

## 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. Significance of the research topic and its relationship with general scientific and national programs**

Vitiligo is a skin disease affecting 0,5 to 1% of the world population. The currently available treatment options are not effective, and the real need for a new treatment strategy still exists. In this Ph.D. dissertation, the author studies phenotypical characteristics of T regulatory cells (Tregs) in peripheral blood of vitiligo patients and presents a novel treatment that is based on using antigen-specific regulatory T cells with a chimeric antigen receptor (CAR) as cell-based immunotherapy. Besides, Mukhatayev Z.Y. studied the effect of using antibiotics for a gut and skin microbiome and subsequently having an impact on depigmentation in vitiligo. The significance of studying Tregs is currently drawing the attention of the research community who is studying autoimmune diseases. Tregs modulate immune tolerance towards self and non-self-antigens and prevent the development of autoimmune diseases. In vitiligo, a local deficiency of Tregs was reported in the skin. This has raised a necessity to restore functional Treg numbers in the skin. The importance of this Ph.D. research is that the author generated CAR based antigen-specific Tregs, and tested them both *in vitro* and *in vivo*. The results show the significant efficacy of CAR Tregs over the untransduced Tregs in the vitiligo mouse model, and the author performed all the necessary confirmatory analyses to prove his claims. Additionally, the author also participated in the project in which the effect of both ampicillin and neomycin was studied for microbiome change in vitiligo prone mouse model, and as well as the T cell redistribution. Therefore, this study provides a potential treatment strategy based on antigen-specific Tregs providing an insight into the characterization of Tregs, and the effect of antibiotics for Treg distribution in vitiligo. According to the abovementioned, this research presented in this dissertation is significant, and corresponds with the global trends of modern immunology and dermatology, and lies with National Healthcare programs. This study was performed as a part of a grant from the National Institutes of Health RO1 AR057643 and a grant AP05131691 "Molecular mechanisms of the influence of T-regulatory cells on the activity of tumor cells", provided by the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan. Thus, this Ph.D. dissertation work is coordinated with the general scientific and national programs of the Republic of Kazakhstan.

## **2. Scientific results and their validity**

In this Ph.D. research, Mukhatayev Z.Y. presented a novel approach to generate antigen-specific Tregs and studied the phenotypical characteristics of peripheral blood Tregs, as well as testing antibiotics to favor microbial diversity to treat autoimmune skin disease – vitiligo. First, the phenotypical characteristics of Tregs have been evaluated, and the number of circulating T regulatory cells that express functional marker CD39 and adhesion/homing receptor CD44 was shown to be significantly reduced in the peripheral blood of vitiligo patients.

In this study, a target antigen named ganglioside D3 (GD3) was identified in the epithelial cells including melanocytes surrounding the vitiligo lesions. With a mind of targeting this antigen, a transduction protocol was developed and optimized for Tregs generating highly transduced GD3-specific CAR Tregs with stable phenotype. Then, Mukhatayev Z.Y. evaluated the suppressive ability of this GD3-specific CAR Tregs toward melanocyte-specific cytotoxic T cells in the presence of human HLA-A2 melanocytes where these GD3-specific CAR Tregs demonstrated a significantly enhanced protection compared to untransduced Tregs. After promising results *in vitro*, he demonstrated the efficacy of using antigen-specific transgenic CAR Tregs in a vitiligo-prone mouse model, which was for the first time applied for autoimmune skin diseases.

Another approach to stimulate Tregs was studied by altering the microbiome in the vitiligo mouse model. To change the microbiome in vitiligo prone mice, antibiotics were administered to drive microbial diversity. The results showed that neomycin promotes the infiltration of vitiligo skin by T regulatory cells and stimulates their immunosuppressive activity resulted in the prevention of depigmentation.

The scientific results generated by the Ph.D. candidate, Mukhatayev Z.Y., are highly valid and significant.

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

The results presented by Mukhatayev Z.Y. are valid and reliable, and were generated using modern techniques from best practices, and these include flow cytometry, fluorescent microscopy, immunohistochemistry, and immunofluorescence staining, retroviral transduction, and IncuCyte live-cell imaging. *In vivo* and *in vitro* experiments are designed according to scientific standards. The sample size, appropriate controls, and mouse numbers per group were well executed to identify significance among the experimental groups. The generated results were processed using the most suitable parametric and nonparametric statistical analysis. The conclusions are correct and well presented.

