Low levels of vitamin D are considered to be the factors contributing to the development of breast cancer and have a prognostic significance. In addition, vitamin D deficiency deepens after the therapies used in this type of cancer. It has now been found that vitamin D supplementation for the treatment of breast cancer delays the onset of disease, prolonging the survival of patients. In our recent studies, we evaluated the effect of calcitriol (active vitamin D_3 metabolite) and its analogs on the growth and metastasis of mouse 4T1 mammary gland cancer in pre-menopausal model (young, 6-8 week old, mature mice, start of the treatment: 7^{th} day after cells inoculation). Surprisingly, the studied compounds increased lung metastasis without affecting primary tumor growth. Furthermore, it was found that calcitriol or its analogs did not affect the proliferation of 4T1 tumor cells *in vitro*. Further analyzes showed increased expression of various factors that accelerated metastases in tumors and plasma of mice under the influence of vitamin D analogs. Calcitriol also increased collagen accumulation in the lung as well as tumor tissue in earlier stages of tumor progression, which is unfavorable from the point of view of the development of metastases. On the other hand, antimetastatic activity of calcitriol was observed in postmenopausal 4T1 model (ovariectomized, 60-week old mice, start of the treatment: 7^{th} day after cells inoculation) in our studies and in studies by other authors, when calcitriol was injected before 4T1 cells inoculation. Therefore, we have found that calcitriol and its analogs affect the metastatic potential of murine 4T1 mammary tumor cells by affecting host cells (such as fibroblasts or macrophages), and the direction of this influence depends on the hormonal status of the organism and on the time of calcitriol supplementation. As the vitamin D receptor (VDR) is detected in nearly all body cells and immunosuppressive effect of vitamin D is described, the aim of this project is to investigate the effect of vitamin D_3 on cancer-associated macrophages and fibroblasts in mice bearing tumors and in breast cancer patients (premenopausal) and the correlation of characteristic features of tumor cells with pro- or anti-cancerogenic stromal cell polarization under the influence of vitamin D.

To explain the involvement of tumor-associated cells in pro-metastatic activity of vitamin D_3, we plan to use young mice (premenopausal model) bearing metastatic breast cancer 4T1 and E0771.LBM - calcitriol does not affect *in vitro* proliferation of both cell lines; and non-metastatic 67NR - this cell line (4T1 and 67NR come from the same BALB/c mouse tumor) is sensitive to vitamin D analogs *in vitro*. Among tumor microenvironmental cells we would like to focus on fibroblasts (CAFs) and macrophages (TAMs) - isolated from metastatic and non-metastatic breast tumors growing in mice exposed to normal (1000 IU) low (100 IU) and high (5000 IU) levels of cholecalciferol (vitamin D_3) in diet. Additionally, to find out, if the effects of supplementation with calcitriol can differ dependently on the basal vitamin D body level, mice with normal and low vitamin D diets will be injected with calcitriol starting from the day 7^{th} after tumor transplantation. In addition, we plan to analyze the phenotype, gene and cytokines expression profiles of CAFs and TAMs isolated from tissue samples of patients with non-metastatic and metastatic breast cancer and their activation profile by incubation with calcitriol *ex vivo*.

Despite many years of research on the possibility of the use of calcitriol or its analogs in the anticancer therapy, clinical trials usually do not provide the expected results. On the other hand, vitamin D supplements are generally recommended. So far there is no exact definition of the molecular factors that determine the sensitivity of cancer cells to calcitriol and VDR expression alone is not enough to define sensitive or not sensitive tumors. Although many studies have been carried out to explain the molecular mechanisms that underlie the effect of vitamin D3 in breast cancer epithelial cells, there are only a few studies of its effects on tumor stromal cells. Our study will help to understand the impact of vitamin D on macrophages and fibroblasts localized in tumors with different characteristics. They will also draw us to a full understanding of the effect of vitamin D on such complex tissue as cancer tissue.