

Dear Reviewers,

Thank you for taking the time to review our manuscript and provide such thoughtful comments and suggestions. We are going to address your comments in a step-by-step manner.

### **Reviewer 1 Comment 1.1**

“Turshudzhyan and Tadros wrote a letter to the editor entitled Cautious optimism in anticipation of hepatitis B curative therapies The title reflects the main subject. The style, language, and grammar are accurate and appropriate. The abstract summarizes and reflects the work described. Finally, the manuscript appropriately cites the latest references. However, a few concerns should be raised. Abstract: - What is meant by healthy patients? - Hepatitis B population is not the opposite of what was termed healthy patients, if appropriate. Keywords: - Many keywords were cited; please summarize the most important Page 4 and page 5: Leowattana et al.----- add the reference " Leowattana et al. [1]"”

### **Response 1.1**

Thank you for your thoughtful suggestions and feedback. We understand the concern and ambiguity of the “healthy patients”, so we wanted to elaborate on that by stating “patients with low viral load and without hepatotoxic injury” instead. We changed it in both core tip as well as the abstract.

Thank you for bringing up the citation of the report by Leowattana et al. The report is cited in the very first paragraph of the letter to the editor.

While there are a lot of keywords, every one of them indicates either a therapy discussed or its abbreviation, which is why we feel strongly that they should be included. No one therapy is more important over others, which makes it challenging to keep some and remove others. Please let us know if you disagree and we will be happy to address your concerns further.

### **Reviewer 2 Comment 2.1**

“I think the author’s viewpoint was acceptable.”

### **Response 2.1**

Thank you for your feedback.

### **Reviewer 3 Comment 3.1**

“Dear authors! Thank you for your thoughtful comments on the paper by Leowattana W et al published recently in World J Virol. The truth is born of argument, and thus any discussion can contribute to a better understanding of the problem. I have only a few minor comments: 1) Page 2, line 25: international consensus on the goals of HBV treatment defines several types of "cure", and "functional cure" is already possible. Please, add "sterilizing" to be consistent with the international definitions. 2) Page 3, line 15: It seems that Blank et al studied plasma bile acids concentration, not "bile production". Please, check and correct appropriately.”

### **Response 3.1**

Thank you so much for your thoughtful comments. We added “**sterilizing**” before cure to help underline that we are discussing a complete loss of HBsAg. We also changed “bile production” to “**bile acid concentration**”, thank you for bringing this to our attention.

### **Reviewer 4 Comment 4.1**

“1. Since Bueviride is effective in patients with HBV/HDV co-infection, why is it not effective in patients with HBV? 2. When taking Bueviride, the bile production of HBV patients increased and the viral load rebounded. The therapeutic effect of Bueviride on patients with HBV/HDV co-infection only inhibits HDV but has no effect on HBV? 3. More literature is needed to search to understand the molecular mechanism of these new treatments and the possibility of curing hepatitis B.”

### **Response 4.1**

Thank you so much for your thoughtful questions. Having patient who have a co-infection with HDV introduces an opportunity for a confounding effect. As a result, it is challenging to determine if the therapy would be successful in HBV infection alone. We hope that further studies that focus on HBV population alone will help us better understand this.

We hope we were able to satisfactory address your comments and that the manuscript can now be considered for publication in the World Journal of Virology.

Kind regards,

Alla Turshudzhyan, D.O.

**Name of Journal:** World Journal of Virology

**Manuscript Type:** LETTER TO THE EDITOR

Cautious optimism in anticipation of hepatitis B curative therapies

Turshudzhyan *et al.* Cautious optimism for HBV cure

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**Abstract:** Despite relative effectiveness of current hepatitis B therapies, there is still no curative agents available. The new emerging approaches hold promise to achieve cure and loss of HBsAg. Studies or clinical trials investigating new therapies remain small

and either focus on **patients with low viral load and without hepatotoxic injury** or patients with hepatitis D co-infection, which makes it challenging to assess their effectiveness and side effect profile in hepatitis B population.

**Key words:** hepatitis B; HBV; HBV entry inhibitor; bulevirtide; transcription activator-like effector nucleases; TALENs; zinc-finger nucleases; ZFNs; and clustered regularly interspaced short palindromic repeats-associated 9; CRISPR/Cas9; Nucleocapsid assembly modulators; HBV transcription inhibitors; HBsAg release inhibitors

**Core tip:** Hepatitis B could become a curable disease in the near future. As our understanding of pathophysiology of hepatitis B infection advances, more therapeutic targets are becoming available. Many new therapies have only been investigated in small groups of **patients with low viral load and without hepatotoxic injury** or in patients with hepatitis D co-infection, which makes it difficult to predict efficacy and side effect profile when applied to the population of interest. Larger clinical trials in hepatitis B patients are needed to further investigate the emerging new therapies, so that more patients can safely benefit from them.

