

REVIEW
of the official reviewer for dissertation work of
Alzhanuly Bakhytzhan on the theme «Development of a cell therapy approach for diabetes by engineering tunable insulin production in β -cells» presented for the degree of Doctor of Philosophy (PhD) in the specialty «6D060700 - Biology».

№	Criteria	Eligibility (one of the options must be checked)	Justification of the position of the official reviewer
1.	The topic of the thesis (as of the date of its approval) corresponds to the directions of development of science and/or state programs	<p>1.1 Compliance with priority areas of science development or government programs:</p> <p>1) The thesis was completed within the framework of a project or target program financed from the state budget (indicate the name and number of the project or program)</p> <p>2) The thesis was completed within the framework of another state program (indicate the name of the program)</p> <p>3) <u>The dissertation corresponds to the priority direction of the development of science, approved by the Higher Scientific and Technical Commission under the Government of the Republic of Kazakhstan (indicate the direction)</u></p>	<p>The dissertation work fully corresponds to the priority direction «Life and Health Science», and the specialized scientific area «Genetic engineering and cellular technologies» approved by the Higher Scientific and Technical Commission under the Government of the Republic of Kazakhstan.</p> <p>The dissertation work also was partially supported by the funds of a grant project AP08857430 "Identification of a new minimally invasive biomarker for the diagnosis and prognostics of diabetic retinopathy based on microRNAs", supported by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan. The project was also supported by administrative resources of M.A. Aitkhozhin Institute of Molecular Biology and Biochemistry.</p> <p>A part of the research was completed at the Diabetes Research Center at the University of British Columbia (Vancouver, Canada) within grant funding of Juvenile Diabetes Research Foundation (now – "Breakthrough T1D").</p>
2.	Importance for science	The work makes/does not make a significant contribution to science, and its importance is well disclosed/not disclosed	The work makes a significant contribution to science, and its importance is well disclosed.

			<p>The author has developed a novel approach for activating insulin gene expression in H1 human embryonic stem cells using the CRISPR-dCas9 system fused to transcriptional activator VP64 and repressor KRAB domains. Stable cell lines expressing these systems were successfully obtained, and their effectiveness was first demonstrated in ordinary human HEK 293 cells and then in H1 stem cells which were later differentiated into insulin-producing β-cells.</p> <p>Importantly, the author demonstrated the ability of the genetic construct to regulate insulin gene expression during cell cultivation and differentiation, which holds promise for future development of cell-based therapies for diabetes.</p>
3.	The principle of independence	<p>Self-reliance level:</p> <p>1) High; 2) Medium; 3) Low; 4) No independence</p>	High.
4.	The principle of inner unity	<p>4.1 Justification of the relevance of the thesis:</p> <p>1) Justified; 2) Partially justified; 3) Not justified.</p>	<p>4.1. Justified (1).</p> <p>The dissertation work is devoted to one of the highly relevant problems of modern medicine – developing a novel cell therapy for type 1 diabetes mellitus using cutting-edge genome editing technology CRISPR/Cas9. The topic is undoubtedly important: despite significant medical progress, type 1 diabetes remains an incurable disease, and existing insulin therapy does not fully prevent long-term complications.</p>
		<p>4.2 The content of the thesis reflects the topic of the thesis:</p> <p>1) Reflects; 2) Partially reflects; 3) Does not reflect</p>	<p>4.2. Reflects (1).</p> <p>The research's content fully reflects the thesis.</p>

