REVISION OF MANUSCRIPT 80613, WORLD JOURNAL OF HEPATOLOGY, RESPONSE TO PRIOR PEER REVIEW, (corrected)

I would like to outline my responses, but first a general statement. Both reviewers had issues with my initial publication title, and I choose a new title that should satisfy both reviews. The new title is **GALECTIN-3 INHIBITION AS A POTENTIAL THERAPEUTIC TARGET IN NASH LIVER FIBROSIS**

The other major issue was a new, original, figure that ties in all the elements discussed in the manuscript. The new figure of immunochemistry staining of liver tissue with a Galectin-3 antibody was featured as a poster presentation at the 2022 AASLD meetings and has never been published. It is from the pathology lab of Dr. Zachary Goodman, one of the world's most preeminent liver pathologists whose work is often featured in NASH clinical trials. I believe it clearly makes the case for Galectin-3 inhibition in liver fibrosis and cirrhosis.

To address, point by point, the reviewer's comments:

Reviewer number 1-

Comments: Liver antifibrotic therapy is a very interesting topic and this review provided some useful information. However, there are some comments to authors. 1. The review included an overview of galectin-3,its role in liver fibrosis and cirrhosis, the galectin-3 macrophage and NASH, current therapeutic approaches and galectin-3 blockers currently in trials. From the content of the review, the current therapeutic approaches and galectin-3 blockers currently in trials only account for a small part. Therefore, it is recommended to modify the title to be consistent with the content. 2. Liver fibrosis is a pathological process of various chronic liver diseases, not just the result of NASH. Why did you focuses on NASH in the abstract and introduction? 3. Because the galectin-3 blockers are newly developed medicine, their adverse effects are more concerned. Please provide some information about them. 4. The annotation format of references need to be modified to meet the requirements of this journal. 5. Please provide the number and title

name of Figures. 6. The content represented by each symbol in figures should be clearly marked.

Answer:

1) Title modified as above
2) NASH was chosen since it is very topical, clearly Galectin-3 inhibition applies to all forms of fibrosis
3) A paragraph is now devoted to safety issues of Galectin inhibition
4) References changed
5) 6) New figure inserted

Reviewer number 2-

Comments: In this minireview author discusses current knowledge on the role of Galectin-3 in non-alcoholic steatohepatitis by analyzing Galectin-3 as a biomarker of liver fibrosis and disease progression. The author gives brief and clear insight into the basic structure and function of Galectin-3, with the basic pathological mechanisms of liver fibrosis mediated by Galectin-3, including the main cells responsible for the initiation and propagation of fibrotic processes, such as liver macrophages. The author also discusses the present knowledge on the use of Galectin-3 inhibition as a potential therapeutic strategy.

Yet, I have some suggestions for the author, which I consider important for improvement of manuscript content.

1. The ***Title*** of the manuscript should be changed since the clinical studies regarding Galectin-3 inhibition in liver fibrosis are still in their infancy. The title in its current form reflects a strong and certain statement, which is still not sufficiently substantiated based on currently available scientific research data. The Title should be changed to ***Galectin-3 inhibition as a potential therapeutic strategy in liver fibrosis.***
2. For the *Abstract* to be clearer, more informative, and to attract the reader, the standard abbreviations (NAFLD, NASH) should be defined in the abstract.