TO THE EDITOR:

We read with great pleasure the article by Leowattana et al. [1] about new emerging therapies in treatment of chronic hepatitis B. They presented a comprehensive review of currently available therapies, pathophysiology of the hepatitis B infection, and developing new therapies. While current therapies, such as nucleosides (NAs), are effective in suppressing viral replication and preventing progression of chronic hepatitis to cirrhosis or hepatocellular carcinoma, they are unable to achieve cure from hepatitis B infection. As a result, new therapies are now being investigated that are aimed at a complete cure and loss of HBsAg. Leowattana et al. presented a comprehensive discussion of developing new therapies, which include agents that

inhibit entry of HBV into hepatocytes, interfere with cccDNA or HBV transcription, alternate nucleocapsid assembly, and prevent HBsAg release from the hepatocytes. The authors are hopeful that given currently available evidence on these emerging therapies, chronic hepatitis B could become a curable disease in the near future. While we share their sentiment and are hopeful for these therapies to be successful in curing hepatitis B infection, we would like to recommend cautious optimism when assessing these new therapeutic agents.

HBV entry inhibitor, bulevirtide, was originally intended to be used for hepatitis D treatment. Wedemyer et al. [2] presented results of a phase 2b trial in 2019 which included 60 patients with chronic HBV/HDV co-infection. While their results were encouraging, the population under investigation was small and all of the patients had both viruses present, which makes it more difficult to apply these results to patients with HBV infection alone. Wedemyer et al. documented increased **bile acid concentration** in patients on bulevirtide and rebound in viral load after therapy discontinuation, which may cause more liver damage. The increase in **bile acid concentration** while on bulevirtide was also investigated by the Blank et al. [3] They confirmed increased **bile acid concentration** associated with bulevirtide without cholestasis, however, their study was limited to 12 healthy volunteers and did not include patients with pre-existing chronic liver disease or with hepatitis B infection, which makes it less applicable to the population of interest. While there are no ongoing clinical trials with hepatitis B patients on bulevirtide, there is phase 3 trial on bulevirtide use in HDV infection which includes 150 adults with HDV infection [4]. It will help reveal long term effects of therapy and help us better understand the adverse events associated with it. The downside of this phase 3 trial is that it is limited to HDV patients. There is still no long-term data on side effect profile of bulevirtide in HBV patients exclusively. We hope there will be new trials to investigate its application in HBV patients.

Gene editing tools such as the transcription activator-like effector nucleases (TALENs), zinc-finger nucleases (ZFNs), and clustered regularly interspaced short palindromic repeats-associated 9 (CRISPR/Cas9) could be a new exciting therapy option in curing chronic hepatitis B. The authors did a comprehensive review of the available options for gene editing. It is important to note, however, that like with any genetic intervention there is a risk of off-target cleavage [5], so more studies and large clinical trials are needed to investigate this therapeutic option.

Nucleocapsid assembly modulators are another exciting modality reported by Leowattana et al. but it is another therapy that should be treated with caution until more data from larger clinical trials is available. Zhang et al. reported that 75% of patients in their study evaluating nucleocapsid assembly modulators experienced elevations in aminotransferases with 4 out of 24 patients requiring to stop therapy and receive glutathione [6].

HBV transcription inhibitors is another emerging therapy that is currently being investigated. There were two clinical trials evaluating HBV transcription inhibitors in phase II [7,8] and one clinical trial in phase I [9] that were discontinued because of the observed lethal toxicity of the EX1 delivery formulation. More studies are needed to investigate the safety profile of this therapy before it can be considered for clinical application. Another practical consideration with any emerging therapy that requires a viral vector to be delivered into the cells is the risk of pre-existing immunity to vectors or development of host immunity to vectors during treatment, which will ultimately render therapy ineffective [10].

Lastly, HBsAg release inhibitors have been under investigation in various clinical trials. Alanine aminotransferase (ALT) flares were observed in 90% of patients treated with HBsAg release inhibitors [11,12]. Additionally, because most of the data came from patients with low viral load, safety and efficacy in patients with high viral load is still to

be determined. Similar to bulivertide, there were reports that discontinuation of HBsAg release inhibitors caused viral rebound precipitating liver decompensation in patient with significant chronic liver disease [13].

We commend Leowattana et al. for their comprehensive review of the emerging new therapies that have the potential to cure chronic hepatitis B. Our goal was to merely add caution to the optimism and hopefully prompt larger clinical trial specific to hepatitis B population, so that more patients can safely benefit from the new therapies in the near future.

#### ACKNOWLEDGEMENTS

No acknowledgements to be made.

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#### **Footnotes**

**Conflict-of-interest statement:** we received no funding.