Despite recent advances in the pharmacotherapy, breast cancer remains a huge burden for the society. Nearly 1.7 million new breast cancer cases were diagnosed worldwide in 2012 (the latest data available). Breast cancer is the second most common cancer worldwide and the first most common in Europe. It represented about 12% of all new cancer cases in the world, and more than 13% in Europe. This is the most frequently diagnosed cancer among women in 140 of 184 countries worldwide. Due to prevalence of effective diagnosis at early stage the mortality rates are lower, however the breast cancer still remains significant problem both worldwide and in Europe, as 5th (6,4%) and 3rd (7,5%) death-causing disease respectively.

Breast cancer is increasingly considered to be not a single disease, but a group of diseases distinguished by different clinical behaviours, risk factors, responses to treatment and most importantly by specific characteristics of molecules distinct for each cancer subtype. Mentioned molecules, called biological markers, are in many cases receptors - proteins involved in transferring messages from the cellular environment into the cell. The latest breast cancer taxonomy is based on hormone receptors, i.e. oestrogen receptors (ER) and progesterone receptors (PR), as well as Human Epidermal Growth Factor Receptor 2 (HER2). Based on these markers, four molecular subtypes of breast cancer have been distinguished: luminal A (ER+ and/or PR+ and HER2-), luminal B (ER+ and/or PR+, HER2+ or with high cell proliferation rates), basal-like (ER-, PR-, and HER2-) and HER2 enriched (ER- and PR-, HER+). Approximately 15% to 20% of breast cancer cases overproduce the growth-promoting HER2 protein. These tumours tend to grow faster, spread more aggressively than other breast cancers, are associated with poorer, short-term prognosis, and generally are more likely to recur than tumours that do not overproduce HER2. Moreover, these cancers are much harder to treat. Nevertheless, there is a therapeutic component - trastuzumab, which combined with a standard chemotherapy significantly increase patients chances for longer life, even 37% compared with chemotherapy alone. Trastuzumab, humanized monoclonal antibody against HER2+ cancers, is one of the best example of targeted therapy approach which was a milestone in cancer molecular medicine. The idea is to block specific targeted molecules needed for carcinogenesis and tumour growth, rather than by simply interfering with all rapidly dividing cells like in case of chemotherapy or radiotherapy. Targeted therapeutics have been established successfully for several cancer types and, in many cases, significantly increase average patient survival. Trastuzumab, also known as HerceptinTM was approved initially for metastatic breast cancer by the U.S. Food and Drug Administration (FDA) in 1998. Finally, in 2006, US FDA accepted trastuzumab for all HER2-positive breast cancers. All invasive breast cancers should be tested for the HER2 gene amplification, or protein overexpression in order to identify women, who would benefit from this therapy. Therapeutic effect of the antibody is to bind to the receptor leading to inhibition of proliferation of cells that overexpress HER2, however mechanism of trastuzumab activity is not completely understood and described. Unfortunately, despite the initial good response, most of the patients eventually develop resistance to trastuzumab. Therefore, there is justified strong need for patients and challenge for researchers to understand the mechanisms underlying Herceptin resistance.

The main aim of proposed study is to get insights into molecular mechanisms underlying the development of trastuzumab resistance. Due to the uncertainty about the exact mechanism of Herceptin influence upon breast cancer cells, it is difficult to describe the resistance mechanism as well. Resistance occur due to natural ability of (cancer) cells to adapt to the changing environment (including different treatment conditions). Present studies treat the resistance as a single phenomenon, while in our opinion it should be investigated as a successively arising events at molecular level of cell function, hence we propose to study resistance development in time. Longitudinal approach will allow us to monitor the stability of the observed changes during the process of evolution. It will give us the unique opportunity to identify the molecular mechanisms, which rapidly change, pinpointing the most significant pathways involved in cell signalling transduction engaged in trastuzumab resistance, especially at the early stage. Cancer is a multiplex, polygenic disease, so even if one pathway is blocked, there is still a lot of other pathways involved in proliferation and surviving of cancer cells. Some cells are able to overcome therapy through subtle changes in signalling pathways. Current studies referring to single pathway–targeted agents reveal their minor effect in clinical trials, even though strong and clear positive outcome have been shown in vitro and in animal experiments. This indicate that efficient signalling pathways targeting must involve multiple directions as well as many components. Moreover, transcriptomic variations (the first level of gene expression changes) are the most obvious and visible target for investigation, lying in the centre of molecular biology of the cell, possibly being cause and/or effect of other changes. Taking all above statements into consideration, we plan to implement a new approach to explore the evolution of resistance in time using innovative method for transcriptome changes investigation combined with computational modelling in order to explain the process.

In order to achieve intended goal, two stable, certified by ATCC (American Type Culture Collection) breast cancer cell lines have been chosen as the initial biological model for the study. Selected cell lines are HER2 positive, confirmed to be trastuzumab sensitive and commonly known to be able to develop Herceptin resistance. These parental cell lines will serve as controls and as a starting point to create Herceptin resistant cell lines. Proposed biological model aims at creating conditions that mimic clinical situation during development of Herceptin resistance. In order to achieve sufficient similarity, it is planned to use long term, constant, relatively small dose of the drug. Based on proliferation rate and morphology characteristics, the most appropriate 6 time points will be chosen for subsequent gene expression changes investigations.

Microarray technique is a novel method for studying gene expression differences between samples e.g. healthy/diseased or gene expression changes e.g. before/after treatment. Due to miniaturization and years of technical improvement it is possible to detect expression changes of tens thousands of genes simultaneously with significantly higher reproducibility. Even though, among platforms available on the market, there are differences in material used for probes immobilization and printing techniques, as well as sample labelling and detection systems, the basic principle of an expression microarray approach is the same. The principle is hybridization of a sample of interest to immobilised set of probes designed for specific organism or to detect specific set of transcripts based on nucleic acid sequence complementarity. The hybridization signal measured from each spot (specific probe) is proportional to amount of particular mRNA molecules bound to the probe, which

allows assessing the intensity of gene expression in comparison with the control. Among other advantages, the array of chosen provides more than 50 000 biological features for detection, including a set of regulatory RNA molecules, which allows to get enough information to build more integrated picture of studied process. Based on gene expression changes in cell line models we will generate a computational model of molecular mechanisms underlying the development of trastuzumab resistance.

We are strongly convinced, that the proposed model will enrich the knowledge in basic science by explaining the mechanisms of trastuzumab resistance development in time at molecular level. This knowledge will serve as source of information for future studies concerning Herceptin resistance and how to overcome this phenomena.