The incidence of cancer diseases is a global problem in countries around the world. According to the research team Merics Health and Evaluation in 2013 was found 14.8 million new cancer cases and was estimated number of deaths due to cancer at 8.2 million. Among men, the most common cancer is prostate cancer (1.4 million cases), while among women is breast cancer (1.8 million cases). An alarming phenomenon is the very large increase in the incidence of various types of cancer. In the 1990-2012 the diagnosed cases of prostate cancer has increased by 300%, breast cancer by 200%, colorectal cancer by 92%, liver cancer by 70% and gastric cancer by 23%. Nowadays, cancer is second leading cause of death in the world after cardiovascular diseases. It is estimated that until 2020 the number of detected cases of people with cancer will reach the number of 17 million. In developed countries, the mortality rate for cancer decreases, which gives hope that easier access to modern therapy will prolongs life.

The cancer cell for development, division and spread needs to change whole metabolism. The most important features of cancer cells are independent signals that cause growth, lack of response to growth signals coming from the organism, no programmed cell death (apoptosis), the formation of new blood vessels (angiogenesis), invasion of tissues (metastasis), immortality. Such far-reaching modifications in the cell require qualitative and quantitative changes of protein. The literature shows that changes in the tumor cell have a base in alternative splicing of genes.

Human has about 26 thousand genes and the number of proteins in the cell is estimated 250 thousand to 1 million. For such a large variety of proteins relative to genes correspond to the processes associated with maturation of pre-messenger RNA molecules - mainly alternative splicing. The human gene consists of sequence which coding proteins and non-coding sequence, while standard (constitutive) splicing relies on non-coding sequences cutting out what causes forming the mature molecule of mRNA (messenger RNA), which is a formula for protein synthesis. Alternative splicing is consist of additional coding sequence skipping or non-coding sequence retention. On the basis of that kind of messenger RNA differing protein is created i.e. isoform of the protein. Besides large variety of proteins in a cell created by this process, alternative splicing produces tissue-specific proteins with different functions. In addition, alternative splicing allows for the differentiation of cells and tissues. One isoform of the protein may be produced, while early development stage where cells have intensively divide while the second isoform is produced in mature cells. Scientific research show that tumor cells pattern of alternative splicing of is similar to immature, embryonic cells, in which there is the uncontrolled growth.

The aim of this project is to improve the effectiveness of bifunctional antisense oligonucleotides for alternative splicing regulation in cancer cells. Bifunctional antisense oligonucleotides are RNA molecules, 20-40 nucleotides long, composed of two parts with different functions: the regulatory part is responsible for the interaction with proteins(splicing factors), which affect alternative splicing, while antisense part binds to the selected region of pre-mRNA molecule. The two-part structure of the molecule allows precise binding to the product of the gene (pre-mRNA), on which we want to change the alternative splicing, and a regulatory part is responsible for the binding of factors regulating alternative splicing. The main aim the proposed project is to optimize regulatory part of bifunctional antisense oligonucleotides which will be based on: selection a sequence that strong and specifically binds to regulating alternative splicing proteins, choosing the most appropriate number of repeats of these sequences, the analysis small structural motifs added to the regulatory sequence and the incorporation of chemically modified analogs instead RNA nucleotides. In addition, it will be analyzed the enzymatic stability of the studied molecules. The next part of the research will involve optimization the full-length bifunctional antisense oligonucleotides using a pre-mRNA SYK gene fragment and *in vitro* splicing assay. The use of such a model will allow for assessment of selected oligonucleotides variants on many levels. The final stage of the research will be based on a synthesis a bifunctional antisense oligonucleotides, which allow for effective regulation of alternative splicing in cancer cell lines. To this part of the study were selected: 1) the human cervical carcinoma cell line (HeLa), in which is disturbed PKM alternative splicing, 2) the human colon carcinoma cell line (SW480), in which is disturbed Ron alternative splicing 3) human breast cancer cell line (MCF7), in which is disturbed SYK alternative splicing.

This complex experiments proposed in project will allow for the construction of bifunctional antisense oligonucleotides which will effectively regulate alternative splicing of each gene in all type of cells. We hope that the results of this study will interest a number of researchers and will be the basis for the use modified bifunctional antisense oligonucleotides in modern cancer therapy.