Cover Letter

Dear Editors and Reviewers,

Authors first appreciate the great effort and comments from the editors and reviewers. The manuscript (Manuscript NO.: 74724, Minireviews) was made minor revisions according to each comment from the reviewers. The following steps outlined below were completed at re-submission.

Please check the responses to each point. All the revisions were tracked in the revised manuscript and were also pointed in the reply to the reviewers' comments in this letter.

Thank you so much.

Please let us know if you have any concerns.

Sincerely,

Ming Yang, PhD

University of Missouri-Columbia

1 MANUSCRIPT REVISION DEADLINE

We request that you submit your revision in no more than **14 days. Please note that you have only two chances for revising the manuscript**.

Response: The revised manuscript was submitted on time.

2 PLEASE SELECT TO REVISE THIS MANUSCRIPT OR NOT

Please login to the F6Publishing system at https://www.f6publishing.com by entering your registered E-mail and password. After clicking on the "Author Login" button, please click on "Manuscripts Needing Revision" under the "Revisions" heading to find your manuscript that needs revision. Clicking on the "Handle" button allows you to choose to revise this manuscript or not. If you choose not to revise your manuscript, please click on the "Decline" button, and the manuscript will be WITHDRAWN.

Response: Author accepted to revise the manuscript according to all the reviewers' comments.

3 SCIENTIFIC QUALITY

Please resolve all issues in the manuscript based on the peer review report and make a point-by-point response to each of the issues raised in the peer review report. Note, authors must resolve all issues in the manuscript that are raised in the peer-review report(s) and provide point-by-point responses to each of the issues raised in the peer-review report(s); these are listed below for your convenience:

Reviewer #1:
Scientific Quality: Grade B (Very good)
Language Quality: Grade A (Priority publishing)
Conclusion: Minor revision
Specific Comments to Authors: The manuscript review the progression of Regulatory T cells and their associated factors in hepatocellular carcinoma development and therapy. The manuscript is well organized and and well scientific written. However, the content of "Tregs in HCC "(line 143) is too simple. The major topic of this paper is the role of Tregs in HCC. Therefore, the paragraph should be dedicatedly introduced.
Response: The authors appreciated the great comments from the reviewer. The role of Tregs in HCC initiation and progression was further discussed. More literature report were references, shown as following paragraph.

'A meta-analysis showed that a higher infiltration of CD3 T cells, CD8 T cells, and natural killer (NK) cells was associated with better overall survival (OS), disease-free survival (DFS), and recurrence-free survival (RFS). In contrast, a higher infiltration of Tregs and neutrophils indicated lower OS and DFS[35]. Another report also showed that an increase of Tregs or a decrease of M1 macrophages (proinflammatory phenotype) were associated with a poor prognosis of HCC patients[36]. $CCR4^+$Tregs are predominant Tregs that are recruited in tumor tissue of HCC associated infection of hepatitis viruses, which is associated with HCC resistance to sorafenib treatment[37]. The frequency of $CD127^{low}CD25^+CD4^+$Tregs was increased significantly in the peripheral venous blood of HCC patients compared to healthy controls[38]. In addition, the serum levels of TGF-β1 and IL-10 in HCC patients were positively associated with the Treg population in the blood, which were decreased post-operation and chemotherapy treatments. CCL5 expression on circulating tumor cells in HCC patients can attract Tregs to induce an immunosuppressive environment, one of the mechanisms for CTC escaping immune surveillance[39].

The expression of immune checkpoint proteins in the HCC microenvironment impacts Tregs and antitumor immunity. $PD\text{-}L1^+$neutrophils, Tregs, and neutrophil to lymphocyte ratio (NLR) were significantly increased in peripheral blood of patients with poorly differentiated HCC with a worse prognosis compared to that in patients with highly-moderately differentiated HCC[40]. Zhou et al. reported that tumor-associated neutrophils (TANs) can induce the infiltration of the macrophages and Tregs from HCC mice or patients via producing C-C motif chemokine ligand 2 (CCL2) and CCL17, resulting in HCC progression and resistance to sorafenib[41]. CTLA-4 on

Tregs in HCC impacts dendritic cell function by downregulating CD80/CD86 on dendritic cells (DCs)[42]. Therefore, blockade of CTLA-4 in HCC can improve DC-mediated anti-tumor immunity.

Treatment with tivozanib, a tyrosine kinase inhibitor, can suppress Tregs by inhibiting receptor tyrosine kinase c-Kit (CD117)/stem cell factor (SCF) axis and increased $CD4^+PD\text{-}1^+$T cells, resulting in a significant improvement in OS of HCC patients[43]. Treatment with Lenvatinib also can inhibit IL-2 mediated Treg differentiation except for decreasing PD-L1 expression in HCC cells[44]. Overall, the balance between Tregs with other T cells plays a vital in liver diseases, including the initiation and progression of HCC (Figure 1).

