

## Response letter

May 12, 2020

Professor Lian-Sheng Ma,

Editor-in-Chief, World Journal of Gastroenterology

Manuscript ref number 56190

Dear Prof. Lian-Sheng Ma,

On behalf of all authors, I would like to express my deep gratitude to you and the reviewers for the constructive comments and suggestions on how to improve our manuscript, entitled “Investigation of immune escape-associated mutations of hepatitis B virus (HBV) in patients harboring HBV drug-resistance mutations.” Accordingly, we have revised the manuscript and responded to your questions and comments in a point-by-point manner as follows.

### Answer to reviewer 1

1. The role of the representative immune escape-associated mutation, sA159V, in the pathological process of chronic hepatitis B infection is suggested to be further clarified. For instance, the clinical outcome of the drug-resistant CHB patients with colocalization of sA159V should be compared with that of patients without the immune escape-associated mutation.

**Answer:** In this study, In Table 3, we compared clinical features of 270 sA159V-positive and 19,170 sA159V-netative patients without seeing significant

difference between the two groups of patients in HBV DNA, ALT, AST, TBIL, and CHE levels. Nevertheless, we really found that sA159V mutation might increase the fitness of LAM/ETV-resistance mutants by decreasing the HBsAg level and increasing the viral replication capacity, thereby influencing clinical outcome of some LAM/ETV-treated patients. We considered that the sA159V was a factor favoring the development of LAM/ETV-resistance mutation, rather than a factor deteriorating clinical outcome after the development of the drug-resistance.

2. With the wide use of the new generation of potent oral NAs such as TAF, the incidence of drug resistance is decreasing rapidly, and its significance should be downgraded accordingly. The authors should mention it.

**Answer:** According to your suggestion, we have supplemented the relevant description and a reference on TAF (No. 8 in References) in the revised manuscript; and references after it were renumbered accordingly.

3. The authors are suggested to provide evidence to show the HBsAg quantitative assay (Roche Elecsys HBsAg) is not subject to the HBsAg mutations. Furthermore, in Table 3, "Analysis of clinical features in sA159V-positive and sA159V-negative patients", the unit of the HBsAg results is "COI". Actually, for the Roche Elecsys semi-quantitative HBsAg assay, results >5000 COI are very likely to reach the plateau, which means the results are beyond the upper semi-quantitative limit. They are not semi-quantitative results any longer. This might affect the statistical result between the two patient groups.

**Answer:** Thank you for you providing expertise in your comments. The COI values obtained for HBsAg Roche detection assay could only roughly reflected HBsAg level and would be less informative for evaluating of samples with higher HBsAg. However, this assay was frequently applied in clinical practice for its higher cost-effectiveness. Limited by retrospective study and availability to get samples for supplementary data of HBsAg quantification for most enrolled patients, we have to use COI values for estimation of HBsAg levels in overall as many other investigators did in their publications. According to your comments, we have removed the word “semi-quantitative” from the description of regular electrochemical luminescent immunoassay.

4. In Fig 2, the sequences from the patient A's samples are marked as "A1", "A2" and "A3". These taxon names are misleading because HBV Genotype A can be subtyped as A1, A2, and A3. Please rename them.

**Answer:** According to the suggestion, we have used A-S1, A-S2, and A-S3 to replace A1, A2, and A3 respectively in revised Figure 2. This modification way is also used for in revised Figure 1 and related statement of the other representative patients.

5. In the Results section, the sentence "HBsAg levels determined by an anti-HBs assay" is not correct; "M2–M5 were partly resistant to ETV sensitivity with 54.9%–64.7% inhibition. " Here the "sensitivity" should be deleted.

**Answer:** Thanks for your careful review. “HBsAg levels determined by an anti-HBs assay” should be “Quantitative HBsAg levels”; and “M2–M5 were partly resistant to

ETV sensitivity with 54.9%–64.7% should be “M2–M5 were partly resistant to ETV, with 54.9%–64.7% inhibition”. We have made the corrections in the revised manuscript.

6. Ref 21, the guideline on prevention and treatment of chronic hepatitis B in China (2005) should be replaced with the version of 2019.

**Answer:** This was a retrospective study and we have to record patients diagnosis based on patient records in clinical practice. Considering that samples studied were collected from 2007 to December 2017, we rephrased the sentence as “The diagnostic criteria were based on the guideline on prevention and treatment of chronic hepatitis B in China (2005), and the updated guidelines were used according to the time of patient enrollment”.

#### **Answer to reviewer 2**

1. It is a good study that made a correlation among immune-escaped and drug-resistant mutations of HBV. You mentioned co.....without sA159V colocalization. Did you mean co-existence? If so, please correct throughout the manuscript.

**Answer:** According to your suggestion, we have replaced “colocalization” with “co-existence” throughout the revised manuscript. “Colocalization” was used for stressing on the co-existence of multiple mutations in the same viral genome, while this word was not commonly used.

#### **Answer to Science Editor**

1.Issues raised: (1) I found the authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s); (2) I found the authors did not provide the original figures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor; and (3) I found the authors did not write the “article highlight” section. Please write the “article highlights” section at the end of the main text.

**Answer:** We have submitted the approved grant application form(s) and the original figures using PowerPoint. The manuscript has contains core tip. In light of the indication for special requirements for article highlights, we independently supplement a file for the highlights with consistent content of core tip. We hope that this manipulation is right.

**Answer to Company Editor-in-Chief:**

However, the quality of the English language of the manuscript does not meet the requirements of the journal. Before final acceptance, the author(s) must provide the English Language Certificate issued by a professional English language editing company. Please visit the following website for the professional English language editing companies we recommend: <https://www.wjgnet.com/bpg/gerinfo/240>.

**Answer:** According to your instruction, we asked an English expert to carefully polish language again, and the certificate will be submitted. As a result, a number of changes in text are there after the language modification in the revised manuscript.

All changes in the text and Tables of the revised manuscript have been highlighted. The revised manuscript contains 5,265 words including abstract (280 words), main body of text (2,508 words), references, tables, and figure legends., 3 tables and 5 figures. We hope that this manuscript meets your requirements.

Thanks again for your time and consideration.

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