

Dear Science Editor and reviewer

Thank you very much for your letter enclosing the reviewer' comments on our manuscript entitled "Antiviral effects of hepatitis B virus S gene-specific anti-gene locked nucleic acid in transgenic mice" (38083). According to the comments of reviewers, we have revised relevant parts in the original manuscript. The amendments have been highlighted in red and commented in the revised manuscript. Point by point responses to the reviewers' comments are listed below. We hope that the revised version of the manuscript is now acceptable for publication in World Journal of Clinical Cases. I am looking forward to hearing from you soon.

Best wishes,

Yours sincerely,

Shu-Rong Xiao, Gui-Dan Xu, Wu-Jun Wei, Bin Peng, Yi-Bin Deng

**Replies to Reviewer's code: 02447091**

1. The legend of Table 1, 2 and Figure 2. ... transgenic mice (n=6, mean±SD) is ... transgenic mice (n=6, mean±SE) according to the description in Statistical analysis.
2. There are four control groups in this experiment. However, the definition of control groups is obscure. Anti-S-LNA group is a kind of positive control. The author should describe the definition of each group more precisely.
3. Immunohistochemistry shown in Figure 3 is not specific. It seems to be so hard or even impossible to quantify the positive signals corresponding to HBsAg in this picture. Please explain in details how the authors quantified the HBsAg-positive cells.

**Response to the comment:**

1. Dear reviewer, we have revised according to the description in Statistical analysis.
2. According to the opinion of the review, four control groups have revised including negative control (blank control, unrelated sequence control), positive control (lamivudine, anti-sense-LNA).

3. Quantified the HBsAg-positive cells: use the image analysis software to select the yellow regions of the immunohistochemical reactant on the picture, and then measure the average light density or the integral light density of these regions.

**Replies to Reviewer's code: 00052926**

However, it would be useful for the authors to describe first the advantages of this novel approach versus the classical anti-sense oligonucleotide approach. Second the limitations of the study should be mentioned. Third it would be useful to consider the possible constraints in order to apply this method in humans.

**Response to the comment:**

Dear reviewer, thank you for your positive comment. We have described the advantages, limitations and useful to humans of this novel approach versus the classical anti-sense oligonucleotide approach in the paper.

**Replies to Reviewer's code: 00503536**

1. The characteristics of HBV transgenic mice, such as transgene and genetic background, are unclear. Moreover, histology of the liver show positive for cytoplasmic HBsAg in all hepatocytes, which is quite different from other HBV transgenic mice (Guidotti LG, Matzke B, Schaller H, Chisari FV. J Virol. 1995 Oct;69(10):6158-69). The authors should describe on those points. 2. Serum HBsAg and HBV DNA were assessed only 7 days after treatment, and it is unclear how long the inhibitory effect of LNA last. 3. The kinetics of HBV DNA in addition to that of HBsAg gene should be shown. Minor point 1. The effect of combination therapy with lamivudine and LNA would be interesting. 2. Immunohistochemical data on HBcAg in the nuclei of hepatocytes with each treatment would be interesting.

**Response to the comment:**

1. HBV transgenic mice were purchased from the Guangzhou Military Air Force Hospital of the People's Liberation Army of China. All mice were

positive for serum HBsAg and HBV DNA. The exogenous gene was directly injected into the fertilized egg by the micromanipulation instrument, and the exogenous gene was integrated into the DNA and developed into a transgenic animal.

2. Dear reviewer, thank you for your positive comment. In this study, the antiviral effect of transgenic mice was observed for only 7 days, and the inhibition rate was not studied for a long time. This is also a deficiency of this experiment. In further research, we will adopt the recommendations of reviewer.

Thank you for your kind suggestion!.