Furthermore, alteration of intrahepatic immunity is associated with HCC prognosis and treatment (Figure 2). An increase of Tregs, Th2, and Th17 T cells, as well as M2 macrophages, is usually and positively associated with HCC progression in patients, whereas an abundance of CD8 T cells, Th1 T cells, and M1 macrophages is associated with HCC therapy and good prognosis for HCC patients[45]. Single-cell RNA sequencing technologies have been applied to investigate the immune landscape of HCC samples to illustrate the subtypes of immune cells in HCC and their gene expressing profiles, as well as immune cell interactions, such as DCs with Tregs or CD8 T cells[46]. ’

Reviewer #2:
Scientific Quality: Grade C (Good)
Language Quality: Grade A (Priority publishing)
Conclusion: Minor revision
Specific Comments to Authors: Chunye Zhang et al report about the potential role of s of Tregs in HCC-inducing factors such as ALD, NALD, cirrhosis, and liver viral infections. The subject is scientifically interesting although still limited to the pure research field. The review is written clearly. Specific comments -Figure 1 and 2 to despite being present at the end of the manuscript, are not mentioned in the text; thus the author should include in the appropriate site the reference to the two figures. -It would be very helpful for non-expert in the field of immunology to introduce an additional figure where the main function of the different are reported (such as CD4/8 T cell, Th1/Th2 cells, Th17, CD8+CD122+Tregs, CD127lowCD25+CD4+Tregs, M2-like macrophages and so on). As it is, the manuscript is not clear in the regard
Response: The authors appreciated the great comments from the reviewer. Figure 1 was discussed in at the end of section ‘TREGS IN CHRONIC LIVER DISEASE’, and Figure 3 (original Figure 2) was mentioned in the section ‘IMPORTANT MOLECULES MEDIATED TREG FUNCTION AND METABOLISM’ in the revised manuscript.
In addition, as suggested by the reviewer, another figure (Figure 2) was added for discussing the role of change of different T cells and macrophages in progression of HCC or its prognosis in the manuscript.

4 LANGUAGE POLISHING REQUIREMENTS FOR REVISED MANUSCRIPTS SUBMITTED BY AUTHORS WHO ARE NON-NATIVE SPEAKERS OF ENGLISH

As the revision process results in changes to the content of the manuscript, language problems may exist in the revised manuscript. Thus, it is necessary to perform further language polishing that will ensure all grammatical, syntactical, formatting and other related errors be resolved, so that the revised manuscript will meet the publication requirement (Grade A).

Authors are requested to send their revised manuscript to a professional English language editing company or a native English-speaking expert to polish the manuscript further. When the authors submit the subsequent polished manuscript to us, they must provide a new language certificate along with the manuscript.

Once this step is completed, the manuscript will be quickly accepted and published online. Please visit the following website for the professional English language editing companies we recommend: https://www.wjgnet.com/bpg/gerinfo/240.

Response: The English grammar was checked by a native English speaker in academic study. The English grammar was scored A with the reviewers.

5 ABBREVIATIONS

In general, do not use non-standard abbreviations, unless they appear at least two times in the text preceding the first usage/definition. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, and mAb, do not need to be defined and can be used directly.

The basic rules on abbreviations are provided here:

(1) Title: Abbreviations are not permitted. Please spell out any abbreviation in the title.

(2) Running title: Abbreviations are permitted. Also, please shorten the running title to no more than 6 words.

(3) Abstract: Abbreviations must be defined upon first appearance in the Abstract. Example 1: Hepatocellular carcinoma (HCC). Example 2: *Helicobacter pylori* (*H. pylori*).

(4) Key Words: Abbreviations must be defined upon first appearance in the Key Words.

(5) Core Tip: Abbreviations must be defined upon first appearance in the Core Tip. Example 1: Hepatocellular carcinoma (HCC). Example 2: *Helicobacter pylori* (*H. pylori*)

(6) Main Text: Abbreviations must be defined upon first appearance in the Main Text. Example 1: Hepatocellular carcinoma (HCC). Example 2: *Helicobacter pylori* (*H. pylori*)

(7) **Article Highlights:** Abbreviations must be defined upon first appearance in the Article Highlights. Example 1: Hepatocellular carcinoma (HCC).

Example 2: *Helicobacter pylori* (*H. pylori*)

(8) Figures: Abbreviations are not allowed in the Figure title. For the Figure Legend text, abbreviations are allowed but must be defined upon first appearance in the text. Example 1: A: Hepatocellular carcinoma (HCC) biopsy sample; B: HCC-adjacent tissue sample. For any abbreviation that appears in the Figure itself but is not included in the Figure Legend textual description, it will be defined (separated by semicolons) at the end of the figure legend. Example 2: BMI: Body mass index; US: Ultrasound.

