Dear Editor-in-Chief,

RE: Manuscript number 86024

On behalf of the authors, I am submitting our revised manuscript by Mak et al entitled "Prevention and management of hepatitis B virus reactivation in patients with hematological malignancies in the targeted therapy era". Changes to the manuscript are shown in red font. Please find below an explanation of how the individual comments have been addressed.

Reviewer #1:

Specific Comments to Authors: No original findings of this manuscript

> ***Response:*** The purpose of the review was to summarize current evidence on risk of HBV reactivation on novel therapies in treating haematological malignancies. Our own recommendations for antiviral prophylaxis in patients receiving specific anticancer therapies are discussed.

Reviewer #2:

Specific Comments to Authors: General comment The topic of the review is highly relevant and timely. The merit of the review is that it covers the entire spectrum of drugs or therapies against hematologic malignancies. Overall, the text is well organized. The tables are informative, but the layout is partly suboptimal. A weakness is the virtual absent description of the HBV replication and the effect of anti-HBV immune reactions on the course of the infection. A small paragraph on the life cycle with its persistent nuclear cccDNA and the role of immune pathogenesis in acute and chronic hepatitis B. The sensitivity and specificity of the HBV screening tests is not even touched. A further

weakness is the inaccurate distinction between HBV infection and HBV induced disease. The nomenclature should be clearer and more consistent.

Response: We have added a paragraph on the life cycle of HBV with its persistent nuclear cccDNA and the role of immune pathogenesis in acute and chronic hepatitis B on page 5 (Ref: Chang Y, Jeong SW, Jang JY. Hepatitis B Virus Reactivation Associated With Therapeutic Interventions. Front Med (Lausanne). 2022 Jan 14;8:770124. doi: 10.3389/fmed.2021.770124. PMID: 35096867; PMCID: PMC8795508.)

A paragraph on the sensitivity and specificity of the HBV screening tests is added under the section of "Screening for hepatitis B" on page 18.

Specific points 1. Title. Spell out HBV in the title

Response: HBV has been replaced by Hepatitis B virus in the title

2. The abstract and core tip should more clearly and explicitly mention that inactive and even "resolved" HBV infection leads virtually always to persistence of HBV genomes in the liver. The expression of those silent genomes is controlled by the immune system. Suppression or ablation of immune cells, most importantly B cells may lead to reactivation of seemingly resolved HBV infection.

Response: Thank you very much for your comment. We have added this piece of important information in the abstract.

3. Introduction, 1st sentence. "Patients with chronic or resolved hepatitis B virus (HBV) infection are at risk of reactivation of the virus if they receive chemotherapy or immunotherapy. " The nomenclature should be more precise.

a. Not all chemotherapies are immunosuppressive. b. Immunotherapy could be used to actively suppress HBV or cancer cells.

Response: The wording has been changed to "chemotherapy or immunosuppressive therapy"

4. Virologic factors. "The identified virologic risk factors for include CHB, high baseline HBV DNA levels and hepatitis B e-antigen (HBeAg) positivity". Again, the wording is unfortunate: a. Risk factor for what? Reactivation or CHB? b. An HBV infection with high HBV DNA and with HBeAg is already very active and cannot be reactivated anymore. However, reactivation of the inactive immune response will lead to an acute flare or even liver failure.

Response: Thank you for your comment. Here, we mean the virologic risk factors for reactivation of hepatitis B virus. We have clarified this in the first sentence of this section.

We totally agree with you that a patient with positive HBsAg with high HBV DNA and positive HBeAg is already having very active infection. However, these patients do not always meet the criteria for HBV reactivation, and therefore these parameters have been identified as predictive factors in clinical studies. We have revised the text as follows to clarify: "The identified virologic risk factors for HBV reactivation include the presence of intrahepatic cccDNA and detectable HBV DNA levels[16-18]. Signs of increased viral replication, such as HBsAg or HBeAg positivity and detectable baseline HBV DNA, before treatment are predictive of the patient meeting the criteria for HBV reactivation during treatment with cytotoxic chemotherapy or autologous stem cell transplantation[19-22]."

5. The work of Salpini et al (ref. 21) is incorrectly described. There, it is indeed mentioned that 5 mutations were in T cell epitopes but most of the mutations were in the B cell epitopes of the HBs antigenic loop. This is in line with the frequent reactivation under B cell suppressive therapy.

Response: The discussion of this research on page 7 has been amended accordingly. The following sentence has been added: "The majority of these mutations resided in the B-cell epitopes of the HBs antigenic loop. Some of the mutations are known to hamper HBsAg recognition by humoral response, which may explain the frequent reactivation of HBV in patients receiving immunosuppressive therapy targeting B cells."

6. "Obinutuzumab is a second-generation anti-CD20 monoclonal antibody. It is a humanized, glycol-engineered type 2 antibody". There is an embarrassing mistake: It has nothing to do with glycol. It has non-fucosylated sugars on the Fc portion.

Response: The sentence on page 10 has been changed to: "It has an engineered fragment crystallizable (Fc) portion and a modified elbow hinge region."

