Name of Journal: *World Journal of Gastroenterology*

Manuscript NO: 39364

Manuscript Type: Review

Please provide specific point-to-point replies to each reviewer's comments.

Response to reviewers:

Reviewer 1: The authors rightfully conclude that several technical limitations including the promiscuous nature of the miRNAs and the lack of specificity preclude their clinical use. Can the authors explain the while secretion of miRNAs in exosomes (e.g. miR-122) can cause an increase in inflammatory response by targeting monocyte/macrophage cells, treatment with antiMIR122 recapitulates and augments the hepatocellular damage and steatosis induced by chronic alcohol and in combination with alcohol worsened ALD pathology as recently reported (Gastroenterology 2018, 154:238). Further, discuss why Miravisen, a miR122 inhibitor shows promising results in chronic hepatitis treatment. This information should be put together into some logical explanation. This is well explained for NAFLD but not for ALD which should be done.

Authors' response: we thank the reviewer for this suggestion. Therefore, we have added a paragraph explaining these apparently contradictory results. We consider that miRNAs have a wide range of actions depending on the targeted cell; thus, in the case of miR-122, the potential regulatory effect could be different inside the hepatocyte when compared with monocytes/macrophages. We also added a new paragraph trying to explain that the effect of Miravirsen could also be due to a disruption of the inter-cellular communication carried out by miR-122 and thus avoiding viral replication. As the reviewer has suggested, we have clarified in our manuscript that the technical limitations and the variability of results of different studies regarding miRNAs actions make difficult their interpretation.

We have added the following paragraphs:

Subheading 2.3- Therapeutic Application of miRNAs in ALD

"A recent study showed that the restoration of miR-122 in hepatocytes could have a protective role against ALD development[33]. These apparently contradictory results could reflect the ability of miRNAs to develop different actions in different cells and also its relevance in inter-cellular communications[32]. In this sense, the therapeutic action of Miravirsen over viral replication could be explained by the interruption of these communications[57]."
Subheading 2.1. (Hepatocytes)
"MiRNAs action and pleiotropic effects could be different depending on the cell in which they act; thus, miR-122 could have a protective role inside the hepatocyte during alcohol-induced liver damage[34]"

Reviewer 2: of the manuscript, under the heading miR-34a, lines 5-6, relative to the effects. of SIRT1 on PGC 1 alpha (or PPAR alpha?).

Authors' response: We thank the reviewer for having pointed out this mistake. We have corrected it.

Reviewer 3: of microRNAs. 3. "SIRT1 inhibits the co-activator 1 alpha of the PPAR-gamma (PPAR-alpha)": the sentence is not clear, PPAR-gamma and PPAR-alpha are two distinct isoforms of PPAR, it would be better to remove "(PPAR-alpha)".

Authors: We thank the reviewer for having pointed out this unclear point. We have corrected it by removing "(PPAR-alpha)" as suggested.

Reviewer 3: 4. General comment (1): the authors should probably provide some further comments on the fact that the role of microRNAs is a critical bridge between genome and environment. They mentioned some SNPs reported in microRNAs that may be associated with increased risk of liver diseases, but more complex regulatory networks are involved at the epigenetic level to modulate the expression of microRNAs and their downstream targets.

Furthermore, dynamic fluctuations of miRNA levels are influenced by environmental factors such as alcohol (discussed), but also diet, drugs, and cigarette smoking. It has been proof that a high-caloric diet and/or chronic smoking predispose to increased oxidative metabolism, leading to a pro-inflammatory profile of released miRNAs and cytokines.

Authors' response: we agree with the reviewer and we have added a paragraph to reflect the variations induced in miRNA profile by many environmental factors, besides alcohol consumption. We have included the following text in the Introduction section: The expression of a wide variety of miRNAs is potentially regulated by many factors, such as alcohol, but also diet, cigarette smoking and other drugs[5].

Reviewer 3: 5. General comment (2): perhaps the authors could provide some more information on the interaction of miRNAs and SIRT1 with the Keap1/Nrf2 system, regulating the anti-oxidant regulatory elements (AREs) and coordinating the cellular response to ROS and oxidative stress. Here is some related literature: a) Boccuto L, Abenavoli L. Genetic and Epigenetic Profile of Patients With Alcoholic Liver Disease. Ann Hepatol. 2017 Jul-Aug;16(4):490-500. b) Yang D, Tan X, Lv Z, Liu B, Baiyun R, Lu J, Zhang Z. Regulation of Sirt1/Nrf2/TNF-α signaling pathway by luteolin is critical to attenuate acute mercuric chloride exposure induced hepatotoxicity. Sci Rep. 2016 Nov 17;6:37157. c) Wan C, Han R, Liu L, Zhang F, Li F, Xiang M, Ding W. Role of miR-155 in fluorooctane sulfonate-induced oxidative hepatic damage via the Nrf2-dependent pathway. Toxicol Appl Pharmacol. 2016 Mar 15;295:85-93. d) Kurinna S, Werner S. NRF2 and microRNAs: new but awaited relations. Biochem Soc Trans. 2015 Aug;43(4):595-601. e) Shi L, Wu L, Chen Z, Yang J, Chen X, Yu F, Zheng F, Lin X. MiR-141 Activates Nrf2-Dependent Antioxidant Pathway via Down-Regulating the Expression of Keap1 Conferring the Resistance of Hepatocellular Carcinoma Cells to 5-Fluorouracil. Cell Physiol Biochem. 2015;35(6):2333-48. f) Yang JJ, Tao H, Hu W, Liu LP, Shi KH, Deng ZY,

Li J. MicroRNA-200a controls Nrf2 activation by target Keap1 in hepatic stellate cell proliferation and fibrosis. Cell Signal. 2014 Nov;26(11):2381-9.

Authors' response: we thank the reviewer for this suggestion and we fully agree that the Keap1/Nrf2 pathway could play a relevant role in miRNA involvement in liver diseases. Thus we have added an explanation of this mechanism and included the majority of the interesting references provided. We have added this text (2.1. subheading): "Oxidative stress and free oxygen radicals generation involved in ALD development are also regulated by miRNAs through different pathways like Kelch-like ECH-associated protein 1 Kelch-like ECH-associated protein 1 (Keap1) / Nuclear factor-erythroid-2-related factor 2 (Nrf2) pathway[16-20]."

And:

"The Keap1/Nrf2 pathway could also be involved in miR-155 role in ALD development and KCs regulation[17]"