Acute lymphoblastic leukemia (ALL) is the most frequent cancer in children. ALL is treated by multi-drug chemotherapy, which cause serious side effects. Chemotherapy is ineffective in ~20% of children, which is related to drug resistance and relapse (disease recurrence). Relapsed ALL is very difficult to treat and often fatal. The majority of relapses occur in patients with T-ALL subtype (T-cell acute lymphoblastic leukemia), which is more aggressive and much less understood than ALL arising from B-cells. Unlike in B-cell ALL, in T-ALL, the genetic prognostic markers of the risk of relapse are still missing.

Pediatric leukemia is a medical problem affecting the whole family, with psychological, societal, and economic consequences. Survival rates of relapsed patients are low, despite intensive treatment. Costs of therapy and those related to rehabilitation due to long-term side effects of therapy, negatively affecting the quality of life, are a significant economic load worldwide. Since ALL is the most frequent childhood cancer, it is of highest importance to expand the knowledge about the mechanisms of leukemia recurrence.

Accurate identification of patients at high-risk of relapse, successful relapse prevention and treatment are currently one of the most important challenges in pediatric oncology. There is an urgent need to identify reliable predictors of relapse and to develop novel therapeutic strategies to prevent the relapse. Yet, this requires an in depth understanding of the mechanisms of disease progression and recurrence.

Based on the results of our previous research and the literature data from other cancers, we hypothesize that the mechanisms contributing to T-ALL relapse include: drug-resistance, cell stemness (related to biological plasticity, which enable the cells to 'escape' the anticancer effects of therapy), senescence-like phenotype induced by chemotherapy, clonal heterogeneity (meaning high diversity among leukemic cells, which make them more prone to evolve towards relapse).

We aim to provide novel knowledge on the genes and biological processes, which drive the survival advantage of T-ALL cells and their evolution from diagnosis to relapse. We will use two state of the art methods to investigate the mechanisms of T-ALL relapse: 1/ single-cell sequencing of the transcriptome (scRNA-seq) of T-ALL cells obtained from patients' samples at diagnosis and at relapse, and 2/ genome-wide dropout screen using CRISPR/Cas9 method. scRNA-seq will enable us to investigate clonal heterogeneity and evolution based on gene expression analysis at a single-cell resolution, while the CRISPR/Cas9 dropout screen will enable to identify genes, which are essential for leukemic cells to survive (the disruption of these genes by CRISPR/Cas9 cause these cells 'dropout' from the cell culture, showing that these genes are indispensable for leukemic cells to survive). From these two tasks, we will select several genes and verify their importance for T-ALL cells – we will activate or inactivate the expression of these genes in T-ALL cell lines and investigate, how these changes impact the ability of leukemic cells to proliferate and survive. Finally, we will use a bioinformatics approach to analyse the data on gene expression and clinical outcome of large cohorts of T-ALL patients, publically available in databases. We will aim to verify, if the alterations of genes' expression observed in our study, are in fact related to the occurrence of T-ALL relapse.

Such an approach to investigate the mechanisms of relapse have not been used in T-ALL research thus far.

The expected outputs of the project include: 1/ overview of genes and processes essential for survival of T-ALL cells; 2/ description of genes and biological processes contributing to T-ALL relapse, with a functional characteristics of several most interesting genes; 3/ characteristics of clonal heterogeneity and clonal evolution in T-ALL relapse; 4/ description of the contribution of the mechanisms related to cell stemness, chemotherapy-induced senescence, and drug resistance to T-ALL relapse; 4/ identification of relapse-associated gene expression features, which might help to predict the risk of relapse and to develop novel therapeutic approaches.

The ultimate goal of the project is to unravel the mechanisms of T-ALL relapse and to pave the way towards precise identification of high risk patients, which will facilitate the determination of the best treatment options to prevent leukemia relapse and improve the survival of T-ALL patients.