Author's response to reviews

Title: Treatment with Pirfenidone for two years decreases fibrosis, cytokine levels and enhances CB2 expression in patients with chronic hepatitis C: open-label clinical trial

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Author's response to reviews: see over
Enclosed please find the revised version of our manuscript entitled “Treatment with Pirfenidone for two years decreases fibrosis, cytokine levels and enhances CB2 expression in patients with chronic hepatitis C: open-label clinical trial” by L. Flores-Contreras and collaborators. It is important to notice that all changes made are in bold and underlined lettering in the new manuscript according to the reviewers’ queries. Also, we have revamped the entire manuscript in order to make it clearer to the reader, corrected grammar errors and added the requested point by point response.

We believe we’ve fulfilled with the reviewers´ queries and we hope that you find now our manuscript suitable for publication in your prestigious journal.

Should you need any more information on this issue, please do not hesitate to contact me.

Best regards.

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Point by point Response

Reviewer #1
Major Compulsory Revisions
In this manuscript, the authors aimed to assess whether two-year treatment with pirfenidone influence necro-inflammation, fibrosis and steatosis, as well as serum markers in patients with chronic hepatitis C (CHC). They found that pirfenidone for two years benefits CHC patients and improves inflammation, fibrosis and steatosis. Although their data seem logical, there are several issues that should be further reconsidered or corrected.

Question: 1. The major criticism raised is the high rate (18%) of early termination from this study. What happened in these subjects with early discontinuation? Was there any severe adverse effect associated with study medication? What were the causes for the mortality?
Answer: As written in the new version of the manuscript, none of the patients dropped out the study due to pirfenidone secondary effects. Patient’s mortality was due to the advanced stage of liver disease and related complications such as hepatocarcinoma. Please see new Tables 5 and 6.

Question: 2. Intention-to-treat analyses should be performed, including liver biochemistry, clinical status, and serum biomarkers.
Answer: In this reply, we are including the Intention-to-treat statistical analysis for biochemical parameters and clinical parameters.

Question: 3. Authors should provide the statistical analyses on the Child-Pugh status and quality of life between before and after treatment (table 2).
Answer: In this reply, we are including the statistical analysis of the Child-Pugh status and quality of life (Table 4).

Reviewer #2
The authors performed this study with the aim to expand their previous pilot experience (from 12 to 24 months) on the treatment of advanced chronic HCV infection with Pirfenidone as anti-inflammatory/anti-fibrotic drug. In this trial they evaluated the change of some pro-inflammatory cytokines as well as the gene expression of cannabinoid receptor (CB1-2).
Overall the study should be of interest and of potential clinical significance, although this study adds few information to the previous study. In addition, the design and the aim of the study are not very clear and the results deserve more considerations and explanations. On these bases the conclusion: “we propose the use of pirfenidone for liver cirrhosis in patients with CHC” is too strong and could be hazardous.

Answer: We have revamped the design and purpose of the study to establish them clearer. Results were expanded in order to provide a better description.

We have eliminated the sentence “we propose the use of pirfenidone for liver cirrhosis in patients with CHC”. We changed it to a concept where it is suggested this pirfenidone treatment for non-responders to SOC that is the actual standard treatment or gold rule for CHC.

Specific point.

Question: 1. The background of the study is not well reported. The study was performed because the authors believed that necroinflammatory activity plays an important role in the progression of the disease. To facilitate the concept to the reader, I suggest to cite the recent publication reviewed this point (HCV and inflammation) (Zampino R. et al Word J Hepatol. 2013:5:528-540.)

Answer: We have re-written the background of the study to make it more comprehensive and we have actually quoted the referred paper. It is important to note that now we have expanded several concepts in order to make easier to the reader the manuscript’s understanding.

Question: 2. The aim of the study should be better stated; in other words, they want to expand their experience, to evaluate the safety, to evaluate the mechanisms, etc. The conclusion should be according to the aim and to the design of the study. It is important to keep in mind that there is a standard of care for CHC and that the virus clearance is necessary to cure the disease. This point is necessary to be discussed. The authors perhaps want to deserve treatment with PDF in non-responders to SOC; if so should be declared.

Answer: We have re-structured the purpose of the study leaving clear from the front end that the aim was to expand our experience and increase the number of patients, along with the measurement of some other key molecules not previously evaluated. We also discussed the standard of care for CHC and mention that the virus clearance is necessary to cure the
Finally, we end our recommendations suggesting that PFD could be used in non-responders to SOC.