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

Mukhatayev Z.Y. developed novel methodological approaches to generate antigen-specific Tregs based on CAR, and to evaluate microbiome diversity caused by

antibiotics in vitiligo. Besides, the author studied the phenotypical characteristics of peripheral blood Tregs in vitiligo patients. The data presented reveal the mechanisms of development of vitiligo, and therefore, can be used to create approaches for immunotherapy of vitiligo, based on the use of antigen-specific Tregs.

### **5. The practical and theoretical significance of research**

The results described in this dissertation contributes to the fundamental and applied science in the field of immunology and dermatology. The described phenotype of circulating Tregs in vitiligo contributes to the fundamental science through the characterization of the main immunoregulatory cells, and their role in mediating the autoimmune reactions. The generated results confirmed the hypothesis of a reduced number and impaired functional activity of Tregs in vitiligo patients.

Along with the theoretical significance of the research, the author is also developed practical approaches to evaluate antigen-specific Tregs in vitiligo mice model. In this study, GD3-specific CAR Tregs were generated and evaluated to suppress effectively melanocyte-specific cytotoxic T cells *in vitro* and halt depigmentation in vitiligo prone mouse model.

Besides, 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 infiltration of Tregs in the skin of the mouse model with vitiligo.

The abovementioned theoretical and practical contributions to the field have a great impact on developing treatment strategies for vitiligo and other autoimmune diseases.

### **6. Comments and suggestions**

1. In provision 2, ganglioside D3 can be specified as a potential target antigen rather than indicating its increased expression in epithelial cells and melanocytes.

2. What was the purpose of measuring other cytokines such as TNF-  $\alpha$ , IFN-  $\gamma$ , and IL-4 besides immunosuppressive IL-10 both *in vitro* and *in vivo*?

3. Why did you use numerous methodologies and techniques? Did you independently interpret the results generated from these methods?

4. The order of tasks and results do not match. The impact of antibiotics in vitiligo can be shifted towards the end of the results section.

5. In provision 6, an additional sentence is recommended to specify that GD3 CAR Tregs delay the rate of depigmentation in the vitiligo mouse model.

6. Why different sets of cytokine panels have been used across the study?

7. Why control group with healthy mice were not included in the studies?

8. It is recommended to write results and discussion sections separately.

9. Provisions should be numbered by the order in the conclusions for clarification.

### **7. Correspondence of the topic and thesis to the specialty**

The results presented in this Ph.D. dissertation contributes to the fundamental and applied dermatology, immunology, and molecular biology. This Ph.D. research

fulfills all the objectives and aims described in the dissertation, and this work has a logical scientific completion. The Ph.D. thesis is fully corresponds to the specialty 6D060700-Biology and highly recommended to proceed for defense.

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

This Ph.D. research presented in this dissertation fulfills all the objectives and aims, and this work has a logical scientific completion. A full literature review regarding the vitiligo research is provided containing a sufficient number of bibliography sources. The applied methods in the thesis research are well performed and contain novel approaches. The provided results in the dissertation were performed Aitkhozhin Institute of Molecular Biology and Biochemistry and at the Department of Dermatology, Feinberg School of Medicine, Northwestern University (Chicago, USA). This dissertation was written independently and proposes a set of new scientific results and provisions that testifies to the author's personal contribution to the field. The practical significance of this Ph.D. dissertation was supported by a provisional US patent "Materials and methods for the treatment of vitiligo" (serial No. 62 / 915.945 in the USA) dated 16.10.2019. The results of the dissertation were published in 13 scientific publications, including 1 article in the International scientific journal "Journal of Investigative Dermatology", which, according to the information base of Thomson Reuters (ISI Web of Knowledge), has an impact factor = 6.29, 4 articles in scientific journals recommended by the Committee for the Control of Education and Science of the Ministry of Education and Science of the Republic of Kazakhstan, 3 abstracts were published in journals with an impact factor (IF = 8.728; IF = 6.29; IF = 4.172) in accordance with the SCOPUS Database and 5 abstracts and reports in materials of international conferences, symposia, and summits.

According to the abovementioned statements, I consider that 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 the specialty 6D060700-Biology complies with all the requirements, and thus, recommended for the defense.

Official reviewer:  
Doctor of Medical Sciences, Professor,  
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at the Scientific Center for Pediatrics  
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Date:

18.11.2020