3. In the sentence *If left untreated, NASH may progress to cirrhosis and hepatocellular carcinoma (HCC), which are major causes of morbidity and mortality* – the author must clarify which population these data refer to, as well as specify the reference.
4. In the *Introduction* part, the sentence that ends with *or ideally both*, the author should add *of these processes*. In the next sentence instead of *No matter what* - the author should use *Regardless of…*
5. In the *Introduction* part also, the author should provide the full term for the abbreviation ECM, since this is its first appearance in the text.
6. In the sentence *The transforming growth factor TGF-β1 has been viewed as the major profibrogenic cytokine released by the liver cell upon injury, turning the* ***Hepatic Stellate Cell (HSC)*** *into a myofibroblast*- there is no need to use both the full term and the abbreviation. The abbreviation (HSC) is quite sufficient, given that it is already explained at the first mention in the text.
7. The sentences *A comprehensive invited review of liver fibrosis has recently been published. The signaling pathways and the drugs targeting the various pathways have been reviewed elsewhere* should be reformulated: *A comprehensive review of liver fibrosis has recently been published, as well as the review of the signaling pathways and the drugs targeting the various pathways in non-alcoholic steatohepatitis.*
8. The subsection *Galectin-3: An overview* should be reformulated into *Galectin-3 basic features.*
9. Generally, the aforementioned subsection lacks references. I suggest for the author add them.
10. Please, also add the reference for this sentence: *In cirrhosis, Galectin-3 has been proven to be a biomarker, in combination with other scores, to discriminate advanced cirrhosis and predict post-transplant infectious complications.*
11. After these sentences *Galectins are proteins that are modified in hom~~o~~eostasis or under pathological conditions by adding glycans to their peptide chains, which in turn modulates their function. These proteins are known as glycoprotein*s,…. I think it

would be useful herein to introduce the term Glycosylation as the process described in the first sentence. (For example – These proteins are known as glycoproteins, and the addition of a carbohydrate molecule to a protein molecule is referred to as glycosylation…).

12. The last three paragraphs in this section (Galectin-3:an overview) should be merged into one, and further changes are needed.

    The sentence *Another source of confusion* should be reformulated into *Another term seen in the literature regarding this versatile molecule is…*

    The sentence *Yet another confusing term for those researching the literature is the MAC2BPGi,* should be reformulated into *The term also seen in the literature is the MAC2BPGi…*

13. The *Introduction* part should contain data not only about the pathology of NASH but also about the galectin-3 itself. Hence, the next paragraphs should be placed in the *Introduction* section: *The number of disease processes where Galectin-3 has been implicated continues to expand. Recent data has have implicated Galectin-3 as a direct causative agent in diverse diseases such as endometriosis, cardiac fibrosis and atrial fibrillation, and Alzheimer's disease. Galectin-3 plays a leading role in cancer progression and in the tumor microenvironment. In HCC, overexpression of Galectin-1 and Galectin-3 has been noted, and Galectin-3 favors tumor metastases via activation of βcatenin (insert dash β-catenin) signaling.*

14. Then, the new paragraph:

    In cirrhosis, Galectin-3 has been proven to be a biomarker, in combination with other scores, to discriminate advanced cirrhosis and predict post-transplant infectious complications. High tissue expression of Galectin-3 was also associated with the risk of chronic liver disease and worse overall survival. Blood levels of Galectin-3 have not correlated as a biomarker in NASH, since other background diseases such as obesity and diabetes as well as heart disease can raise Galectin-3 levels on their own. Then,

the line *This review will focus on the role of Galectin-3 in liver fibrosis,* at the end of this part.

15. The subsection termed *GALECTIN-3 ROLE IN LIVER FIBROSIS AND CIRRHOSIS* should be changed into *GALECTIN-3 IN LIVER FIBROSIS AND CIRRHOSIS* and provide a little bit more data regarding the link between the Galectin-3 and liver fibrosis. The author should mention the results from the preclinical studies, which demonstrated that upregulation of galectin-3 is observed in several pre-clinical models of hepatic fibrosis, NASH, and primary biliary

    (1. I. Jeftic, N. Jovicic, J. Pantic, N. Arsenijevic, M.L. Lukic, N. Pejnovic. Galectin-3 ablation enhances liver steatosis, but attenuates inflammation and IL-33-Dependent fibrosis in obesogenic mouse model of nonalcoholic steatohepatitis. Mol Med, 21 (2015), pp. 453-465.

    2. N. Pejnovic, I. Jeftic, N. Jovicic, N. Arsenijevic, M.L. Lukic
    Galectin-3 and IL-33/ST2 axis roles and interplay in diet-induced steatohepatitis
    World. Gastroenterol., 22 (44) (2016), pp. 9706-9717.

