Format for ANSWERING REVIEWERS

December 10, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 13987-Review.doc).

Title: Progress and prospects of engineered sequence-specific DNA modulating technologies for the management of liver diseases

Author: Samantha A Nicholson, Buhle Moyo, Patrick B Arbuthnot

Name of Journal: *World Journal of Hepatology*

ESPS Manuscript NO: 13987

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 The article has been revised to address the constructive criticisms of the reviewers. Details of the 'point by point' changes to the manuscript are provided below and the changes are indicated in red font in the revised version of the manuscript.

Reviewer 1:

1 Overall, this is an excellent article; however, this article could be improved if the authors would have addressed specific targeting of foreign nucleic acids to specific organs and cell types.
Authors' response:
The focus of the review is on delivery of the gene editing sequences to liver cells and targeting of hepatocytes is therefore the primary goal. To address the suggestion of the reviewer the following statement was included on page 24-25.
"The vectors used for delivery of sequences encoding gene editors may be engineered to target specific tissues. Using different serotypes, receptors or lipids it is possible to ensure specific delivery of the transgene payload. This flexibility also allows for modification of the delivery vehicle to avoid immune detection, should repeat administrations be necessary in a clinical setting."

2 In addition, the authors should have discussed the potential influence of the development of immune reactions to the various delivery methods.
Authors' response: Concerns about an immune response to the gene editors and vectors is indeed very relevant. However, the lack of clinical and empirical data on the subject means that definitive discussion in a review format is difficult. Nevertheless, the points about of immunogenic effects of gene editing sequences are addressed in the paragraph immediately preceding the conclusions section (page 25).
"The potential of an immune reaction to gene editors may be a concern for application of the technology. This immunostimulation may result from either the gene modifier itself[116] or from the mechanism of delivery[117]. In either case it is important to assess immune activation as it may diminish efficacy following repeat administrations and cause toxicity. Immune stimulation has significantly hampered gene therapies in the past[118] and will be important for clinical application of gene editing."

3 A few concerns are present that should have been addressed by the author's a discussion of potential immune reactions to the various gene delivery methods would have been useful. Also, when introducing sequence-specific gene editing segments, would these result in "off target" effects? Overall, this is a very good review article.
Authors' response:
The authors agree that these are important topics. The concern about immunostimulatory effects is dealt with above. Discussion of off-target activity of the gene editing technologies is already provided in the review. We feel that expansion of the topic will not provide the reader with valuable additional information. For this reason extra discussion of off target effects is not provided.

4 A final concern is that in many of the cell culture studies, the efficiency of gene reduction is not that impressive, about 50% or so, which might prove very problematic for this introduction of these procedures in clinical practice. Would multiple treatments be necessary to rid the cells of the harmful nucleic acids?
Authors' response:
This was addressed by adding the following statement to the section addressing delivery concerns associated with gene modifying technologies (page 24).
"This specific delivery may be further complicated by the need for multiple administrations of a targeted therapy due to their lower efficacy rates (around 50%) in a clinical setting."

Reviewer 2
This is an interesting paper which may improve our knowledge in the field. The subject matter is suitable for the intended audience and it fits the journal scope. Article is mostly clearly written, but Title is suggestive of the article's content. Article is appropriately organized and the headings are indicative of content I suggest to accept this paper in the present form.

Reviewer 2 made no suggestions for revisions to the manuscript.

Reviewer 3
1 Taken all together, it is a very good paper, just I think the last section should be shortened and focused on conclusions.
Authors' response:
Reviewer 3 raised the point that the conclusions are lost within the final portion of the paper. To address this, the authors have introduced a final subheading to highlight the conclusions (page 25). We believe that this is an improvement and clarifies the main points of the article.

We hope that these changes to our manuscript address the reviewers' concerns adequately and that the article is now acceptable to the *World Journal of Hepatology*.
Yours sincerely,
Patrick Arbuthnot.