SUMMARY - **Heterogeneity immunocompetent cells during chimeric antigen receptor T cell (CAR-T) CD19 immunotherapy.**

Acute Lymphoblastic Leukemia (ALL) is the most common cancer disease in children, with the occurance at 2-5 years of age. The morbidity rate is around 200-250 children per year. This disease is related to the dysfunction of the bone marrow, which produces large numbers of immature lymphocytes. The prognosis in children is very good, and the cure rate is about 90%. Nevertheless, ALL remains a significant cause of death in the pediatric population. Recently, new type of immunotherapy start to be more available, it was first used in children in 2012, the name of that is CAR-T cells therapy (T cells with a chimeric antigen receptor). In Poland, the therapy was used for the first time in March 2020, and from September 2022 it is also reimbursed. At present, the only approved preparation in children is Tisagenlecleucel (Kymriah). CAR-T immunotherapy is a last change for some children, unfortunately a few patients fail to obtain CAR-T cells, unfortunately we still do not know why.

Side effects like cytokine release syndrome are quite common after drug infusion, or neurological complications such as encephalopathy, confusion or delirium. These events most often occur in the first weeks after the administration of the preparation. The overall remission rate in the Kymriah® registration study was estimated to be over 80%. Unfortunately, a certain percentage of patients experience a relapse that occurs within the first months after infusion and is correlated with early CAR-T cell loss. The reason why some patients lose valuable lymphocytes so quickly, while in others they last more than 2 years, requires further study. Perhaps it is related to the so-called antigenic escape and loss of CD19 antigen expression on leukemic cells.

Research project is based on assessment of lymphocyte subpopulations by using the most modern analytical technique, mass cytometry (CyTOF - cytometry by Time-of-Flight). This method combines the advantages of flow cytometry and mass spectrometry, it consists in using stable isotopes of transition metals (mainly lanthanides) as markers for antibodies, DNA probes or small-molecule chemical markers. About 30 patients undergoing CAR-T therapy will participate in the project. These will be patients under 25 years of age, suffering from acute lymphoblastic leukemia.

Patients who will take part in the study will have access to the most modern immunological test, which gives us very accurately assess the lymphocyte subpopulation during the use of CAR-T immunotherapy. Not only will this help us learn more about the immune system’s response to this modern therapy, like why some patients develop severe side effects, but it may also help us identify the group of patients at risk of CAR-T loss and early relapse. In addition, the collected data will contribute to a deeper understanding of the preparation and may allow for the improvement of this modern therapy.