Patients suffering from metastatic disease or those who develop metastases after successful treatment of the primary tumor generally have poor prognosis. During the multistage process of metastasis, the tumor cells must evade an attack of the immune system. However, continued tumor-associated inflammation, widely recognized as potentiating primary tumor development, is also favorable to the neoplastic metastasis. Interleukin 17 (IL-17), produced mainly by Th17 cells is a poor prognostic factor when increased in the breast cancer microenvironment. This proinflammatory cytokine among others promotes proliferation and chemo-resistance of breast cancer cells. Our preliminary data concerning the impact of vitamin D_3 active form (calcitriol) and its metabolite tacalcitol (PRI-2191) on Th17 cells during the progression of mouse 4T1 breast cancer showed increased number of this cells in spleen of young mice in which the pro-metastatic and pro-angiogenic effect of calcitriol and its analog was observed. On the other hand in aged ovariectomized mice (OVX; as a postmenopausal model) transient anti-metastatic effect of these compounds was accompanied with decrease of the IL-17 secretion from Th17 cells. The last effect (inhibition of proinflammatory Th17 cell as a consequence of calcitriol treatment) was reported by others in young mice with experimental autoimmune encephalomyelitis (EAE). Th17 cells differentiation is stimulated by osteopontin (OPN). In our research models calcitriol and its analogs regulate OPN secretion age-dependently: in young 4T1 tumor-bearing mice its tumor level significantly increased whereas in aged OVX mice – decreased.

Therefore, we hypothesize that Th17 cells stimulation (young mice) or inhibition (old OVX mice) after vitamin D treatment of tumor bearing mice, may be dependent on VDR (vitamin D receptor) interaction with *Spp1* (OPN encoding gene) and further impact of OPN through its receptors on Th17 cells in an age-dependent manner.

The two main topics of our studies will focus on age-dependent effect of calcitriol and tacalcitol treatment on the expression of OPN receptors and Th17/Treg cells subsets in the tumor bearing mice. Analyzes performed on breast tumors with different ability to metastasize will give the answer about the participation of Th17/Treg cells in the previously observed pro- or anti-metastatic effects of vitamin D compounds (VDCs; calcitriol and tacalcitol) depending on the age of tumor-bearing host. Moreover we will analyze for how much the action of VDCs varied between sister breast cancer models with different ability to metastasize (4T1 – disseminated disease, 168FARN – lymph nodes metastases, 67NR – non-metastatic) and also between two various metastatic cell lines (4T1 and E0771). We will studied also age-dependent influence of OPN on Th17 cells differentiation upon calcitriol or tacalcitol treatment using knock-out (*Vdr-/-* and *Spp1-/-*) mouse models. These experiments will answer the question about the impact of tumor-derived factors on the differentiation process of Th17 cells upon VDCs treatment and can indicate how important is the effect of vitamin D directly on Th17 cells.

In the light of the growing number of studies suggesting on one hand the benefits or the lack of effect or on the other hand, the harm resulting from the use of vitamin D or its derivatives during breast cancer treatment and increasing usage of diet supplements containing vitamin D, it will be important to carry out the studies analyzing the effect of calcitriol and one of its metabolites tacalcitol not only in one selected tumor model, but using the set of cell lines (with various characteristics) transplanted to immunocompetent mice. Moreover planned studies will expand the knowledge about the role of OPN in unfavorable effects of calcitriol and tacalcitol during breast cancer treatment.