31st May 2016

Dr Jin-Xin Kong,

Scientific Editor

Baishideng Publishing Group Inc

8226 Regency Drive, Pleasanton, CA 94588, USA

Dear Dr. Jin-Xin Kong,

Re Manuscript No: 26452 - Cell therapy using human insulin-releasing 1.1B4 cells configured as pseudoislets improves glycaemic control in streptozotocin diabetic SCID mice, Green AD, Vasu S, McClenaghan NH and Flatt PR.

Thank you for the email of 19th May 2016, with editorial evaluation of our manuscript. We are pleased that you would consider our manuscript suitable for publication pending revision.

We have now revised our manuscript according to Reviewer's comments which are highlighted in red underlined text in the revised manuscript. Our replies to each of the points raised by the Reviewers plus details of revisions to the text are attached.

We hope that you will consider the revised version of our paper acceptable for publication in *World Journal of Diabetes.* I look forward to hearing the outcome of your review in due course.

Yours sincerely,

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Reviewer 1: We thank the Reviewer for his/her comments and for remarking that the topic is of interest. The Reviewer's comments are addressed below:

1. The Reviewer comments that, '*On page 5, line 20 the statement: few days when grown in suspension culture." Indicate how many days this information relates*'. We have now included this information in text on page 6, line 18.

2. The Reviewer comments that, '*The objective of the study is unclear, both in the abstract, as the end of the introduction. These points what is shown is the main finding of the study, not the question that generated the hypothesis of the study*'. We have now included a statement in the Introduction section on page 6, line 23 to 25, that reads, 'Transplantation of cells configured as pseudoislets may represent an attractive means to improve graft survival, function and resistance to hyperglycaemia'. This is also stated in the opening line of the Abstract.

3. The Reviewer comments that, 'On page 6, methods section: "*Animal and surgical procedures". Why that for this study was necessary to use the immunodeficient SCID mice? In this section, I suggest insert a picture with the experimental design, specifying the groups of rats with their treatments and the analysis period'*. We would like to clarify that we used mice and not rats and for transplantation studies, SCID mice are widely used to avoid the problem of graft rejection. Thus transplantation of human 1.1B4 cells into normal mice without immunosuppression would result in tissue rejection. We have added the following (page 6, line 28 – 29): 'These immunodeficient mice were used to prevent rejection of human 1.1B4 cell implants'. We have also added a new figure outlining the timeline of experiments and inserted a statement on page 8, line 5, that reads, 'Timeline of the procedures is depicted in Figure 1'.

4. The Reviewer comments that, '*Both for biochemical analysis as histological were used the same extracted tissue from a single mouse? Or to these approaches were used separate*

groups of mice? Specify in the text'. Histological and biochemical analyses were carried out on same samples. We have now included this information on page 8, line 4, that reads, '…implants and pancreata were collected for both histology and hormone content assessment'.

5. The Reviewer comments that, '*The statistical analyses are inappropriate. Why was used Student's unpaired t-test if there four groups with two interventions (diabetes and implant)? To the measures with endpoint the statistical analyses are ANOVA. And to the measure with several points in the same rats (ex. Food intake), the statistical analyses are repeated measures ANOVA. Thus, analysis data is confusing. It clearly appears only the effect of diabetic or may not be on the implant. And is not shown a difference between the types of implants (cell or pseudoislet), to characterize the fact that the results are different between the implants and the like. For analyzing the way in which it appears, it seems similar results between the therapies'*. We believe our method of statistical analyses is appropriate, with effects of transplantation on diabetes clearly evident from the way data are presented. Statistical review of the study was performed by a biomedical statistician.

6. The Reviewer comments that, '*In figure 1 there are errors: in B, C and D the insulin therapy begins on day 6, but in A, this starts on day 9. The final dates are different between measurements in A and B is on day 45, and C and D on day 48. If the diabetic rats treated with cell implants had severe hypoglycemia after 21 days of transplantation, and the sequence of analysis was interrupted, the others groups should also be. All groups must have the same comparison period*'. We thank the Reviewer for this comment and we have now edited Figure 1A, with correct information regarding start of insulin therapy (day 6). We did not terminate the study 21 days post transplantation for all treatment groups to better understand the effects of pseudoislet transplantation. As clearly seen from the graphs, pseudoislet transplantation resulted in a gradual improvement in blood glucose levels.