7. HBV screening. The text should cover rare cases as well. Fig. 1 is indeed helpful. a. HBsAg may be false negative due to diagnostic escape mutations. b. Anti-HBc may be false negative after a previous B cell depleting therapy. (Gärtner et al. Permanent loss of anti-HBc after reactivation of hepatitis B virus infection in an anti-HBs and anti-HBc-positive patient after allogeneic stem cell transplantation. J Clin Virol. 2007 PMID: 17182277. c. Isolated anti-HBs without anti-HBc may be present in pretreated patients without previous HB vaccination. d. For all three cases a sensitive test for HBV DNA is advisable.

Response: A discussion of these points was added to the manuscript on page 17. It now reads: "This starts with screening for the presence of HBsAg and anti-HBc in blood. The commercial immunoassays usually capture HbsAg having specificity for epitopes present on the antigenic α determinant. The enzyme-linked immunosorbent assay (ELISA) method used in HBsAg detection has around 80% of sensitivity and specificity, compared with more than 90% using the immunochromatographic test[130]. Complete loss of anti-HBc with chronic and high viremic HBV infection after allogeneic stem cell transplantation has been reported[131]. However, there might be some rare scenarios where HBsAg or anti-HBc might be falsely negative. For example, mutations within or outside of the α determinant may affect conformational epitope recognition or HbsAg secretion or expression, resulting in diagnostic escape [132]. Moreover, there has been a case report reporting complete loss of anti-HBc after allogeneic stem cell transplantation in a patient with resolved HBV infection who previously had positive anti-HBs and anti-HBc prior to the stem cell transplant[131]. Isolated anti-HBs without anti-HBc may be present in pretreated patients without previous hepatitis B vaccination[133, 134].

Thus, a more sensitive combined screening strategy is advisable, including serological testing with HBsAg, anti-HBc, anti-HBs and a sensitive HBV DNA."

8. HBsAg-positive patients without hepatitis at baseline. The problem here is that the immunosuppressive therapy may initially increase the viral load without ALT rise. However, if immune reconstitution occurs, a fulminant hepatitis may result which cannot be treated with NAT anymore. The same can occur with HBsAg negative patients.

Response: Content has been added to page 6. It reads: "In patients receiving immunosuppressive therapy, the loss of immune control may result in viral replication inside the hepatocytes without any increase in ALT levels. Nevertheless, upon immune reconstitution, sometimes during immunosuppressant tapering or withdrawal, the immune system will target the hepatocytes aiming to clear the virus, resulting in liver damage[10]."

With regard to the HBsAg-negative patients, we have added the following discussion on page 20:

"It is rare to have HBV reactivation in patients with isolated anti-HBs, but HBV reactivation has been reported in patients who were seropositive for anti-HBs alone[133, 134].

A patient with follicular lymphoma without record of hepatitis B vaccination was positive for anti-HBs but negative for anti-HBc before chemotherapy. He later developed high viremia (HBV-DNA of 1.8×10^8 copies/mL) with an HBV escape mutant, which was difficult to detect by HBsAg assays[133]. The escape mutants of HBV carry mutations in the major antigenic region of HBsAg and they are able to grow in the presence of anti-HBs. Anti-HBc may appear very late.

Another patient with diffuse large B-cell lymphoma without a record of hepatitis B vaccination was negative for HBsAg and anti-HBc, and positive for anti-HBs (127 IU/mL) before chemotherapy. She had HBV reactivation after completion of rituximab-based chemotherapy. Antiviral treatment with entecavir was started after HBV reactivation was detected. Despite that, she had clinical deterioration with development of hepatic

encephalopathy and died of liver failure finally[134].”

9. CHOICE OF ANTIVIRAL THERAPY. It should be mentioned that previous lamivudine therapy often leads to selection of mutants which may become rapidly resistant to entecavir. Geipel A et al. Entecavir allows an unexpectedly high residual replication of HBV mutants resistant to lamivudine. Antivir Ther. 2015;20(8):779-87. PMID: 25560463.

Response: This reference has been added as reference 147.

10. Tables 1-5 report the number of deaths caused by reactivation as zero throughout. However, many historical case reports exist with a lethal course of HBV reactivation under immunosuppressive therapy, e.g.: Foont JA, Schiff ER. Avoid the tragedy of hepatitis B reactivation in immunosuppressed patients. Nat Clin Pract Gastroenterol Hepatol. 2007 PMID: 17262070.

Response: Discussion of this issue was added in the manuscript on pages 21-22. It reads: “Foont and Schiff [154] performed a systematic review on the use of lamivudine for the prophylaxis of HBV reactivation in patients on chemotherapy. In the 10 trials with 173 patients included in the analysis, two patients taking lamivudine prophylaxis developed fatal HBV reactivation[154].”

We have also cited a case report by Ifuku and colleagues as reference 153 (Ifuku H, Kusumoto S, Tanaka Y, Totani H, Ishida T, Okada M, Murakami S, Mizokami M, Ueda R, Iida S. Fatal reactivation of hepatitis B virus infection in a patient with adult T-cell leukemia-lymphoma receiving the anti-CC chemokine receptor 4 antibody mogamulizumab. Hepatol Res. 2015 Dec;45(13):1363-7. doi: 10.1111/hepr.12513. Epub 2015 Apr 15. PMID: 25753008.)

We hope that, with these revisions, the article is now suitable for publication in your journal. I look forward to hearing from you soon.

Yours sincerely,

Dr Man Fai Law