

## 25786-Answering reviewers

Comment	Response
<p><b>Reviewer 1</b></p> <p><b>General comments (1)</b> The importance of the research and the significance of the research findings This research is important in terms of reporting the result of clinical research using human embryonic stem cells.</p> <p>(2) The novelty and innovative nature of the research This is an innovative research describing about possibility of human embryonic stem cells in type-2 diabetes mellitus.</p> <p>(3) The quality of the manuscript's presentation and readability It is well written, however, the content of the article may be appropriate for clinical category such as Case Report or Clinical Trials study, etc. other than Basic study.</p> <p>(4) The ethics-related aspects of the research Numbers of informed consent documents and patients in the manuscript does not match. Please confirm the informed consent.</p> <p><b>Specific comments</b></p> <p>Title: It accurately reflects the major topic and contents of the study.</p> <p>Abstract: It appropriately describes about the content of the manuscript.</p> <p><b>Introduction:</b> There are some characters that are not shown appropriately. Please correct them in overall manuscript.</p>	<p>We have now sent Informed consent forms for all the 95 patients enrolled.</p> <p>We have again formatted the MS properly. We have corrected the missing characters.</p>

<p><b>Materials and Methods:</b> Protocol and patient selection criteria should be explained more in detail.</p> <p><b>Results:</b> Please explain the meaning of gm% for HbA1c levels.</p> <p><b>References:</b> Please check reference citations carefully.</p> <p><b>Figure and Table:</b> The real numbers showing the improvement in each patient should be indicated.</p>	<p>It is corrected now and has been highlighted in MS. The detailed procedure of culturing has been elaborated in previous studies. References have been given.</p> <p>We have removed gm.</p> <p>It is checked</p> <p>In both tables and figures we have given the absolute number of patients improved as well as their percentages.</p>
<p><b>Reviewer 2</b></p> <p>This research article is excellently written. This work has been performed as an advance step for the previous case studies by Dr. Shroff G (J Diabetes Mellitus 5, 313-318, 2015). The current report will make a great contribution to the promotion of the hESC-based regenerative medicine in the field of Diabetes. However, there are several issues to be addressed before publication in World Journal of Stem Cells. I do hope that the author would appropriately revise the manuscript for readers in broad fields. Points to be addressed</p>	

**Materials and Methods** 1) In page 8, lines 4-5, the sentence "... so that to allow the recipient's immune system to be active, ..." seems rather confusive for non-expert readers. The aim of the intramuscular administration of smaller numbers of hESCs is to induce immune tolerance, rather than to provoke active immune reactions. Therefore, it would be better to rewrite the sentence as "... so as to induce immune tolerance against hESCs, ..." and so on. 2) In page 8, line 6, the description "... via intravenous (i.v) route to "home in" the required area ..." lacks concreteness. Actually, it is a copy of the sentence in the previous report (Shroff G, J Diabetes Mellitus 5, 313-318, 2015), which also lacks the concrete description regarding the procedure. For readers to correctly understand the work, the detailed transplantation procedure should be written. For example, readers cannot understand which vein (portal vein or splenic vein?) or which artery (dorsal pancreatic artery or inferior pancreatico-duodenal artery or others?) was used as a route for transplantation.

### Results:

1) Author should describe the effects of hESC-based transplantation therapy on the incidence of hypoglycemia by summarizing the results in a Table. I think that it is highly possible that the therapy effectively reduced the incidence of hypoglycemia.

This paragraph has been modified as suggested and has been highlighted in manuscript.

We have explained this in discussion section (page 11) that hESC therapy overcomes the limitation of hypoglycemia that is usually associated with other conventional drugs along with relevant references and we also have reported in Results that during our study, we did not observe any incidence of hypoglycemia in our patients. The