    3. J. Tian, G. Yang, H.Y. Chen, D.K. Hsu, A. Tomilov, K.A. Olson, A. Dehnad, S.R. Fish, G. Cortopassi, B. Zhao, F.T. Liu, M.E. Gershwin, N.J. Torok, J.X. Jiang
    Galectin-3 regulates inflammasome activation in cholestatic liver injury
    FASEB J., 30 (12) (2016), pp. 4202-4213)

16. The subsection ***THE GALECTIN-3 MACROPHAGE AND NASH***, should be termed *Galectin-3, macrophage activation, and liver fibrosis*
17. Please, provide the references for the whole paragraph:

    This fibrosis lattice process appears to be occurring in many disease states such as kidney fibrosis, cardiac fibrosis, and pulmonary fibrosis. In the lung fibrosis associated with Covid19, a macromolecular assembly on the surface of epithelial and mesenchymal cells that clusters pro-fibrotic factors has been discovered. The researchers coined the

term the "Gal-3-fibrosome" to describe how Galectin-3 oligomerizes via its N-terminal domain and binds modified glycan chains on glycoproteins on cell surfaces. The Galectin-3 interactions anchor two complexes, TGF-βRII and the (CD98hc):a β1-integrin complex that mediates inflammatory and fibrotic cellular responses to extracellular stimuli. TGF-βRII is a key receptor for the profibrotic cytokine TGF-β1. The CD98 heavy chain (CD98hc): β1-integrin complex mediates inflammatory cytokine responses to extracellular factors. This discovery was made in pulmonary fibrosis, and needs to be proven in hepatic fibrosis, but disruption of this 'Gal-3-fibrosome' appears to be a promising target for new anti-fibrotic therapies.

18. The sentence *A YouTube video representation of these cell membrane Galectin-3 lattice structures is available online* should be excluded from the text.
19. The subsection *CURRENT THERAPEUTIC APPROACHES* should be changed to *Galectin-3 in current therapeutic approaches.*
20. The author needs to add the reference and discuss the sentence: *Galectin-3 inhibitors employ either small molecules that can be given orally, or large molecules that are administered parenterally.*
21. The subsection *GALECTIN-3 BLOCKERS CURRENTLY IN TRIALS* should be changed into *Galectin-3 inhibitors currently in trials.*
22. I have also a couple of important remarks regarding the Figure. First of all, the Figure does not reflect the core of this manuscript. The Figure is very general and shows the structure of Galectin-3, with general data regarding its extracellular oligomerization and intracellular signal transduction. The appropriate Figure needs to summarize data presented in the manuscript, which is the role of Galectin-3 in liver fibrosis, regulation of macrophage, along with its inhibitory potential.

    The second remark considers the top of the Figure. The same Figure can be seen in the article: *Suthahar N, Meijers WC, Silljé HHW, Ho JE, Liu FT, de Boer RA. Galectin-3 Activation and Inhibition in Heart Failure and Cardiovascular Disease: An*

Update. Theranostics. 2018 Jan 1;8(3):593-609. doi: 10.7150/thno.22196. PMID: 29344292; PMCID: PMC5771079.

I would kindly ask the author to make new, original Figure, which would summarize the key information presented in this paper.

23. The author should insert in Key Words: Galectin-3, Macrophage
24. The author should number the references in Arabic numerals.

Answer:

1) Title changed as above
2) Abbreviations defined
3) Population at risk for HCC defined
4) Changed to "Regardless "
5) ECM defined
6) Modified
7) Modified as per reviewer's suggestion
8) Modified
9) References inserted
10) Reference inserted
11) Glycosylation introduced
12) Merged and modified as per reviewer
13) Introduction now contains data in Galectin-3
14) New paragraph as per reviewer
15) Subsection title changed; new preclinical data inserted
16) Modified as per reviewer
17) References inserted
18) Sentence removed
19) Sentence modified
20) References inserted and discussion of Galectin-3 expanded with intracellular and extracellular blockade and rationale of blockade discussed

21) Subsection title changed

22) Figure changed, discussion as above

23) Key words inserted

24) Arabic numerals inserted

Reviewer number 2 re-revision:

Comments: The author has addressed all the raised issues in the manuscript and the manuscript can be accepted for publication in the present form.

Answer:

Thanks for your comments.