RESPONSE LETTER

The authors would like to thank the reviewer's for the comments made to our manuscript *'INT-767 improves histopathological features in a diet-induced ob/ob mouse model of biopsy-confirmed non-alcoholic steatohepatitis (NASH)'.* We have carefully addressed the criticisms point-by-point, our response is indicated below in italics. Changes in the manuscript are highlighted in yellow.

REVIEWER 1

This is an interesting and complex study the evaluates INT-767 a FXR and TGR5 agonist improvement on biopsy proven *ob/ob*-NASH mice. In addition, it compares INT-767 with OCA and evaluates its genetic and drug levels expression in intestinal and liver setting.

1. Major comments: Abstract: The authors should clarify the three steps of the study in the abstract, it is very confused regarding time of drug use, gourds that are compared and doses.

Response: We agree with the suggestion and within the abstract have grouped each study with its respective results. This has helped clarify the various studies and treatment groups.

2. In addition, it is not clear in the abstract that there are different steps in the study. Why time on INT-767 was different between INT -767 only and INT vs OCA? Also, why were the doses different in the two steps? Would this have any impact on the results? In fact, the authors decided to double the time on INT-767 when comparing with OCA. Did they compare eight weeks of INT as well to OCA? They could do this analysis since they have this result.

Response: We appreciate the question and it highlights an important point re: appropriate OCA dosing in preclinical NASH models. To address, we have added justification for our dosing/duration in the drug treatment methods section (page 6, 1st paragraph). "A longer duration of administration was selected for this second study to (a) examine the durability of INT-767 NASH histological improvements, and (b) because in our experience OCA requires extended dosing (e.g., at least 12 weeks at 10-30 mg/kg) to elicit histological anti-fibrotic responses (data on file) and shorter treatment periods with OCA are not always sufficient to promote antifibrotic effects in ob/ob-NASH mice (20)".

3. How many mice were lost to the experiment?

Response: A total of 6 mice were lost in the experiment(s) due to health issues unrelated to drug treatment. Experimental data from these mice were not included in the present paper.

4. Regarding NASH parameters, NAS scoring system and liver fibrosis, what was considered as improvement by the authors?

Response: As noted in the legends to figure and now added to the statistical analysis methods, for qualitative histological endpoints efficacy was defined as ≥1 point improvement in the respective parameter. For improvement in quantitative fibrosis we considered an improvement to be a statistically significant difference based on ANOVA with Bonferroni's post-hoc test. The therapeutic/biological significance of a drug-induced quantitative percent change is up to the reader to attribute, but we suggest that the marked

FXR agonist-induced reductions in steatosis from baseline coupled with a clear prevention in fibrosis progression should be considered an improvement.

5. The authors should better clarify that lean mice were included as controls only for biochemical parameters.

Response: We have added the word "only" to the results discussion of drug concentrations to clarify that this applies only to chow-fed C57Bl/6 mice (page 14, 2nd paragraph).

6. Discussion: The authors state that the administration of INT-767 for 16 weeks confirms that INT-767 has durable histological benefits. I don't agree with this conclusion since mice were still under drug use. This should be concluded it those mice had stopped drug use and a liver biopsy was performed to evaluate the permanent improvement in liver histology. This phrase might be excluded or modified.

Response: We have added the caveat that durability was evident in the presence of sustained drug administration. Thus, per the reviewer's suggestion the statement is revised as: "These findings confirm durable histological benefits of INT-767 with continued drug administration in a preclinical model of NASH and suggest that INT-767 may exert greater efficacy than OCA at both matched and (in vitro) potency-adjusted doses." (page 20, last paragraph).

REVIEWER 2

Authors indicated the effect of INT-767 on pathological condition in NASH model mice in detail, and INT-767 has more positive effect than OCA on NASH phenotype. The experimental design will be helpful to improve the screening of candidate drugs for NASH in preclinical trials. Thus, it will be suitable for the publication in this journal. There are some minor questions in this manuscript.

1. It is well known that the lipid droplet size is smaller by the activation of PPARγ signaling. In Fig. 3, INT-767 reduced lipid droplet area, and are FXT and TGR5 signaling also involved in the lipid droplet size?

Response: This is an interesting observation and we agree is reminiscent of PPAR effects. To highlight this, we have added the following to the results section (page 13, line 23-26): "In line with these morphometric changes, and similar to PPAR agonists, INT-767 10 mg/kg significantly reduced mRNA levels of cell death-inducing DNA fragmentation factor alpha-like effector c (CIDEC; a lipid-droplet associated protein that promotes intracellular storage) relative to vehicle (in RPKM, 109.4 ± 10 (vehicle) vs. 40.8 ± 10.8 (INT-767), p=0.0018)".).

2. In Fig. 1A, what is the asterisk indicated?

Response: As noted in the legends to figures, an asterisk signifies a significantly different ($p<0.05$) responder rate from baseline to endpoint histology relative to vehicle controls using a Chi Square test. We have also clarified this in the statistical analyses portion of the methods (Page 9, 1st paragraph).

3. There are some English spelling mistakes.

Response: We have re-reviewed that manuscript and corrected any additional spelling mistakes that we found.