7. The Reviewer comments that '*n page 13, line 4 the statement "energy and fluid balance, body weight, blood glucose tolerance were normalized…" That is, to state that the measures were standardized in the group with pseudoislet implant should be given the differences in all groups compared to the control. And if there is no statistical difference between the groups with implant pseudoislet vs. the non-diabetic control group, and the values were similar, the word normalize can be used'*. Weapologize for this confusion and have now rephrased the statement on page 13, line 11, that reads, 'Furthermore, energy and fluid balance, body weight, blood glucose and glucose tolerance improved gradually in these mice'.

Reviewer 2: We thank the Reviewer for his/her comment that our manuscript is interesting. The Reviewer's comments are addressed below:

1. The Reviewer comments that, '*First, the authors need to verify that the enhanced insulin in the SCID mice is derived from the human cells by at least showing human C-peptide levels*'. Unfortunately, we did not have the opportunity to measure human C-peptide alongside that of glucose, insulin and glucagon due to small volume of plasma obtainable from these mice. However, it is hard to imagine that the insulin was due to spontaneous regeneration of beta cells because analysis of pancreatic tissue at end of study revealed severe loss of islet beta cells and cellular insulin in both 1.1B4 cell implanted groups similar to diabetic controls. To accommodate the Reviewer we have added the following (page 12, line 26 to page 13, line 1 – 2): 'We did not have the opportunity to measure human C-peptide for confirmation but we assume that this insulin was derived from extra-pancreatic source because analysis of pancreatic tissue at end of study revealed severe loss of islet beta cells and cellular insulin in both 1.1B4 cell implanted groups similar to untreated diabetic controls. Furthermore, human insulin and C-peptide were readily detectable in 1.1B4 cells [25]'.

2. The Reviewer comments that, '*The histological analyses are poor, they need to be improved by performing immunohistochemistry showing markers for the 1.1B4 cells*'. We believe that these images are of acceptable standard and regret not being able to add better ones. To accommodate the Reviewer, we have removed Figure 3 and associated text. The other Figures have been renumbered as appropriate. We have already published data in JBC concerning markers for 1.1B4 cells: human insulin, C-peptide etc (McCluskey et al 2011; reference 25).

Reviewer 3: We thank the Reviewer for his/her comments that our study is interesting. The Reviewer's comments are addressed below:

1. The Reviewer comments that, '*The authors showed that plasma insulin levels were increased in human 1.1B4 cells or pseudoislets transplanted STZ diabetic mice. Can human insulin be detected in human 1.1B4 cells or pseudoislets transplanted STZ diabetic mice? How to distinguish the human insulin or mouse insulin secreted from transplanted STZ mice? Moreover, the human C peptide levels should be considered to detect'*. We thank the Reviewer for this comment and refer to our reply to Reviewer 2 above regarding necessary changes made to the text. Our earlier paper in JBC (McCluskey et al 2011; reference 25) showed that human insulin and C-peptide were all detectable in 1.1B4 cells. To further accommodate this Reviewer, we have added the following after the other addition starting on page 12, line 26 to page 13, line 1 – 2: 'We did not have the opportunity to measure human C-peptide for confirmation but we assume that this insulin was derived from extra-pancreatic source because analysis of pancreatic tissue at end of study revealed severe loss of islet beta cells and cellular insulin in both 1.1B4 cell implanted groups similar to untreated diabetic controls. Furthermore, human insulin and C-peptide were readily detectable in 1.1B4 cells [25]'.

2. The Reviewer comments that, '*In the methods, the cells or pseudoislets were administered in 500 ul serum free RPMI medium subscapularly using a needle. What is the region for subscapular injection? Are fats or muscles?*'. We would like to clarify that the cells or pseudoislets were injected into the neck fat pad in the subscapular region. We have added the comment (page 7, line 25): '..subscapularly into adipose tissue deposit at back of the neck using a 25G needle.'

3. The Reviewer comments that, '*In Figure 3, the hematoxylin and eosin and insulin staining of cell masses are not convincing. It should be revised and explained in detail*'. Please see response to Reviewer 2, point 2 above.