

## REVIEW OF PhD-DISSERTATION

in Biology (6D060700)

author: Zhussipbek E. Mukhatayev

title: «Development of approaches for stimulation of T-regulatory cells for immunotherapy of vitiligo»

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Autoimmune diseases represent a heterogeneous group of disorders consisting of more than 80 different chronic inflammatory diseases that affect specific target organs or multiple organ systems. A breakdown in the immunological surveillance and the inability of immunoregulatory cells to maintain immune tolerance are considered to be the main factors in the development of autoimmune diseases. T regulatory (Treg) cell population is one of the main immunoregulatory subsets that suppress immune responses by inhibiting proliferation, differentiation, activation, pro-inflammatory cytokine production, and cytotoxic activity of a wide range of effector cell populations, and, thereby, contribute to the maintenance of immune homeostasis. Impaired Treg cell activity is recently considered to play a crucial role in a breakdown of the immune tolerance towards self-antigens and autoimmune diseases progression. Nevertheless, phenotypical and functional characteristics of Treg cells in autoimmune diseases are still not fully revealed. It is of particular importance to identify the mechanisms of impaired suppressive activity and migration of Treg cells in autoimmune lesions and develop the approaches of restoring their activity for treating autoimmune diseases.

The Ph.D. thesis is dedicated to developing the new approaches to restore Treg cell immunosuppressive activity to treat vitiligo, an autoimmune skin disorder. The main objectives of the thesis included the phenotypic analysis of circulating Treg cells in a comparative aspect, in healthy people and vitiligo patients, to reveal the mechanisms of their impaired functional activity and insufficient migration into vitiligo foci, development of methods of Treg cell stimulation by the indirect effect of antibiotics on the intestinal microflora and development of approaches to generate antigen-specific Treg cells *via* transduction of chimeric antigenic receptor (CAR), as well as *in vivo* and *in vitro* analysis of effectiveness of developed approaches as immunotherapy of vitiligo. Thus, the relevance of the thesis is very high and is in line with global trends in modern immunology and dermatology.

The main objectives of the work have been fulfilled successfully, as shown through the evaluation sections in the different contributions, and in the overall case study. During the thesis implementation, a significantly reduced proportion of circulating Treg cells and their decreased expression of suppressive marker – CD39 and adhesion and homing molecule – CD44 were found. It is possible that a decrease in the expression level of these molecules may be involved in the violation of the immunosuppressive properties of Treg cells and their infiltration of vitiligo foci. The thesis has also provided valuable evaluation of the influence of antibiotics on the skin and intestinal microbiome, and the indirect effect on the functional activity of Treg cells *in vivo*. Oral administration of neomycin was shown to abrogate depigmentation in association with enhancement of Treg cell infiltration of vitiligo lesions. The results of this section are published in the highly indexed journal “Journal of Investigative Dermatology”, which, of course, testifies to their high significance.

An important contribution to the field is the disclosure of the melanocytes antigen that overexpressed in vitiligo and the development of the method of generation of antigen-specific Treg cells transfected with CAR to that antigen. The experimental results showed the efficiency

of the adoptive transfer of the generated Treg cells in vitiligo treatment *in vivo*. The merit of this approach has to be emphasized, because many authors just rely on simulation experiments for the evaluation of new immunotherapy approaches. Thus, the during the research an actual implementation for the treatment of vitiligo has been developed. This, of course, represents a lot of work that pays off with very relevant evaluation results.

The methodology used in the thesis is considered appropriate. In the frame of the thesis, new approaches to assess the suppressive activity of Treg cells against self-reactive, melanocyte-specific cytotoxic T cells in the presence of melanocytes have been developed. Furthermore, Zhussipbek E. Mukhatayev developed a new method for the CAR transduction into Treg cells and the cell culture conditions allowing to enhance their subset and maintain immunosuppressive phenotype.

In particular all the contributions are well founded on solid theoretical grounds, and are proven through exhaustive evaluation and, more important, through actual implementation. The thesis satisfies the conditions of a creative scientific work, as is shown through the publications of some of its results in peer-reviewed journal and conferences of significant impact in the embedded real-time systems community.

The author of the thesis proved his ability to perform research and to achieve scientific results. I recommend the thesis for presentation with the aim of receiving the Degree of Ph.D. in Biology.

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