<p><b>Discussion: 1)</b> In page 11, lines 7-9, the sentence “hESCs are pluripotent and derived from human fertilized eggs, therefore they are free from the risk of immunosuppression and transplant rejection by recipients[16, 31, 32]” contains erroneous expressions. Since hESCs are allogenic to recipients, they cannot be free from the risk of immunosuppression and transplant rejection by recipients. Author should rewrite this sentence as “hESCs are pluripotent and derived from human fertilized eggs, therefore they have a much lower risk of tumorigenesis than iPSCs. Moreover, hESCs reportedly possess immune-privileged properties#1, #2, whose beneficial effects are further strengthened by the advanced intramuscular administration” and so on. #1 Li L et al. Stem Cells 22:448-456, 2004. #2 Drukker M et al., Stem Cells 24: 221–229, 2006</p>	<p>changes have been highlighted in MS.</p> <p>It is corrected now and the mentioned references have also been incorporated. All the modified lines are highlighted in Manuscript</p>
<p><b>For the Comments given in Edited Manuscript (25786)</b></p>	
<p><b>Comment 1</b></p> <p>Any research study (clinical trial) that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects on health outcomes must be registered. Authors have 6 mo from the first patient enrollment to register the trial, but BPG recommends registration prior to enrollment. This registration policy applies to prospective, randomized, controlled trials only. Authors must provide the</p>	<p>As it was a retrospective study, there was no registration for the clinical trial. Therefore no URL or registration number can be added in Footnote.</p>

<p>registration identification number and the URL for the trial's registry. In addition, the registration information must be provided in a PDF format, and the registered URL and registration identification number must also be mentioned as a footnote in the manuscript text.</p>	
<p><b>Comment 2</b></p> <p>Please offer signed pdf files of conflict of interest statement</p>	<p>The doc 25786-Conflict-of-interest statement has been attached</p>
<p><b>Comment 3 (Biostatistics)</b></p> <p>Any manuscript describing a study (basic research and clinical research) that used <b>biostatistics must include a statement in the Materials and Methods section affirming that the statistical review of the study was performed by a</b> biomedical statistician. Statistical review is performed before the submission or after peer-review. The author invites an expert in Biomedical Statistics to evaluate the statistical method(s) used in the study, including but not limited to the <i>t</i>-test (group or paired comparisons), chi-square test, ridit, probit, logit and regression (linear, curvilinear, or stepwise) modeling, correlation, analysis of variance, and analysis of covariance. The review by the biomedical statistician is conducted with respect to the following points: (1) Statistical methods are adequately and appropriately described when they are used to verify the results; (2) Whether the statistical techniques are suitable or correct; (3) Only homogeneous data can be averaged. Standard deviations are preferred to standard errors. The number of observations and subjects (<i>n</i>) is given. Losses in observations, such as drop-outs from the study, are reported; (4) Values, such as ED50, LD50 and IC50, have the 95% confidence limits calculated and have</p>	<p>No statistics is involved; the data has been presented as per the counting frequencies (Number and percentage of patients' improvement).</p>

<p>been compared by weighted probit modeling (using the functions described by Bliss and Finney); and (5) The word “significantly” is replaced by its synonyms (if it indicates extent) or the <i>P</i> value (if it indicates statistical significance). In addition, a copy of any approval document(s)/letter(s) or waiver should be provided to the BPG in PDF format</p>	
<p><b>Comment 4 (Data sharing statement)</b></p> <p>Basic research and clinical research studies require a data sharing statement. The data sharing statement will be provided in the title page, and will be presented in the following form: Technical appendix, statistical code, and dataset available from the corresponding author at Dryad repository, who will provide a permanent, citable and open-access home for the dataset. In addition, a copy of the signed statement should be provided to the BPG in PDF format.</p>	<p>Our all publications are pubmed indexed and are available online (open access). We shall not be taking the membership of Dryad Repository.</p>
<p><b>Comment 5</b></p> <p>Audio core tip</p>	<p>Have given in text (~250 words) in manuscript itself in Title page and a MP3 FILE has also been sent</p>
<p><b>Comment 6</b></p> <p><b><i>Writing requirements for each subsection</i></b></p> <p><b>(1) Background</b></p> <p>To summarize concisely and accurately the relevant background information so that readers may gain some basic knowledge about your study’s relevance and understand its significance for the field as a whole.</p>	<p>Type 2 Diabetes mellitus (T2DM) is the most common lifestyle disorders nowadays. At present, there is no cure for diabetes. Human Embryonic stem cells (hESCs) is a new approach to treat it. hESC can produce unlimited number of</p>

