SUMMARY

T-cell acute lymphoblastic leukemia (T-ALL) is a rare subtype of acute lymphoblastic leukemia, the most common childhood cancer. Nowadays, around 80-90% of children with T-ALL achieve lasting remission. Nevertheless, still about 20% of T-ALL patients relapse or have a primary resistant disease and face particularly poor prognosis of surviving 3 years not exceeding 15%. To identify these patients early during treatment, new prognostic markers are needed. Such prognostic markers, including genetic prognostic markers, could help to early recognize, already at the time of diagnosis, the patients with very high risk of relapse. High risk patients could be offered hematopoietic stem cell transplantation or alternative therapies with targeted drugs or immunotherapy, aimed to minimize the risk of disease recurrence, which is very difficult to treat.

Here, I aimed to identify genetic prognostic markers in children with T-ALL, diagnosed and treated in Poland with I-BFM (*International Berlin-Frankfurt-Münster Study Group*) treatment protocols. I assessed the prognostic value of t(8;14)(q24;q11) with *MYC* proto-oncogene rearrangement, as well as mutations and copy number alterations of recurrently mutated T-ALL oncogenes and tumor suppressor genes, namely*: NOTCH1*, *FBXW7, PTEN, WT1, IL7R, STAT5B, FLT3, RUNX1, DNMT3A*, *SIL-TAL1, LEF1*, *CASP8AP2, MYB, EZH2, CDKN2A/B, MLLT3, NUP214-ABL1, LMO1*, *LMO2*, *NF1, SUZ12, PTPN2,* and *PHF6*. These alterations were correlated with clinical data: features at diagnosis, response to therapy and long-term survival.

The study group included 162 children, consecutive patients diagnosed with T-ALL, treated with I-BFM trials: ALL IC-BFM 2002 and ALL IC-BFM 2009. Mutations and copy number alterations were detected with the use of multiplex ligation-dependent probe amplification (MLPA), high resolution melt (HRM) analysis, Sanger sequencing and shallow whole genome sequencing (sWGS). t(8;14)(q24;q11) was detected with G-banding karyotyping and fluorescent *in situ* hybridization (FISH).

The obtained results indicate that the leukemic clone characterized by the presence of t(8;14)(q24; q11) with *MYC* proto-oncogene rearrangement and the co-occurring deletion of *PTEN* and *CDKN2A/B* genes represent a clone that is resistant to standard chemotherapy and undergoes clonal selection during treatment. This molecular profile, namely the presence of t(8;14)(q24; q11) with *PTEN* and *CDKN2A/B* deletions might be indicative of high risk T-ALL and might represent a candidate for negative prognostic marker in T-ALL. Moreover, I have

shown that *PTEN* mutations and/or deletions represent another candidate for negative prognostic marker in T-ALL. In the group of T-ALL patients treated with ALL IC-BFM 2002 and ALL IC-BFM 2009, *PTEN* mutations and/or deletions correlated with inferior outcome and high risk of relapse. Furthermore, the *PTEN* status (mutations and/or deletions vs wild type *PTEN*) added prognostic information within risk groups defined based on the level of minimal residual disease assessed by flow cytometry (FC-MRD), allowing for more accurate identification of T-ALL patients at high risk of relapse. Finally, I have shown that mutations in *DNMT3A* gene have limited contribution to T-ALL pathogenesis, which hinders their utility as prognostic markers in T-ALL. Nevertheless, the methods I implemented to study *DNMT3A* mutational status: combined high resolution melt analysis and Sanger sequencing might be used as an alternative to next generation sequencing in screening of *DNMT3A* status in hematologic malignancies characterized by high frequency of mutations in this gene (e.g. acute myeloid leukemia, AML).