	<p>4.3. The purpose and objectives correspond to the topic of the thesis: 1) <u>correspond</u>; 2) partially correspond; 3) do not correspond</p> <p>4.4 All sections and provisions of the thesis are logically interconnected: 1) <u>completely interconnected</u>; 2) the interconnection is partial; 3) there is no interconnection</p> <p>4.5 The new solutions (principles, methods) proposed by the author are reasoned and evaluated in comparison with the known solutions: 1) <u>there is a critical analysis</u>; 2) partial analysis; 3) the analysis does not represent one's own opinions, but quotes from other authors</p>	<p>4.3. Fully corresponds (1).</p> <p>4.4. Completely interconnected (1).</p>
5.	<p>5.1 Are the scientific results and provisions new? 1) <u>completely new</u>; 2) <u>partially new (25-75% are new)</u>; 3) not new (less than 25% are new)</p> <p>5.2 Are the dissertation findings new? 1) <u>completely new</u>; 2) <u>partially new (25-75% are new)</u>; 3) not new (less than 25% are new)</p> <p>5.3 Technical, technological, economic or management decisions are new and reasonable: 1) <u>completely new</u>; 2) <u>partially new (25-75% are new)</u>; 3) not new (less than 25% are new)</p>	<p>5.1. Completely new. Insulin transcription modification studies using genome editing technology has been only a few times earlier, therefore the scientific results and provisions can be evaluated as completely new.</p> <p>5.2. Completely new.</p> <p>5.3. Partially new. Some technics and research decisions are quite popular ones in biomedical research studies, so they can be evaluated as partially new without affecting the significance of this work.</p>
Scientific novelty principle		

6.	The validity of the main findings	All main conclusions are/are not based on scientifically significant evidence or well-grounded (for qualitative research and areas of training in the arts and humanities)	All main conclusions are based on scientifically significant evidence. The author correctly interprets the experimental data, draws justified and well grounded conclusions, and adequately discusses the study's limitations, including reduced glucose responsiveness of the obtained β -cells compared to native islet cells, which is logical considering their in vitro origin.
7.	The main provisions for the defense	It is necessary to answer the following questions for each provision separately: 7.1 Is the provision proven? 1) proven; 2) rather proven; 3) rather not proven; 4) not proven 7.2 Is it trivial? 1) yes; 2) no 7.3 Is it new? 1) yes; 2) no 7.4 Application level: 1) narrow; 2) medium; 3) wide 7.5 Is it proven in the article? 1) yes; 2) no	7.1. Proven. 7.2. No. 7.3. No. 7.4. Medium 7.5. Yes
8.	The principle of reliability Reliability of sources and information provided	8.1 Choice of methodology - is justified or the methodology is described in sufficient detail 1) yes; 2) no	8.1. Yes. The dissertation is based on a full spectrum of modern molecular biology and cell technologies, including cell culture, molecular cloning, lentiviral vector construction, transduction, transfection, immunostaining, quantitative PCR, Western blotting, and others. Of particular note is the successful design

		<p>8.2 The results of the thesis were obtained using modern methods of scientific research and methods of processing and interpreting data using computer technologies: 1) yes; 2) no</p> <p>8.3 Theoretical conclusions, models, identified relationships and patterns have been proven and confirmed by experimental research (for areas of training in pedagogical sciences, the results have been proven on the basis of a pedagogical experiment): 1) yes; 2) no</p> <p>8.4 Important statements are confirmed / partially confirmed / not confirmed by references to current and reliable scientific literature</p> <p>8.5 Used literature sources are sufficient/not sufficient for a literature review</p>	<p>of guide RNAs targeting the insulin promoter and construction of the CRISPR-dCas9 complexes. The combination of these approaches ensured reliability of the obtained results.</p> <p>8.2. Yes.</p> <p>8.3. Yes. The author correctly interprets the experimental data, draws justified conclusions, and adequately discusses the study's limitations, including reduced glucose responsiveness of the obtained β-cells compared to native islet cells, which is logical considering their in vitro origin.</p> <p>8.4. Statements are confirmed.</p>
9	Practical value principle	<p>9.1 The thesis has theoretical value: 1) yes; 2) no</p>	<p>Yes. The research provided obtaining new fundamental knowledge on cell biology of HEK 293 human cells as well as on the biology of H1 stem cells. These findings hold significant theoretical knowledge in further understanding the behavior of these cells in studies using genome-editing technology.</p>

