviations in your manuscript and do like this when they were used firstly.

Response: Thank you. We have searched all abbreviations in the manuscript to make sure that abbreviations and acronyms were defined the first time they were used within the main text and then used throughout the remainder of the manuscript.

3. Please check that there are no repeated references!

Please add PubMed citation numbers and DOI citation to the reference list and **list all authors.** Please revise throughout. The author should provide the first page of the paper without PMID and DOI.

PMID (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=PubMed) DOI (http://www.crossref.org/SimpleTextQuery/) (Please begin with DOI: 10.**)

Response: Thank you. We checked that there are no repeated references and we updated all the references according to the Format for references guidelines. (Page 16-19, line 428-544)

4-6 List all abbreviations used in the table and explain them below please.

Response: Thank you. All abbreviations are listed and annotated below all tables. (Page 21-24)

Reviewed by 00030389,

The authors investigated serum IL-38 level and its clinical role in predicting virological response to telbivudine (LdT) in patients with chronic hepatitis B. They found that higher serum IL-38 levels before treatment indicate a greater probability of VR to LdT treatment and that elevated serum IL-38 levels in untreated patients with CHB reflect ongoing liver injury. This is an interesting paper.

Major comment 1. There are papers indicating that cytokines inhibit HBV replication. Their discussion should refer those papers such as below. Galun E, Nahor O, Eid A, Jurim O, Rose-John S, Blum HE, Nussbaum O, Ilan E, Daudi N, Shouval D, Reisner Y, Dagan S: Human interleukin-6 facilitates hepatitis b virus infection in vitro and in vivo. Virology 2000;270:299-309. Bouezzedine F, Fardel O, Gripon P: Interleukin 6 inhibits hbv entry through ntcp down regulation. Virology 2015;481:34-42. Kuo TM, Hu CP, Chen YL, Hong MH, Jeng KS, Liang CC, Chen ML, Chang C: Hbv replication is significantly reduced by il-6. J Biomed Sci 2009;16:41-49. Zhao XM, Gao YF, Zhou Q, Pan FM, Li X: Relationship between interleukin-6 polymorphism and susceptibility to chronic hepatitis b virus infection. World J Gastroenterol 2013;19:6888-6893.

Response: We appreciate your constructive comments. In response to your concerns, we have revised our discussion and included the suggested references (Page 13, line 359-372).

Minor comments 1. Page 9, line 9. What is PR?

Response: Thank you. PR is the abbreviation of primary response. We have added the definition of PR when it was first introduced in the main text. (Page 9, line 252)

Minor comments 2. Table 3. The serum IL-38 level which determined low or high should be indicated.

Response: Thank you. We now include the cutoff values for low and high level of IL-38 below the table. (Page 23)

Reviewed by 02943023,

This manuscript demonstrated that higher pretreatment serum IL-38 levels are associated with a greater probability of VR to LdT treatment. Authors

suggested that elevated serum IL-38 levels reflect ongoing liver injury, which is an indicator of endogenous clearance of HBV infection. These findings are interesting because of it might provide a novel predictors for good response to antiviral therapy. However, there is still need to be revised in several points.

Comments. 1. As authors suggested, if serum IL-38 levels could predict a vigorous endogenous HBV clearance and better response to LdT therapy, it would be better to show treatment outcomes at week 52 including the markers for serological response (HBsAg levels or reduction, HBeAg less or seroconversion), biochemical response (serum ALT normalization) as well as virological response (HBV DNA reduction or undetectablity).

Response: We appreciate your constructive comments. We have revised the section of Results and included treatment outcomes of the two group at week 24 and week 52, including HBsAg loss, HBeAg loss, ALT normalization as well as virological response in Table 4. However, HBeAg elimination, ALT normalization and HBsAg loss did not show difference between HG and LG (Table 4). No significant difference in serum HBsAg levels was noted between HG and LG during the LdT therapy ($P > 0.05$ at week 12, 24, 36, and 52, Fig. 2B). Elevated serum IL-38 level at baseline could predict better virological response. (Page 11, line 302-307)

Comments. 2. What is the reference for dividing serum IL-38 level into high and low by cut-off level 250 pg/ml?

Response: Thank you. We generated Receiver operating characteristic curves to assess effectiveness of using baseline serum IL-38 levels to predict VR at 24 and 52 weeks. The optimal cut-off value at 24 weeks was 250.54 pg/ml, which generated 81.3% sensitivity and 53.3% specificity; the optimal cut-off value at 52 weeks was also 250.54 pg/ml, resulting in 72.0% sensitivity and a 57.1%specificity. Using cut-off level 250 pg/ml, all patients were divided into

low and high IL-38 groups (Page 10, line 282-283).

Comments. 3. Analyze the factors including serum IL-38 levels for virological response at week 52 by multivariate logistic regression model.

