	«С.Ж. АСФЕНДИЯРОВ АТЫНДАҒЫ ҚАЗАҚ ҰЛТТЫҚ МЕДИЦИНА УНИВЕРСИТЕТІ» КЕАҚ НАО «КАЗАХСКИЙ НАЦИОНАЛЬНЫЙ МЕДИЦИНСКИЙ УНИВЕРСИТЕТ ИМЕНИ С.Д. АСФЕНДИЯРОВА»	
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REVIEW

of an official reviewer for the dissertation of Mukhatayev Z.Y. on the topic: "Development of approaches for stimulation of T-regulatory cells for immunotherapy of vitiligo", for the degree of Doctor of Philosophy (Ph.D.) in specialty 6D060700-Biology.


1. Relevance of the research topic and its relationship with general scientific and national programs

This Ph.D. dissertation is dedicated to studying the phenotypical characteristics of T regulatory cells (Tregs) in the periphery of vitiligo patients, studying the effect of antibiotics to control depigmentation in vitiligo mouse model, and develop the approaches to stimulate and generate antigen-specific Tregs transfected with chimeric antigen receptor (CAR) as a cell-based immunotherapy of vitiligo.

Tregs are a subpopulation of T cells, which play a key role in the regulation of the immune response. By controlling the immune response to self and foreign antigens, Tregs can prevent the development of autoimmune disorders. Nevertheless, in the majority of autoimmune disorders, as well as vitiligo, the regulation of the immune response is impaired, including deficiency or impaired activity of Tregs. The percentage of immunosuppressive Tregs in vitiligo skin is greatly reduced compared to healthy skin, suggesting that autoreactive cytotoxic T cells remain uninhibited, contributing to depigmentation in vitiligo. Increased Treg infiltration was directly associated with repigmentation in a spontaneous mouse model of the disease, which is suggestive of a role for Tregs in preventing autoimmunity towards melanocytes. Thus, the detailed characterization of Tregs in line with vitiligo course and development of approaches of stimulation of Tregs potentially benefit to treat vitiligo and other autoimmune diseases. The topic of the dissertation is very relevant and is in line with the global trends of modern immunology and Government's Healthcare programs.

2. Scientific results and their validity

Ph.D candidate – Mukhatayev Z.Y. evaluated the phenotypical characteristics of Tregs and showed that the number of circulating Tregs that express functional marker CD39 and adhesion/homing receptor CD44 was shown to be greatly

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reduced in the peripheral blood of vitiligo patients. Also, the candidate proposed novel approaches to generate antigen-specific transgenic Tregs. He demonstrated the efficacy of using antigen-specific transgenic CAR Tregs in a vitiligo-prone mouse model and for the first time for autoimmune skin diseases. Mukhatayev Z.Y. also tested the suppressive ability of this GD3-specific CAR Tregs toward melanocyte-specific cytotoxic T cells *in vitro* and showed their immunosuppressive activity.

Another approach to prevent depigmentation in vitiligo using antibiotics to drive microbial diversity change was developed. Obtained results showed that Neomycin promotes the infiltration of vitiligo skin by Tregs and stimulates their immunosuppressive activity that resulted in the prevention of depigmentation in the vitiligo-prone mouse model.

The scientific results obtained by the Ph.D candidate are highly valid and significant.

3. The validity and reliability of the results and conclusions presented in the dissertation


Mukhatayev Z.Y. used novel and innovative methods to achieve the results, including fluorescent microscopy, immunohistochemistry, flow cytometry, viral transduction, and live-cell imaging. Moreover, the dissertation includes the methodological approaches that were developed by the Ph.D candidate.

The research was performed using the adequate objectives, experimental models, and reliable sampling. The statistical analysis of the obtained results was performed using the most suitable methods, and *in vivo* results were verified by a statistician at the core facility at Northwestern. The provided figures are well-prepared with reasonable interpretation.

Thus, the results of the dissertation research are reliable and valid. The results explanations and presentation are correct and satisfactory.

