T-cell acute lymphoblastic leukemia (T-ALL) is a rare subtype of acute lymphoblastic leukemia (ALL), the most common childhood malignancy. This disease is characterized by high aggressiveness and bad prognosis. Its treatment is based on chemotherapy, which has a number of serious and often long-lasting side effects, which is of particular importance in pediatric patients. Diagnosis and treatment of T-ALL generate high costs, yet in as much as 20% of cases the therapy turns out to be ineffective, which leads to relapse or death.

T-ALL in children is a serious medical problem with psychological and socio-economic consequences. Currently, the idea of universal anti-cancer therapies is being abandoned. The activities of researchers focus on the search for the so-called targeted therapeutic options, adapted to the biological characteristics of tumor cells and intended for well-defined groups of patients with a similar "molecular profile" of the tumor. Targeted therapy, which allows to switch off the overactive mTOR pathway in patients, is a potential therapeutic strategy in T-ALL. This pathway is crucial for the function of healthy cells, it mediates the transmission of signals from the environment, and it lies at the nexus of many signaling pathways within the cell. In tumors, including T-ALL, this pathway is overactive, contributing to the uncontrolled tumor growth. Currently, attempts are made to use inhibitors of the mTOR pathway in the treatment of many cancers, including hematological cancers. However, in order for patients with T-ALL to benefit from this type of medical solutions in the future, it is necessary to carefully characterize the mTOR pathway deregulation mechanisms in this leukemia.

For many years, research on T-ALL has been focused on finding changes in protein-coding sequences. As a result, knowledge about oncogenes and tumor suppressor genes involved in the pathogenesis of T-ALL is relatively broad. Currently, more and more attention is paid to the importance of epigenetic mechanisms (mainly DNA methylation) and the role of miRNA molecules, which are involved in the regulation of gene expression, in T-ALL. DNA methylation is a chemical modification that adds methyl (-CH3) groups to the nitrogen bases of the nucleotides that build the DNA strand. Methylation does not change the DNA sequence, but only the availability of DNA for proteins responsible for gene expression. miRNAs are short RNAs that silence gene expression by directly interacting with gene transcripts. It is now known that both the expression level of miRNAs and the level of DNA methylation in leukemic cells are altered relative to normal cells. These changes may have a direct impact on tumor development, as these mechanisms reduce the expression of genes that inhibit neoplastic transformation and increase the expression level of oncogenes. So far, the (epi-) genetic mechanisms of regulation of the mTOR pathway have not been studied in the context of the biology of T-ALL.

The aim of the proposed project is: 1/ functional characterization of 5-7 miRNA-mRNA interactions directly related to mTOR signaling and determining the impact of these interactions on cell survival T-ALL in *in vitro* studies 2/ functional characterization of the influence of DNA methylation on the expression of 4-6 genes related to mTOR pathway signaling and verification of the impact of disturbances in expression of these genes on T-ALL cell survival in *in vitro* studies.

The research will be based on various modifications of the CRISPR-dCas9 technique, which will allow for targeted and precise changes in gene expression in T-ALL cell lines. The oncogenic/suppressor potential of the studied interactions and genes will be assessed in functional tests assessing the survival of T-ALL cells under the influence of the introduced epigenetic modifications.

The miRNA-mRNA interactions and the genes undergoing aberrant DNA methylation were selected for study based on multi-omic data derived from the integration of data from several Next-Generation Sequencing platforms of T-ALL patient samples. We undertook the characterization of several levels of regulation of the mTOR pathway in order to obtain the most complete picture of its deregulation in T-ALL leukemia. The combination of several state-of-the-art research approaches will significantly advance the knowledge of mTOR signaling disorders in leukemic cells.

The results obtained in the proposed project will contribute to extending the knowledge about the regulatory dependencies of the mTOR pathway. The identified miRNA-mRNA regulatory axes and disturbances in the DNA methylation process will be a starting point for further functional studies (in the murine T-ALL model) aimed at confirming their oncogenic/suppressor role *in vivo*. They can also potentially be a starting point for the development of new therapeutic strategies for patients with T-ALL aimed at inactivating the mTOR pathway.