# ANSWERING REVIEWERS

January 25, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 6697-review.doc).

**Title:** Molecular diagnosis and treatment of drug-resistant hepatitis B virus.

**Author:** Jeong Han Kim, Yong Kwang Park, Eun-Sook Park, Kyun-Hwan Kim

**Name of Journal:** *World Journal of Gastroenterology*

**ESPS Manuscript NO: 6697**

The manuscript has been improved according to the suggestions of reviewers:

1 Revision has been made according to the suggestions of the reviewer

(1) Reviewed by 02444774

This is a very well-written review article. Just some minor comments which may be useful to further enrich the article. 1. What is the current evidence of de novo combinations of NAs to prevent development of drug resistance mutants? 2. What is the view of the authors on the future of this area, molecular diagnosis and treatment of drug-resistant hepatitis B virus, in the presence of potent NAs (e.g. ETV, TDF, or even TAF which will be coming up soon) with very low risk of drug resistance? 3. The most recent estimate of HBV infection was around 250 millions (Vaccine; 2012;30:2212-2219) 4. The tables and figures should be placed at the end of the manuscript. 5. Please state the reference if the figures are adapted from other papers or textbooks.

: High priority for publication

***Answer:*** 1. In case of HBV, the de novo combination of NAs is not recommended to prevent the development of drug resistant HBV since there was no known beneficial to prevent the resistant mutations. 2. We newly added a sentence in Conclusion. 3. The estimate of HBV infection was updated to 250 million in Introduction with a new reference. 4. The tables and figures are placed at the end of the manuscript as recommended. 5. All figures in this manuscript are newly made, not adapted, for this review.

(2) Reviewed by 01136482

The present paper in well written and the references are updated. Only minor points are requested for Author revision: 1. The Author can present all the resistance, for any drug divided by paragraphs (i.e. L-nucleosides; acyclic phosphonates; d-cyclopentane group) and after the multidrug resistance 2. About the treatment to better understand the data and the conclusion reported in this review, analyzed the response to the treatment by a paragraph on the response and partial response to antiviral therapies.

: Minor revision

***Answer:*** The detailed resistance mutations corresponding to every drug are well reviewed in other papers which are already cited in this review. Due to the size and scope of this review, we introduced the representative resistance mutations and response to antiviral therapies. However, according to reviewer's comment, we supplemented "5-year cumulative resistance rate" and our "comments" in Table 1 and 2, respectively.

(3) Reviewed by 02860577

It is a very good manuscript but it somehow lacks the input of the authors as expertise in this field (handling this issue critically) which could appear in them postulating a certain treatment guideline for patients that have resistant HBV...etc

: High priority for publication

***Answer:*** Most of the reviewer's comment were reflected and revised in manuscript. Revisions were made according to the reviewer's comments in Introduction. A graph in Fig. 2 was drawn only for this review, not adapted. In addition, we added additional columns; "5-year cumulative resistance rate" and our "comments" in Table 1 and 2, respectively. Recommendations for patients were also newly added in Conclusion section.

(4) Reviewed by 02860585

In the present article, Kim et al. aimed to discuss the currently available molecular diagnosis tools, in vitro phenotypic assays for validation of drug-resistant HBV, and treatment options for drug-resistant HBV. Drug-resistant HBV is an emergency concern and, therefore, it is clinically relevant. The paper is easy-reading and is well-written. Minor comments: -In the Table 1, it would be desirable to add a column with resistances at 5 years of treatment-experienced and percentage of seroconversion. -It is mentioned that tenofovir has no resistances 3 years after starting the therapy. In the last congresses, it has been reported this fact at 5 years. -There is an interesting point of view related to this topic, which is the fact of higher risk of developing hepatocellular carcinoma in subjects with drug-resistant. Please, add this part in the manuscript with new references. (i.e. World J Gastroenterol. 2013 Dec 7; 19(45): 8373-81). -Recently, it has been published a very interesting prospective study related to this topic (regarding to mutations in HBV DNA polymerase in treatment-na?ve patients) that should be mentioned (Clin Gastroenterol Hepatol. 2013 Dec 13. pii: S1542-3565(13)01892-2.)