<p><b>(2) Research frontiers</b></p> <p>To introduce briefly the current hotspots or important areas in the research field as related to your study.</p> <p><b>(3)Innovations and breakthroughs</b></p> <p>To summarize and emphasize the differences, particularly the advances, achievements, innovations and breakthroughs, as compared to other related or similar studies in the literature, which will allow the readers to assimilate the major points of your article.</p> <p><b>(4) Applications</b></p> <p>To summarize the practical applications of your research findings, so that readers may understand the perspectives by which this study will affect the field and future research.</p>	<p>pancreatic islet cells and can be easily transplanted while tissue culturing directly to endoderm and then to pancreatic and islet precursor cells, where they produce <math>\beta</math> cells. They have less chances of immune mediated rejection.</p> <p>Stem cell therapy, Transplantation, Diagnostic scales and parameters for diabetes</p> <p>Undoubtedly hESC have better potential than other treatments. Being pluripotent and derived from human fertilized eggs, they have a much lower risk of tumorigenesis than iPSCs. They are easily transplantable.</p> <p>The patients were treated with hESC therapy have shown remarkable improvement. This innovative treatment approach have overcome the life long insulin dependence of patients and have also reduced the intake of oral hypoglycemic and other conventional drugs</p>
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<p><b>Comment 7</b></p> <p><i>Writing requirements for each subsection</i></p> <p><b>(1)Case characteristics</b> Please summarize main symptoms in one sentence.</p> <p><b>(2) Clinical diagnosis</b> Please summarize main clinical findings in one sentence.</p> <p><b>(3)Differential diagnosis</b> Please summarize thoughts and methods for differential diagnosis in one sentence.</p> <p><b>(4) Laboratory diagnosis</b> Please summarize laboratory testing methods and major findings in one sentence.</p> <p><b>(5) Imaging diagnosis</b> Please summarize imaging methods and major findings in one sentence.</p> <p><b>(6) Pathological diagnosis</b> Please summarize pathological methods and major</p>	<p><b>General symptoms:</b> Insulin resistance, diabetic neuropathy, weakness in limbs, poor vision</p> <p>Decreased insulin and HbA<sub>1C</sub> level and reduced intake of oral hypoglycemic drugs with and without insulin</p> <p>A) hESC transplantation via different routes using standard protocol.</p> <p>B)Measurement of improvement levels for 11 different parameters using NFS scale (Grade 1-5 in direction Bad to Good)</p> <p><b>After treatment:</b> <b>Hb1Ac:</b> 70.5% of patients had levels <math>\leq 6.5</math> (WHO cut off range) <b>Insulin:</b> 65.9%of patients reached the normal range Overall, 94.8% patients showed improvement by at least one grade of NFS scale at the end of treatment</p> <p>There were no such findings</p> <p>No such studies were carried out</p>
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<p>findings in one sentence.</p> <p><b>(7) Treatment</b></p>	<p>Patients with a previous history of diabetes and its associated complications were enrolled and injected hESC lines as per the defined protocol. The patients were assessed using Nutech functional score (NFS), a numeric scoring scale to evaluate the patients for 11 diagnostic parameters. Patients were evaluated at baseline and at end of treatment period 1 (T1). All the parameters were graded on NFS scale from 1 to 5. Highest possible grade (HPG) of 5 was considered as the grade of best improvement.</p>
<p><b>Comment 8</b></p> <p>Please add PubMed citation numbers and DOI citation to the reference list and list all authors. Please provide PubMed citation numbers for the reference list, e.g. PMID and DOI, which can be found at <a href="http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed">http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed</a> and <a href="http://www.crossref.org/SimpleTextQuery/">http://www.crossref.org/SimpleTextQuery/</a>, respectively. The numbers will be used in the E-version of this journal. Thanks very much for your co-operation.</p> <p>Such as: 1 <b>Nayak S</b>, Rath S, Kar BR. Mucous membrane graft for cicatricialelectropion in lamellar ichthyosis: an approach revisited. <i>OphthalmolPlastReconstrSurg</i> 2011: e155-e156 [PMID: 21346670 DOI: 10.1097/IOP.0b013e3182082f4e]</p>	<p>The references are corrected as suggested</p>



