Breast cancer is the most common cancer in women. Number of deaths from breast cancer have declined over past two decades, but remain the second leading cause of cancer death among women overall. There are four main subtypes of breast cancer - luminal A, luminal B, HER2 positive and triple negative breast cancer, which differ in sensitivity to therapies and are associated with differences in the survival of patients. Despite significant therapeutic progress, prognosis for the last three of the aforementioned breast cancer subtypes remains poor. Systemic therapies used in the treatment of breast cancer are mainly based on chemo-, hormone therapy and anti-HER2 treatment. However, despite initial response, progression might occur leading to formation of metastases and death of patients. Consequently, new therapy as well as better understanding of processes underlying resistance and metastases are needed. Modern targeted and immunotherapies hold the promise for improvement of treatment of breast cancer. However, response to these therapies requires presence of specific biomarkers (i.e. actionable/druggable mutations) that can be detected by genetic testing. Identification of actionable mutations in primary tumors (PTs) may reveal potential therapy targets allowing additional treatment. However, genetic instability of PTs as well as primary and acquired resistance arising from intratumor heterogeneity are recognized as the most important reasons for therapy failure. Basing therapy decision only upon characteristics of PTs before therapy ignores changes in mutational profiles acquired during treatment or in systemically spreading cancer cells found in blood (i.e. circulating tumor cell, CTCs) or other organs, which may genetically differ from PTs bulk. Therefore, in the our project we will analyse CTCs and circulating tumor DNA (ctDNA) – biomarkers representative of a highly aggressive cancer cell population – for the presence of actionable genetic alterations. We hypothesize that CTCs and ctDNA inform best about actionable changes emerging during/after treatment. To validate that, we will collect and analyse treatment-naïve PT and isolate CTCs and ctDNA at different stage of systemic therapy (before and during/after) from a cohort of 15 metastatic breast cancer patients and 60 non-metastatic, high-risk breast cancer patients. For this purpose, we will utilize our proprietary method for isolation of a wide spectrum of CTCs subtypes and adopt the FDA approved MSK-IMPACT assay to identify druggable oncogenic mutations in CTCs before and during/after treatment. MSK-IMPACT allows mutational screening of 410 cancer associated genes and quantification of tumor mutational burden in PTs. We will adjust this method to analysis of CTCs during the course of our project. Complementary to this, ctDNA will be isolated from blood samples collected before and during/after treatment. Data resulting from profiling of PTs and CTCs will be used to determine patients-specific sequence markers, which will be specifically searched for during the screening of ctDNA.

Analysis of CTCs remains challenging due to their phenotypic diversity connected with activation of epithelial-mesenchymal transition process. Presence of CTCs displaying a mesenchymal phenotype is linked to an aggressive course of the disease and poor prognosis in both early and metastatic breast cancer patients. However, mesenchymal CTCs are frequently overlooked by conventional detection methods. To assess the clinical value of CTCs for therapy selection, we will determine actionable somatic mutations in both epithelial and mesenchymal single CTCs.

As genetic background of patients may have an important impact on the outcome of treatment, in this project we will analyze not only the landscape of somatic actionable mutations but also the profiles of pharmacogenetic (PGx) germline polymorphisms (sequence alterations in non-tumor cells associated with altered response to treatment) of each patient. The MSK-IMPACT method will be modified to enable detection of germline variants annotated as drug metabolism modifiers. Parallel analysis of different samples types (PT, CTC and cfDNA) will inform about the most relevant source(s) of information to predict clinical outcome and personalized selection of therapy options for patients at high risk of progression. Moreover, analysis of CTC and cfDNA collected after treatment may uncover mechanisms associated with drug resistance and relapse. Results of our study may pave the way for development of novel companion diagnostics test allowing to treat breast cancer patients more effectively.