UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Wits/SAMRC Antiviral Gene Therapy Research Unit
School of Pathology, Faculty of Health Sciences
University of the Witwatersrand
Private Bag 3, WITS, Johannesburg, 2050, South Africa
https://www.wits.ac.za/agtru

AGTRU

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March 18, 2021

Dr Lian-Sheng Ma

Editor-in-Chief

World Journal of Gastroenterology

Dear Dr Lian-Sheng Ma

Manuscript Submission- Manuscript NO.: 63470 - Silencing HBV cccDNA - the potential of an epigenetic therapy approach

We would like to thank you, the reviewers, and editorial staff for the positive feedback regarding the submission of our review entitled 'Silencing HBV cccDNA - the potential of an epigenetic therapy approach' (ID: 03761988, Manuscript NO. 63470) to the *World Journal of Gastroenterology*. Please see below a point-by-point responses based on the peer review report. We hope that your find the amendments made to the manuscript acceptable.

This review is not under consideration by another journal and has not been published elsewhere. All authors have approved the manuscript for submission. Declaration of potential conflicts of interest: none.

Thank you for considering our revised manuscript for publication.

Yours sincerely

Kristie Bloom.

Point-by-point response:

Reviewer #1:

Scientific Quality: Grade B (Very good)

Language Quality: Grade A (Priority publishing)

Conclusion: Accept (General priority)

Specific Comments to Authors: This paper is very interesting and well compiled. Few minor comments are indicated below: 1. In introduction, the authors could include some recently published works of CRISPR/Cas9 effect on cccDNA. 2. HBx and Needylation interactions towards HBx stability can be explained a bit details, or can be referred to published paper. 3. Role of MLN4924 on needylation and HBV replication can be added too.

Response:

Thank you for your positive feedback. Minor comments have been addressed as follows:

1. We have supplemented the CRISPR/Cas9 (lines 76 to 96) section in the introduction to include recent data demonstrating viral vector-mediated delivery of CRISPR/Cas systems to disrupt cccDNA. The following text was added: '*Viral vectors, including adeno-associated viruses (AAVs) and high-capacity adenoviral vectors (HCAdV) have recently been explored as hepatotropic delivery vehicles [13, 21, 22]. To overcome the packaging limitations of AAVs, the smaller Staphylococcus aureus (Sa) endonucleases have been combined with HBV specific guide RNAs to demonstrate cccDNA-targeting and antiviral efficacy in hNTCP-HepG2 cells [22] and transgenic mice [21]. On the other hand the large packaging capacity of HCAdVs have been exploited to accommodate multiple HBV guide RNAs along with the larger Streptococcus pyogenes Cas9 [13], which may improve the efficacy of this gene editing approach.*'
2. The stability of HBx conferred through NEDDylation is now described in lines 433-435: '*NEDDylation-dependent reduction of ubiquitination and subsequent degradation of HBx by E3 ligases, such as Siah-1, may account for the increased stability of this viral protein.*'

3. The role of MLN4924 on NEDDylation and HBV replication is now described in lines 435-442: '*MLN4924 (pevonedistat), a NEDD8-activating enzyme inhibitor, has been shown to impede HBV replication by reducing cullin [147] and HBx NEDDylation [146]. In addition, MLN4924 promotes the upregulation of phosphorylated extracellular signal-regulated kinases (ERKs) resulting in reduced HNF1α, HNF4α and C/EBPα transcription factor levels [147], which are known activators of HBV transcription. Overall, the maintenance of persistent HBV infection by HBx contributes to HCC, by inducing host epigenetic modifications implicated in cancer*.'

EDITORIAL OFFICE'S COMMENTS

Issues raised:

(1) The "Author Contributions" section is missing. Please provide the author contributions;
Response: The author contributions have now been added to the manuscript.

(2) 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);
Response: We could not locate the grant application forms, however as funding received is non-statutory, we have moved this section to the acknowledgements.

(3) The authors did not provide original pictures. 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 (10) Please obtain permission for the use of picture(s). If an author of a submission is re-using a figure or figures published elsewhere, or that is copyrighted, the author must provide documentation that the previous publisher or copyright holder has given permission for the figure to be re-published; and correctly indicating the reference source and copyrights.
Response: The figures in this manuscript are original and have not been published elsewhere. Figures were generated using BioRender, a relatively new online software illustration tool that allows you to create your own figures (similar to CorelDraw) but also has pre-designed icons to simplify the process. Our laboratory has an academic licence that allows us to create and publish figures using the software. To avoid confusion, we have adopted the 'citing' of the software at the end of the figure legend, which is the method currently preferred by Nature. Please let

us know if you would prefer a different method of citation for the software. In addition, as the figures were not generated using PowerPoint, they can not be decompensated. We have therefore added high resolution versions of the images in PowerPoint.