For many years, the application of various types of immunostimulators (among which cytokines have proved to play a crucial role) has been one of the ways to enhance anti-tumor immunity. The one of the therapeutic tools to allow changes in the tumor microenvironment are dendritic cells (DC) - generally recognized as the most effective antigen presenting cells. DC genetically modified to produce cytokine such as IL-12 and IL-18, may promote the activation of anti-tumor response. IL-12 is involved in the induction of cellular and humoral immune response. Interleukin 18 is cytokine functionally similar to the IL-12. The effect of IL-18 on the modulation of the immune response to a large extent depends on the presence of IL-12 in the environment. The synergistic action of these two cytokines leads to the induction of Th1-type immune response. In contrast, during the absence of IL-12 in the environment, IL-18 promotes a Th2-type of immune response. Owing to the significant impact of both DC and cytokines such as IL-12 and IL-18 on the formation of this response, it seems reasonable to combine these elements in order to elaborate of new strategies of cancer-treatment. For this purpose, in this proposal BM-DC cells genetically modified for long term co- production of IL-12 and IL-18, stimulated with tumor antigens will be employed.

The main objective of the project is to determine the impact of the application of murine bone marrow-derived dendritic cells (BM-DC) genetically modified to simultaneous production of interleukin (IL) 12 and IL-18, stimulated with tumor antigens (TAg) in MC38 colon cancer immunotherapy. The study will also include the development and characterization of these cells vaccines.

The project provides two stages of research. During the first part, a new genetic modification of dendritic cells for the co-production of IL-12 and IL-18 (BM-DC/IL-12/IL-18) will be developed. For the construction of vectors the latest and most efficient third generation lentivirus vector system will be used. In addition, the phenotype and functional characteristic of BM-DC cells modified to co-produce IL-12 and IL-18 after their stimulation with TAg will be performed. The cells are intended for use as cellular vaccines. In the second stage of the study, the effect of immunotherapy with DC-based vaccines consisted of BM-DC/IL-12/IL-18/TAg on tumor growth and stimulation of the immune response against subcutaneously growing MC38 colon cancer, will be analyzed. Moreover, the ability of modified dendritic cells (DCs) to migrate to sentinel lymph nodes and to influence on changes in spleen cell-activity as well as infiltration of tumor tissues will be determined.

The study performed during this project will allow us to assess whether the use of third generation lentiviral vector system to transduce DCs will increase their efficiency in therapy of MC38 bearing mice. The antitumor cellular response after administration of BM-DC genetically modified for the co-production of IL-12 and IL-18, stimulated with TAg (BM-DC/IL-12/IL-18/TAg) will be determined and compared to the effect of independently administered transduced BM-DC (BM-DC/IL-12/TAg and BM-DC/IL-18/TAg).

The first stage of the research is to construct the co-expression vectors carrying Il12 and Il18 gene and elaborate of new variants of genetically modified dendritic cells with enhanced co-production of IL-12 and IL-18 (BM-DC/IL-12/IL-18, JAWS II/IL-12/IL-18). Subsequently, transduced bone marrow-derived dendritic cells (BM-DC) and dendritic cell of JAWS II cell line will be stimulated with tumor antigens (TAg) (BM-DC/IL-12/IL-18/TAg, JAWS II/IL-12/IL-18/TAg). The phenotypic and functional characteristic of such modified DCs will be evaluated. In the second part of the research, fully characterized BM-DC/IL-12/IL-18/TAg will be used as components of cellular vaccines for anti-cancer immunotherapy of murine MC38 colon carcinoma. Mice with advanced subcutaneously growing MC38 tumor will be treated with vaccines containing BM-DC/IL-12/IL-18/TAg. The effect of their application on tumor growth as well as the level of anti-cancer immune response will be evaluated. Lymphoid organs and tumor tissue will be obtained from treated and untreated mice for further analysis. The ability of modified DCs to migrate to sentinel lymph nodes and their influence on changes in the tumor tissue and in the spleen cells activity will be determined.

Understanding of the phenomenon, associated with the interaction of tumor cells with the immune system, allows the identification of immune cells participating in fight against cancer. In generation of an effective anti-tumor responses, a range of immune cells are involved, among which the most important role is played by dendritic cells, T helper cells (CD4+), cytotoxic T lymphocytes (CD8+) and NK cells. However, administration of cytokines such as IL-12 and IL-18, resulted in increased activity of these cells in the fight against cancer.

Immunotherapy is one of the methods of treating cancer. It is usually used as a supplement to conventional cancer treatments. Current researches on the efficacy of immunotherapy are primarily focused on the induction of specific anti-tumor responses. Owing to the significant impact of both DCs and cytokines such as IL-12 and IL-18 on the formation of this response, it seems reasonable to combine these elements in order to elaborate of new strategies of anti-cancer treatment. For this purpose, in this proposal BM-DC cells genetically modified for long term co-production of IL-12 and IL-18, stimulated with tumor antigens will be employed.