Response: Thank you. We analyzed serum ALT, HBsAg, HBV DNA and IL-38 levels at baseline as well as age with multivariate logistic regression model to determine which ones can predict VR. However, none of them were associated with VR at week 52 of LdT treatment, as shown in the table below. (Page 11, line 300-302)

Table. Logistic regression model of probability of virological response at week 52 of LdT treatment.

Variable	OR(95%Cl)	P value
Age	0.939(0.873-1.010)	0.092
Basement IL-38	1.002(0.999-1.005)	0.217
HBV DNA	1.152(0.513-2.588)	0.732
HBsAg	0.472(0.115-1.939)	0.298
ALT	1.000(0.994-1.005)	0.869

HBV, hepatitis B virus; ALT, alanine transferase; HBsAg, hepatitis B surface antigen; Normal values: ALT ≤ 50 IU/L; HBV DNA ≤ 1.78 log10 IU/mL (60 IU/mL); HBV DNA in log IU/mL; HBsAg in log IU/mL.

Comments. 4. Because of higher genetic barrier and lower antiviral resistance, the first recommend oral NAs for CHB treatment are entecavir or tenofovir. But, in this study, patients were treated with telbivudine. Please comments about the background for this point.

Response: Thank you. Several oral antiviral agents have been approved for treatment of chronic hepatitis B, and both entecavir and tenofovir are recommended as first-line therapy by major international guidelines [European Association For The Study Of The Liver. EASL clinical practice

guidelines: Management of chronic hepatitis B virus infection. J Hepatol. 2012 Jul;57(1):167-85]. However, tenofovir has yet to be approved in China, and the majority of CHB patients may not be able to access to first-line therapy due to economic issues. The GLOBE trial had established the superiority of telbivudine over lamivudine in CHB patients [Lai CL, Gane E, Liaw YF, Hsu CW, Thongsawat S, Wang Y, et al. Telbivudine versus lamivudine in patients with chronic hepatitis B. N Engl J Med 2007;357:2576–2588]. Comparing efficacy, frequency of antiviral resistance and cost between telbivudine and lamivudine, telbivudine was selected for treating CHB patients.

Reviewed by 02943023,

Many of our clinical experiences suggest that replication of HBV is affected by host immune response. Nucleotide analogues are not so potent to eradicate HBV; therefore the potency of HBV is also affected by host immunity. It has been reported that several cytokines have some effect on HBV replication. In this paper authors describe newly discovered IL-38 has crucial effect on anti-viral treatment. Relationship between IL-38 and HBV replication is vague in this report. Authors described here the relationship AMONG IL-38, IL-6 and IL-12. Please clarify the role of IL-38 in cytokine cascade and host immunity that is related to HBV replication.

Response: We appreciate your constructive comments. We analyzed the relationship between IL-38 and HBV DNA loads prior and during the treatment. Unfortunately there was no positive correlation. (Page 10, line 276-278) Our data showed positive correlations between IL-38 and IL-6 or IL-12, suggesting the functional association between cytokine IL-38 and TH1/TH2 type cytokines IL-6 and IL-12. Further studies are needed to address underlying association between these cytokines and the involvement of IL-38 in host's response to HBV replication.

Previous studies indicated that cytokines were involved in the pathogenesis of CHB. For example, it has been reported that IL-37, an

anti-inflammatory cytokine, was the part of the immune response in patients with CHB who showed HBeAg seroconversion, and IL-37 levels were associated with liver injury in CHB patients. [Li C, Ji H, Cai Y, Ayana DA, Lv P, Liu M, Jiang Y. Serum interleukin-37 concentrations and HBeAg seroconversion in chronic HBV patients during telbivudine treatment. J Interferon Cytokine Res. 2013 Oct;33(10):612-8]. A high pre-treatment serum IL-23 level may predict the therapeutic response in HBeAg-positive CHB patients during PegIFN therapy.[Yu C, Gong X, Yang Q, Lian J, Xu K, Ruan B, Li L. The serum IL-23 level predicted the response to pegylated interferon therapy in patients with chronic hepatitis B. Liver Int. 2015 May;35(5):1549-56]. And IL-32 was associated with HBV-related liver inflammation/fibrosis. [Xu Q, Pan X, Shu X, Cao H, Li X, Zhang K, Lu J, Zou Y, Li X, Liu H, Zhang Y, Yang D, Ning Q, Shen G, Li G. Increased interleukin-32 expression in chronic hepatitis B virus-infected liver. J Infect. 2012 Oct;65(4):336-42].

To our knowledge, this is a first study of kinetic changes in serum IL-38 levels in CHB. Our study demonstrated that higher pretreatment serum IL-38 levels were associated with a greater probability of VR to LdT treatment. Elevated levels of serum IL-38 in untreated patients with CHB reflected ongoing liver injury, which is an indirect indicator of vigorous endogenous clearance of HBV infection. A favorable response may be observed if the LdT antiviral effect is combined with a strong endogenous viral clearance. These findings might provide a novel predictor for favorable response to antiviral therapy.