Breast cancer is the cancer that forms in the cells of a breast. It is the most common type of mammary gland carcinoma derived from epithelial tissue. It comprises around 23% of all cancer types in women and is responsible for 14 % of deaths. In Poland 18000-19000 new cases are being diagnosed yearly and 6000 of patients die. The most important risk factors are sex (99% of all cases apply to women), genetic background (inherited mutations in *BRCA1* and *BRCA2* genes), estrogen replacement therapy, contraceptives, high fat diet, smoking and alcohol abuse. Regular mammographic screening especially for those at highest risk in many cases allows for immediate intervention and elimination of the tumor at early stages. However due to the proximity of lymphatic system yet on very early stage of the disease it can invade surrounding lymphatic nodes and proceed to furthest organs in the body like lungs, brain, liver or bones. Breast cancer is usually classified primarily by its histological appearance. Moreover breast cancer cells have receptors on their surface and in their cytoplasm and nucleus. Chemical messengers such as hormones bind to receptors, and this causes changes in the cell. Breast cancer cells may or may not have three important receptors: estrogen receptor (ER), progesterone receptor (PR), and HER2. Estrogen receptors are found in 80% of cases and progesterone receptors in 40-70% of all cases. New molecular classification based on receptor status marks out luminal A and B, HER2+, basal and triple negative breast cancer giving the worst prognosis and treatment possibilities as there is no direct treatment possibility. Frequently, after tumor removal and overall chemotherapy, relapse and metastasis of the disease is being observed.

Studies regarding metastasis suggest high impact of epithelial-to-mesenchymal transition (EMT) in this process. EMT is a process by which epithelial cells lose their cell polarity and cell-cell adhesion, and gain migratory and invasive properties to become mesenchymal stem cells. Those cells can shed into the vasculature or lymphatic from a primary tumor and are carried around the body in the blood circulation. Circulating tumor cells (CTCs) can travel via cardiovascular system or lymphatic system to distinct organs like lungs or liver and reversely transform to epithelial state causing development of a new metastatic site. This process is still poorly described.

Alpha-catulin is a protein that is coded by *CTNNAL1* gene. It has been described by our laboratory to be highly expressed in very invasive carcinoma cells. Moreover the results showed that upregulation of α-catulin expression correlates with the transition of tumor cells from an epithelial to a mesenchymal morphology, as well as an upregulation of EMT markers. Preliminary data showed that alpha-catulin is also highly expressed in breast cancer tissue and that expression level correlates with higher grade and aggressiveness of cancer.

The goal of this project is to characterize circulating tumor cells using xenograft model. Immunocompromised mice will be injected into mammary gland with human triple negative breast cancer cell lines. Those cell lines will be labelled with the genetic reporter including green fluorescence protein (GFP). In this project I will also investigate the role of alpha-catulin in the process of circulating tumor cells formation via depleting alpha-catulin from those cell lines injected into mice. Circulating tumor cells will be isolated from whole blood with a use of flow cytometry and sorting. Then single cell RNAseq analysis will be performed on those isolated cells what will allow us for the first time to describe the heterogeneity of the population of circulating tumor cells and what transcriptional changes occur during their formation. Proposed experiments will contribute to better understanding the process of formation of circulating tumor cells and how diversified is this population. This will contribute to better understanding of a metastatic process what may lead to enhancing antitumor breast cancer therapy in the future.