4. The novelty of the scientific results and conclusions presented in the dissertation

Undoubtedly, the performed results are novel and will attract the attention of the scientists that specialize in the field of immunology and autoimmune pathology. Mukhatayev Z.Y. at the first time described the phenotypical characteristics of Tregs that provide the mechanisms of decreased

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immunosuppression in the vitiligo sites and their distribution define the disease progression. The observations regarding the reduced number of Tregs prompted the author to develop new methods and approaches for repopulating the number of Tregs to the vitiligo lesions. One approach was proposed to use Neomycin to drive microbial diversity change to prevent depigmentation in vitiligo. Another approach is based on the adoptive transfer of GD3-specific CAR Tregs that provide significantly enhanced immune tolerance in vitiligo mice in comparison with untransduced Tregs. Of note, for this, Mukhatayev Z.Y. for the first time identified the target antigen - ganglioside D3, which is overexpressed on epithelial cells including melanocytes.

5. The practical and theoretical significance of research

The research contains several practical and theoretical significances, which contribute to the fundamental and applied immunology. The theoretical significance of the research is performed as a new knowledge of decreased subpopulation of peripheral blood Tregs expressing immunosuppressive marker CD39 and adhesion/homing receptor CD44 in vitiligo patients that contributes in understanding of autoimmune pathology.

Mukhatayev Z.Y. also developed practical approaches to stimulate Tregs in vitiligo mice model. Mukhatayev Z.Y. performed *in vivo* experiments using antibiotics to drive microbiome change in favor of halting depigmentation during the vitiligo progression. The author demonstrated that Neomycin induced the accumulation of Tregs in the skin and stimulated the activity of these cells in mice with vitiligo.

The practical significance described in this dissertation also includes the discovered overexpression of ganglioside D3, which was further used in the development of GD3-specific CAR Tregs. Moreover, during the implementation of the thesis, the author developed several methodological approaches to analyze the suppressive activity of GD3-specific CAR Tregs toward melanocytes-reactive T cells *in vitro* and to detect melanocyte apoptosis in real-time. Mukhatayev Z.Y. showed *in vitro* and *in vivo* that GD3-specific CAR Tregs can effectively suppress melanocyte-specific cytotoxic T cells and reduce depigmentation. Thereafter, developed approach can be used as a platform in vitiligo immunotherapy contributing in applied dermatology and immunology.

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The abovementioned developed methodological and treatment approaches have a great impact on developing a treatment options for vitiligo and other autoimmune diseases.

6. Comments and suggestions

1. In the process of peer review, a number of questions primarily related to the sample size arose. During the discussion, the applicant clarified these issues.
2. Suggestions are mainly related to the structuring of the dissertation:
 - In the section "Conclusions" it is advisable to more clearly formulate the conclusions for each task, since during the study some extra conclusions were made, which were indicated in the "provisions for the defense"
 - Scattered order of tasks, conclusions and “provisions for the defense” complicated analysis of dissertation.
 - Provisions for the defense #3 sounds like a practical significance.
 These remarks are not essential and do not diminish the scientific value of the study.


7. Correspondence of the topic and thesis to the specialty

The provisions described in this dissertation are reliable to proceed for defense for the specialty 6D060700-Biology, and overall the novelty of the research greatly contributes to the modern immunology.

8. Compliance of the thesis to the p. 5.6.7 Doctoral degree requirements

This Ph.D. dissertation research of Mukhatayev Z.Y. named as “Development of approaches for stimulation of T-regulatory cells for immunotherapy of vitiligo” satisfy all the required elements for Ph.D. dissertation completion, and this performed research is sufficient for gaining a Ph.D. degree in specialty 6D060700-Biology.

The authors provide original results of their investigations. The applied methods and the interpretation and presentation of results correspond to international standard. The given illustrations underline the presented results and facilitate understanding reported experiments. References of concerned literature are extensive and cover the present state of knowledge of Tregs in autoimmune pathologies and modern approaches of immunotherapy based on Tregs transfer. The results of the study have been published in several high-impact peer-reviewed

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journals, indicating the high degree of scientific value of this study.

Summarizing, the thesis addresses interesting and relevant topics of the Tregs contribution in vitiligo and vitiligo immunotherapy demonstrates the author's ability of critical thinking and scientific teamwork in this specific field. The thesis meets the requirements imposed on a PhD. dissertation in Biology. Thereby, I certainly recommend its acceptance.

Official reviewer:

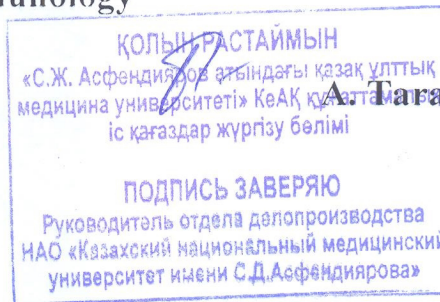
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Date *11/11/2020*