Noncommunicable conditions such cancer and cardiovascular diseases, are the leading causes of death worldwide. It is estimated that over the next three years, the number of diagnosed cancer patients will exceed 17 million. Development of new therapies can improve the quality of life of patients and reduce mortality. In humans, genetic information is encoded in about 30,000 genes and about 85,000 different mRNA molecules that are templates for protein synthesis. The sequencing of the human genome in the HUGO Project (Human Genome Project) was a milestone for the development of modern pharmacy. Identification of the gene responsible for a given genetic disease is the basis for the design of drugs targeted at a specific genetic condition at the level of the mRNA or gene. The best example of this therapeutic approach is the use of antisense oligonucleotides (ASOs). Antisense oligonucleotides are single-stranded DNA or RNA molecules, designed to be specifically bound to target mRNA by Watson-Crick base pairing embedded in the regular duplexes. The formation of ASO/mRNA heterocomplex provides stearic hindrance for the ribosomes that lead to a change in gene expression. Gene inhibition based on antisense mechanism was for the first time demonstrated by Zamecnik and Stephenson in 1978 on chicken fibroblast culture infected with Rous sarcoma virus. Introduction of synthetically prepared 13mer ODNs complementary to the 3'-terminal sequence of the Rous sarcoma virus significantly inhibited viral production and prevented fibroblasts to form sarcoma cells. After 35 years, the US FDA approved mipomersen as antisense agent targeted at apoB-100 for the treatment of homozygous familial hypercholesterolemia, which was a major breakthrough for this type of biological therapy. Currently, six antisense oligonucleotides are already approved as drugs and several others are in clinical trials and soon will reach decisive phases.

Translation is the fundamental process regulating the flow of genetically encoded information. G-quadruplex formation with the participation of mRNA could be an expedient tool to control gene expression. G-quadruplexes are four stranded helical structures formed by DNA and RNA sequences that are rich in guanine. These structures occur naturally in human cells and can be suitable targets for treatment of cancers and neurodegenerative diseases. Additionally, a wide range of small-molecule ligands have been identified to exhibit selectivity towards G-quadruplexes over single- or double stranded nucleic acids. Taking advantage of this property, our goal was coupling of ligands with ASO to control the introduction of quadruplex-duplex hybrid structures into mRNA. The designed ligand–hybrid structures would enable the construction of artificial gene regulation systems, which may provide very sensitive and specific strategies for both basic biological study and clinical research. This methodology is based on sequence-guided ligand tethered to G-rich-ASO that bind to mRNA and modulate its structure. For instance, the model antisense oligonucleotides consist of three functionally independent domains: the duplex domain (ASO) is responsible for duplex formation, the quadruplex domain (Q) with two contiguous runs of three guanine residues is designed to assemble into intermolecular G-quadruplex by incorporation of a guanine rich region of the other RNA strand and the ligand domain (L) which stabilizes the G-quadruplex structure. We expect that the effective concentration of L-Q-ASO required to silence gene expression can be significantly lower than that for ASO. We are going to study the systemic structure–activity relationship of the L-Q-ASO/mRNA complexes because it has been found that the level of suppression is proportional to the thermodynamic stability of the G-quadruplex motif. L-Q-ASO acts like an ASO by binding to the target mRNA and inhibiting the translation process by providing stearic hindrance for the ribosomes. As a result, the ribosomes cannot traverse along the mRNA and hence the protein synthesis is altered.

The model antisense oligonucleotides will be tested *in vitro*. The complex formation of quadruplex-duplex hybrid with a ligand will be monitored using NMR spectroscopy. It allows unambiguous identification of the formation of such structures in solution because Watson-Crick base pairs and G-quadruplexes give characteristic NMR spectra. To get additional information about structural properties of the quadruplex-duplex hybrids in solution we will employ additional experimental methods like UV, circular dichroism spectroscopy, fluorescence or native gel electrophoresis. For L-Q-ASO, the effect of gene silencing in rabbit reticulocyte lysate will be examined using *Renilla* Luciferase (RL) model system and the selected human mRNA such as EGFR, EGFR-L858R or c-MYC.

The novel hybrid systems based on the linkage of ligands with G-quadruplex domains can provide opportunities for regulation of biological processes and lead to a promising arena for cancer therapy.