(9) Tables: Abbreviations are not allowed in the Table title. For the Table itself, please verify all abbreviations used in tables are defined (separated by semicolons) directly underneath the table. Example 1: BMI: Body mass index; US: Ultrasound.

Response: The manuscript was formatted according to the above rules.

6 EDITORIAL OFFICE'S COMMENTS

Authors must revise the manuscript according to the Editorial Office's comments and suggestions, which are listed below:

(1) Science editor:

This is a mini-review by Zhang and collaborators, on the role of Regulatory T cells and their associated factors in the development of hepatocellular carcinoma (HCC) and potential influence on therapeutic aspects. The subject is relevant as HCC is a leading cause of cancer-related death worldwide. In addition, the authors highlight the importance of non-alcoholic fatty liver disease (NAFLD), one of the critical factors capable of inducing HCC initiation and of promoting its progression. The increasing prevalence of NAFLD with the accompanying progressive increase in the incidence of obesity and type 2 diabetes is addressed in the review as a crucial point in terms of global health. The review also points towards issues regarding therapeutic limitations

and presents data to support important new players in the pathogenesis of HCC. T regulatory cells (Tregs) and their associated factors are appreciated in several aspects, including the question regarding immune tolerance of the tumor microenvironment in HCC. In addition, the article gathers information and presents in a summarized manner data on the role of Tregs in HCC-inducing factors including alcoholic and non-alcoholic fatty liver diseases, liver fibrosis, cirrhosis, and viral infections. The reviewers who analyzed the manuscript raised questions to be addressed by the authors. 1. The reviewers suggest that the authors should further explore and give more details on Tregs in HCC, the major topic of the paper. 2. In addition, they also suggest that figures should be mentioned in the text and; 3. that authors should make an effort to introduce the main function of the different immune cells involved in the immune response and surveillance, possibly adding a new figure to summarize that specific point. In addition, the editorial office raised several points in the manuscript that will need further attention and corrections from the authors.
Language Quality: Grade A (Priority publishing)
Scientific Quality: Grade B (Very good)
(2) Company editor-in-chief:

I have reviewed the Peer-Review Report, the full text of the manuscript, and the relevant ethics documents, all of which have met the basic publishing requirements of the World Journal of Gastroenterology, and the manuscript is conditionally accepted. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office's comments and the Criteria for Manuscript Revision by Authors. Before final acceptance, uniform presentation should be used for figures showing the same or similar contents; for example, "Figure 1Pathological changes of atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: ...". Please provide decomposable Figures (in which all components are movable and editable), organize them into a single PowerPoint file. Please authors are required to provide standard three-line tables, that is, only the top line, bottom line, and column line are displayed, while other table lines are hidden. The contents of each cell in the table should conform to the editing specifications, and the lines of each row or column of the table should be aligned. Do not use carriage returns or spaces to replace lines or vertical lines and do not segment cell content. In order to respect and protect the author's intellectual property rights and prevent others from misappropriating figures without the author's authorization or abusing figures without indicating the source, we will indicate the author's copyright for figures originally generated by the author, and if the author has used a figure published elsewhere or that is copyrighted, the author needs to be authorized by the previous publisher or the copyright holder and/or indicate the

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Response: Thank you for the comments. The Tables and Figures were revised according to the editorial officer's suggestions.

7 STEPS FOR SUBMITTING THE REVISED MANUSCRIPT

Step 1: Author Information

Please click and download the Format for authorship, institution, and corresponding author guidelines, and further check if the authors names and institutions meet the requirements of the journal.

Step 2: Manuscript Information

Please check if the manuscript information is correct.

Step 3: Abstract, Main Text, and Acknowledgements

(1) Guidelines for revising the content: Please download the guidelines for Original articles, Review articles, or Case Report articles for your specific manuscript type (Minireviews) at: https://www.wjgnet.com/bpg/GerInfo/291. Please further revise

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(3) Requirements for Article Highlights: If your manuscript is an Original Study (Basic Study or Clinical Study), Meta-Analysis, or Systemic Review, the "Article Highlights" section is required. Detailed writing requirements for the "Article Highlights" can be found in the Guidelines and Requirements for Manuscript Revision.

(4) Common issues in revised manuscript. Please click and download the List of common issues in revised manuscripts by authors and comments (PDF), and revise the manuscript accordingly.

Step 4: References

Please revise the references according to the Format for References Guidelines, and be sure to edit the reference using the reference auto-analyser.

Reminder: It is unacceptable to have more than 3 references from the same journal. To resolve this issue and move forward in the peer-review/publication process, please revise your reference list accordingly.

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Response: The revised manuscript was submitted according to the above seven steps, with all the required documents.

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Response: Copyright was signed and uploaded.

9 CONFLICT-OF-INTEREST DISCLOSURE FORM

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Response: The Conflict-of-Interest Disclosure Form was signed and uploaded.

All the best,

Ming Yang, PhD

University of Missouri