Question: 3. This is an open study. If the intention of the authors was to make a therapeutic trial why they did not perform a randomized trial, in which patients could be randomized according to age, sex and disease stage. Thus, this study do not permit to give therapeutic conclusions and it should modify accordingly.

Answer: It was not the authors´ intention to perform a randomized trial. Instead, the primary aim was to expand our experience, increase the number of patients, and to extend the period of treatment with PFD up to 24 months. We decided to carry out this open study since we reasoned we could find a better effect on fibrosis score and necro-inflammation index. On the other hand, our secondary objectives were aimed to elucidate gene expression of key molecules not previously evaluated. Thus, we modified the conclusions accordingly with this purpose.

Question: 4. The enrollment of patients is not clear. What was the criteria of enrollment? All consecutive patients or there were other criteria.

Answer: The criterion for enrollment was consecutive patients. We have pointed that in patients´ description section.

Question: 5. The authors declare that they have enrolled only patients with advanced liver disease/cirrhosis. Figure 1 shows a mean score of 4.2, that means that a large proportion of patients did not have cirrhosis. The histological score of patients must be clearly reported. Fig. 1 is confused what means HAI Fibrosis stage? Is necro-inflammatory activity or fibrosis stage? The population studied must be better defined:

Answer: In Figure 1 C is represented the necro-inflammatory activity and in D) fibrosis stage is depicted. We have changed the text in the graphics to be clearer. Also, we describe better the population of study, adding criterion for enrollment and have corrected in the text that patients were in different stages of liver disease not exclusively with advanced liver disease/cirrhosis.

Question: 6. The most critical point, that must be cleared, is the six patients who were excluded from evaluation. The authors generically declare that “were non compliant or dead”. The reason of non-compliance must be reported. More
important, the cause of death as well as the eventually relation with treatment must be clearly reported.

Answer: Now we reported the reasons for non-compliance, as well as the cause of death. It is important to specify that none of the patients dropped out the study due to Pirfenidone secondary effects. Patient’s mortality was due to the advance stage of the liver disease and related complications such as hepatocarcinoma (Tables 5 and 6).

Question: 7. It is not clear the data on cannabinoid receptors, the results should better discussed also in relation with the recent publication on the matter (Coppola N, et al.. Cannabinoid receptor 2-63 QQ variants associated with severe necroinflammation in chronic hepatitis C. Clin Gastroenterol & Hepatol 2013; 2013 May 21. doi:pii: S1542-3565(13)00687-3. 10.1016/j.cgh.2013.05.008.).

Answer: In this study, we reported only mRNA levels for CB receptors. We did not test any polymorphism. Since we had no idea of the prevalence of the Cannabinoid receptor 2-63 QQ variants in our population, we believe discussion of this paper is not adequate. However, it could be a good point to analyze in the future in our population.

Reviewer #3:

Contreras et al have investigated the efficacy of Pirfenidone in the treatment of chronic hepatitis C. This molecule used in the treatment of pulmonary fibrosis was given for 2 years in 34 HCV patients with different stage of disease severity. Control liver biopsy showed a significant improvement of steatosis, fibrosis and inflammation. Some cirrhotic patients improved their Child score. Molecular analysis showed modulation of CB receptors expression.

Question: Authors published similar results in a previous small pilot study (Gut 2006). Accordingly, I don't understand why they did not perform a randomized controlled study rather than a second pilot study. Moreover, in the era of DAAs, the relevance of this antifibrotic treatment is likely out of relevance. I have also major concerns with the safety of this molecule.

Answer: It important to establish from the very front end that was not the intention of the authors to perform a randomized trial, instead the primary aim was to expand our experience and increase the number of patients, and extend the period of treatment with PFD to 24 months. We decided to carry out this open study since we reasoned we could find a better effect on fibrosis score and necro-inflammation index. On the other hand, our
secondary objectives were aimed to elucidate gene expression of key molecules not previously evaluated.

There is a great deal of information regarding of DAAs, some of them with proven efficacy; however, these kinds of treatments are not available for population outside the social services in countries like Mexico, and others. Then, we believe antifibrotic treatment can have a potential used in this population due to its low cost compared to some DAAs. Besides, most of the DAAs have low efficacy in certain HCV genotypes like 1 and 2. Thus, patients infected with this type of virus could achieve improvement in their health and quality of life with an antifibrotic therapy. Besides, the combination of pirfenidone with DAAs could be useful in patients infected with genotypes that can be hardly eliminated with standard therapies.

**Question:** Results should be presented in intent to treat (6 patients excluded of the efficacy analysis)

**Answer:** We have added the ITT analysis for biochemical and clinical parameters.