		<p>9.2 The thesis is of practical importance and there is a high probability of applying the results obtained in practice:</p> <p>1) yes; 2) no</p>	<p>Yes.</p> <p>The findings lay the foundation for the development of innovative cell-based therapies for type 1 diabetes, offering a potential solution to the shortage of healthy and functional donor β-cells. The work is fully aligned with global efforts to develop gene editing and regenerative approaches for compensating insulin deficiency.</p>
		<p>9.3 Are the practice suggestions new?</p> <p>1) completely new; 2) partially new (25-75% are new); 3) not new (less than 25% are new)</p>	<p>Completely new.</p>
10.	The quality of writing and design	<p>Academic writing quality:</p> <p>1) high; 2) average; 3) below average; 4) low.</p>	<p>1. High.</p> <p>The dissertation is well-structured, with clear presentation of objectives, methodology, results and discussion, and conclusion. The work fully complies with academic standards and demonstrates the candidate's competence in scientific research and academic writing.</p>
11.	Notes on a thesis		<p>1. Further studies should focus on more detailed functional improvement and also better evaluation of the derived β-cells, particularly their insulin secretion dynamics in response to glucose stimulation.</p> <p>2. In vivo testing on animal models would be a logical next step for assessing safety and viability of the generated cells though it is understood that before the in vivo step the candidate will most probably focus on improving the functionality of the cells first.</p> <p>3. Broadening the panel of transcription factors may potentially enhance the differentiation efficiency. In particular, VP64 domain use is getting "older" now and there are quite a few modern modifications for that. But considering that those new versions are stronger than VP64 alone, reaching these results with insulin transcription modulation using VP 64 only is already a good result.</p>

		<p>4. I would also bring the attention of the candidate to another challenge in all this type of research which is immediate attack of the transplanted cells or tissue by immune system of the host. We should not forget that type 1 diabetes is autoimmune disease. Therefore, it will be advisable to also keep this challenge in your mind when you will be continuing your research.</p> <p>5. Another recommendation is to modify the differentiation protocol for the hope to improve the glucose-responsive function of the final cells, because we see that most of the ability of your cells were lost during the differentiation process. What has been gained is still very good, but the candidate might also need to take a deep look at the differentiation steps and protocols.</p>
12.	<p>Scientific level of the doctoral student's articles on the topic of research (in case of defense of the dissertation in the form of a series of articles, the official reviewers comment on the scientific level of each article of the doctoral student on the topic of research)</p>	<p>The scientific level of the articles are quite high. This is logical because the significance of the research is very high as diabetes type 1 still remains as non-curing disease worldwide.</p>
13.	<p>Decision of the official reviewer (pursuant to paragraph 28 of the present Model Regulations)</p>	<p>The PhD dissertation of Alzhany Bakhytzhan is an independent, highly relevant, methodologically sound, and scientifically significant work that contains elements of scientific novelty and clear practical implications. The dissertation fully meets the requirements for the PhD degree. Therefore, in my opinion, the candidate fully deserves to be</p>

		awarded the Doctor of Philosophy (PhD) degree in specialty «6D060700 – Biology»
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In reviews, official reviewers indicate one of the following solutions:

1) to award the degree of Doctor of Philosophy (PhD) or Doctor of Specialization;

2) send the thesis for revision (except for cases of thesis defense in the form of a series of articles);

3) refuse to award the degree of Doctor of Philosophy (PhD) or Doctor of Specialization.

Copies of the reviews of the official reviewers are handed over to the doctoral student no later than 5 (five) working days before the defense of the thesis.

Official Reviewer:

Candidate of Medical Sciences,
President of the Kazakhstan Society for the
Study of Diabetes
Member of the Executive Committee of the Asian
Association for the Study of Diabetes

(place of work, academic title)



Zhanay A. Akanov
(FULL NAME)