: Minor revision

***Answer:*** According to reviewer's comments, we added additional column "5-year cumulative resistance rate" in Table 1. A recent study demonstrating the higher risk of developing hepatocellular carcinoma in subjects with drug-resistant was introduced in Introduction with citation (World J Gastroenterol. 2013 Dec 7; 19(45): 8373-81). Also, a study regarding to mutations in treatment-naïve patients was also mentioned in Introduction with citation (Clin Gastroenterol Hepatol. 2013 Dec 13. pii: S1542-3565(13)01892-2.).

(5) Reviewed by 01221188

Kim JH described the details of nucleotide/nucleoside analogue drugs for hepatitis B virus. The information such as diagnosis and treatment was very important and helpful for clinicians.

: Accept

(6) Reviewed by 02860814

The paper is dealing with the molecular methods that are available for the detection of resistant HBV-strains and with the treatment options of drug-resistant HBV. It is a comprehensive and up to date work and needs a few revisions. One point to be mentioned is that nowadays lamivudine monotherapy is not recommended as a first-line treatment due to induction of high drug resistance, especially under long-term administration, with resistance developing in 71% of the patients after 4 years. Lamivudine has no place in the era of new antiviral drugs and all the CHB patients should be treated with the newer and potent antiviral drugs, TDF and ETV. The other three NAs may only be used in the treatment of CHB if more potent drugs with high barrier to resistance are not available or appropriate. This is in line with the guidelines of all associations for the study of liver diseases (EASL, AASLD, APASL and KASL). Another one point that must be analyzed further is that large trials with patients receiving tenofovir for long periods showed actually no emergence of resistance mutations [Marcellin NEJM 2008 and Snow-Lampart Hepatology 2011]. Resistance to TDF has been proven so far in in-vitro studies. Regarding the methods described herein (PCR-based direct or cloning sequencing, Ultra-deep pyrosequencing, RFMP), the authors must clear that these methods are not a part of everyday clinical practice and up to now are used mainly in the research field. They are expensive, labor-intensive and demand an experienced laboratory.

: Minor revision

***Answer:*** According to the reviewer's comments, we newly added the following sentence in TREATMENT part; "LMV is not recommended as a first-line treatment due to high resistance development. Currently, the newer and potent drugs, TDF and ETV are recommended[13, 16-18]." The references describing TDF resistance were newly added in "ADV and TDF resistance" part with citation (ref 93 and 94). "Resistance to TDF has been proven so far in in-vitro studies" was also added.

(7) Reviewed by 02861401

The paper is well written and clinically relevant. There are only minor points to be conveyed to the authors: 1) It will be clearer to specify the drug-resistance is for oral antiviral agents in the title. Resistance is rare for IFN-based therapy. 2) It will be better to explain in the introduction section what the first-line and second-line antiviral agents for HBV are. According to AASLD, tenofovir or entecavir are recommended for the first-line oral antiviral medications. Therefore, more attention and discussion should be paid to these two agents. 3) The discussion of drug resistance is mainly drug orient. It would be nice if the authors can add a section and a table to do the mutation orient discussion regarding drug resistance, diagnosis and treatment. 4) Immunosuppressive therapy on HBV resistance and treatment might be relevant and worth discussion. Resistance in the HIV-HBV co-infected patients might also be worth discussion.

: Accept

***Answer:*** 1) The drug-resistant HBV is generally recognized as HBV resistant to oral antiviral agents, NAs. 2) We newly mentioned the first-line antiviral agents for HBV in Introduction. The current guidelines recommending tenofovir (TDF) and entecavir (ETV) as first-line antiviral agents were also added with citations [13, 16-18] in Introduction.

(8) Reviewed by 02860874

I congratulate the authors. In my opinion this is an example of a good review article. It provides to the reader a complete and clear information about a pathology that nowadays constitutes a important clinical challenge for clinicians treating CHB. I think the authors were able to synthesize key aspects

that clinicians must know about potential resistance to antiviral agents when we treat CHB.
: Accept

(9) Reviewed by 02860895

This excellent review article written by Kim et al. thoroughly covers recent topics concerning therapeutic resistance of HBV, including its molecular mechanisms and diagnostic tools. It is a concise and well-organized paper, in which I cannot point out any weak-points.
: Accept

(10) Reviewed by 02441729

I think that this review article is well-written and will be valuable for the readers.
: Accept

2 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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