

Point by Point Reply to Reviewers

Manuscript Title: Genome engineering and disease modeling in pancreatic beta cells via programmable nucleases for insulin gene therapy; promises of CRISPR/Cas9 technology

Manuscript ID: 63308

We thank the reviewers for their insights and helpful suggestions, and the Editor for the overall evaluation. Below are listed the related comments and replies/actions taken regarding each comment. All changes were marked red in the manuscript.

Response to the Comment of Reviewer 1:

Reviewer's comment: Please enjoy the reading.

Response: We thank the Reviewer for reviewing our manuscript and for his/her comment.

Responses to the Comments of Reviewer 2:

1) I cannot get the focus of this manuscript from the abstract. The abstract should focus on the main content of this review, rather than introducing the history of gene-editing technology, which can be removed to the introduction section.

Response & Action Taken: Thank you for the comment. We redesigned the abstract as suggested by the Reviewer. We believe that in its current form, our abstract directly reflects the content of our manuscript, which covers the main types and general features of programmable nucleases and finally focuses on CRISPR/Cas technology particularly in beta cell development and function, and disease modeling/testing of novel therapeutic approaches in diabetes:

Abstract section, page 2:

*Targeted genome editing is a continually evolving technology employing programmable nucleases to specifically change, insert, or remove a genomic sequence of interest. **These advanced molecular tools** include meganucleases, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and RNA-guided engineered nucleases (RGENs), which create double-strand breaks at specific target sites in the genome, and repair DNA either by homologous recombination in the presence of donor DNA or via the error-prone non-homologous end-joining (NHEJ) mechanism. **A recently** discovered group of RGENs **known as** CRISPR/Cas9 gene-editing **systems** allowed precise genome manipulation revealing a causal association between disease genotype and phenotype, **without the need for the reengineering of the specific enzyme when targeting different sequences**. CRISPR-Cas9 has been successfully employed as an ex vivo gene-editing tool in embryonic stem cells and patient-derived stem cells to understand pancreatic beta-cell development and function. RNA-guided nucleases also open the way for the generation of novel animal models for diabetes **and allow testing the efficiency of various therapeutic approaches in diabetes, as summarized and exemplified in this manuscript.***

2) Many pages in the article describe the development history of gene editing technologies, including ZFN, Talen and CRISPR-cas9. I suggest that some of the

content can be deleted and the paper needs to focus on the content as your title demonstrated.

Response & Action Taken: We thank the Reviewer for his/her comment. We give a brief history of each gene-editing technology as an introductory context for the corresponding sections. We believe that this provides precise and clear introductory information making the usually complex topic of programmable nucleases easier to comprehend; and complies with the title of our manuscript which refers to the programmable nucleases in general, while specifically referring to the CRISPR/Cas9 system: “Genome engineering and disease modeling in pancreatic beta cells via programmable nucleases for insulin gene therapy; promises of CRISPR/Cas9 technology”.

3) The article described in detail the application of lentivirus vector to transfection the edited insulin gene into mice, but at present, there are many controversies about the application of lentivirus in vivo, and the application of AAV may be safer and more effective.

Response & Action Taken: Thank you for this comment. It is true that different viral vector systems can be used for testing our strategy specified in this manuscript, AAV being one of the suitable vectors. As expected, each vector system has its advantages and disadvantages and may be associated with different controversies regarding *in vivo* applications. As a group with years of experience on lentiviral vector systems, we are comfortable to state that lentiviruses are one of the most frequently preferred vectors in many *in vitro* as well as *in vivo* settings for their efficiency, long-term expression they provide, and the high safety of the latest generations. In fact, lentiviral vectors were preferred as transgene carriers for FDA-approved Kymriah and EMA-approved Zynteglo gene therapy medications ^[1]. Many clinical trials test strategies based on lentiviral vector-mediated gene transfer for diseases such as X-SCID, transfusion-dependent beta-thalassemia, sickle cell anemia, and Fanconi anemia ^[1]. Overall, lentiviral vectors are considered as one of the safest gene therapy vectors, and no integration-induced mutagenesis has been reported in the clinical trials performed to date. We discuss our strategy in general within the text (page 18), and specify the vector in the corresponding figure legend.

4) Have you experimented with what is depicted in Figures 3 and 4 or was it just conceived and never worked on?

Response & Action Taken: The strategy depicted in Figures 3 and 4 has been tested by our research team with successful results. The study continues in different aspects, the results of which will be reported upon completion.

5) The figures are well prepared. A copyright license may be required if specific software was used in the creation of the figures.

Response & Action Taken: Thank you for this comment. All figures were designed and prepared by members of our team, Salih Sanlioglu, and Yunus Emre Eksi, respectively, outside the scope of a copyright license.

Responses to the comments of the Editor:

1) The “Author Contributions” section is missing. Please provide the author's contributions.

Response & Action Taken: Author Contributions is included in the manuscript:

Page 20, line 1:

Author Contributions. YEE drafted the manuscript and prepared the figures; ADS prepared and revised the manuscript; BA and BEO revised the manuscript; SS designed both the study and the figures.

2) The authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s).

Response & Action Taken: Approved grant application forms are uploaded to the system.

3) Please obtain permission for the use of picture(s). If an author of a submission is re-using a figure or figures published elsewhere, or that is copyrighted, the author must provide documentation that the previous publisher or copyright holder has given permission for the figure to be re-published; and correctly indicating the reference source and copyrights.

Response & Action Taken: None of the pictures provided in our manuscript requires permission, as they were originally designed and prepared by our research team. We have referred to two different articles for Figures 1 and 2, because of partial similarity in some parts of the design.

References:

- 1 Milone MC, O'Doherty U. Clinical use of lentiviral vectors. *Leukemia* 2018; 32: 1529-1541 [PMID: PMC6035154 DOI: 10.1038/s41375-018-0106-0]