Endometriosis (EMS) is an estrogen-dependent, chronic disease of women of reproductive age. Even **190 million women are suffering from this disease around the world**. EMS is connected with chronic pelvic pain or menstrual disorders. It is estimated that EMS is diagnosed in about 50% of women with the problem of getting pregnant and is considered as one of the most important causes of infertility. It is worth emphasizing that EMS significantly reduces the quality of life of women, increasing the risk of depression. **Despite significant progress in research, the exact pathogenesis of EMS development still remains unexplained.** There is also no effective treatment for this disease that would prevent its recurrence. One of the factors involved in the development of endometriosis is complex disorders in the functioning of the immune system. Recent evidence has shown that endometriosis is associated with changes in systemic and local immunity including quantitative and functional disorders of effector (neutrophils, T cells, natural killer cells), and antigen-presenting cells (monocytes/macrophages, B cells, dendritic cells). A few studies indicated that improper expression of immune checkpoints (ICPs) may also be involved in the pathogenesis of this disease.

Due to the action profile ICPs can be divided into co-inhibitory like programmed cell death receptor-1 (PD-1), T-cell membrane protein-3 (TIM-3), and co-stimulatory molecules such as OX40. ICPs and their ligands are key players in maintaining self-tolerance and modulating the immune responses of effector cells in normal tissues. In numerous cancers, the increased expression of ICPs and their ligands is a known mechanism of tumor cell escape from the control of the immune system. Elevated expression of inhibitory ICPs is associated with reduced T cells' effector function, leading to their apoptosis or 'exhaustion'. Exhausted T cells exhibit defective proliferative capacity, and their effector functions, such as cytotoxicity and cytokine production are impaired. In opposite to inhibitory receptors, co-stimulatory ICPs are responsible for T-cell activation by providing the co-stimulatory second signal.

Taking into account the available literature data on ICP expression in tumors and our research experience, **we hypothesize that disturbances in the proper activity of immune cells, the presence of "exhausted" T cells, and immunosuppression in patients suffering from endometriosis are the result of an imbalance in the expression of co-inhibitory and co-stimulatory immune checkpoints (ICPs).**

The presented project aims to verify the following research hypotheses:

Hypothesis 1: Development and progression of endometriosis are associated with both local (peritoneal fluid, PF) and systemic (peripheral blood, PB) immunosuppressive niches.

Hypothesis 2: Both upregulation and downregulation of the expression of co-inhibitory (TIM-3 and PD-1) and co-stimulatory (OX40) receptors and their ligands (Gal-9, PD-L1, PD-L2, OX40L) on immune system cells may lead to serious disturbances in the immune response in patients with EMS.

Hypothesis 3: Elevated expression of ICPs on peritoneal cells is associated with reduced effector function of T cells leading to their anergy, apoptosis, or 'exhaustion' results in the ineffective removal of endometrial implants from the peritoneal cavity in patients suffering from EMS.

Hypothesis 4: Systemical decreased expression of ICPs in patients with EMS may lead to immunodeficiency, decreased activation of effector cells, and improper antigen presentation process on the periphery.

To verify hypotheses we will conduct a multiparametric analysis of the expression and co-expression of co-inhibitory (TIM-3/Gal-9, PD-1/PD-L1/PD-L2) and co-stimulatory (OX40/OX40L) ICPs on the effector (T cells, NK cells) and antigen-presenting cells (monocytes/macrophages, B cells, and dendritic cells) using flow cytometry in two environments in patients with endometriosis.

Next, we will perform an analysis of the level of RNA of the proposed molecules and miRNA, which regulate the function of T cells in patients with EMS and PB of healthy donors. We will also analyze the cytotoxic activity of T and NK cells, the secretory activity of dendritic cells, the immunosuppressive potential of monocytes/macrophages, and T-cells apoptosis in patients with EMS and the control group.

The results of the presented project will allow a deep understanding of the role of ICPs in the formation of complex disorders of the immune system in patients with endometriosis, including disorders of the proper activity of effector and antigen-presenting cells, mechanisms leading to the formation of "exhausted" T lymphocytes, and development of the immunosuppression in this disease. The obtained data will open the door for innovative ways of endometriosis treatment based on the modulation of the immune response, restoring the activity of effector T cells, and initiating the systemic treatment of this troublesome disease.