In the presented project the primary aim is to investigate the mechanisms of daratumumab-induced and natural killer (NK)-cell-dependent cell death of T-cell acute lymphoblastic leukemia (T-ALL) blasts.

An additional aim of the proposed project is to compare the cytolytic activity of NK cells directed against T-ALL blasts in the presence of daratumumab in an in vitro setting.

Basing on the literature and our experiences we present a hypothesis that CD38(+) T-ALL cells undergo two types of cell death dependent on daratumumab and NK cells. The first type is programmed cell death (PCD) directly induced by binding of CD38 by daratumumab cross-linked by FcγR receptors present on NK cells. The second mechanism is based on antibody-dependent cell-mediated cytotoxicity (ADCC) triggered by daratumumab binding to activating FcgR receptors present on NK cells and directing them to T-ALL cells via interaction with CD38.

T-ALL is aggressive hematologic malignancy and accounts for 25% of adult cases of ALL. Treatment of T-ALL with conventional cytotoxic chemotherapy results in high cure rates in pediatric cases but is suboptimal in the treatment of adult patients. Unsatisfactory results of conventional chemotherapy in adult patients are the effect of excessive induction mortality, chemotherapy resistance, higher risk factors present at diagnosis, more comorbidities and need to reduce doses of key drugs because of the increasing age and adverse effects of the treatment. Only a few immunotherapies have been developed for treatment of T-ALL in comparison to effective therapies used in B-ALL. The main problem with advanced immunotherapy for T-ALL is the difficulty in identifying surface antigens uniquely expressed on leukemic blasts but not on normal T-lymphocytes which could possibly act as a target for immunotherapy.

CD38 is a type 2 transmembrane protein which functions as an adhesion molecule. It is expressed highly and uniformly on multiple myeloma (MM) cells and at low levels in normal lymphoid and myeloid cells, NK cells as well as in non-hematopoietic tissues (prostate, smooth muscle, eye). CD38 distribution ranges from discrete expression during lymphocyte differentiation to an extremely limited presence during the normal physiological life of both T and B cells. CD38 is highly expressed on the surface of T and B ALL blasts which makes it an ideal target for daratumumab.

Daratumumab is a human immunoglobulin G1 (IgG1) monoclonal antibody (mAb) that targets CD38. Daratumumab induces tumor cell death through multiple mechanisms, including ADCC, complement-dependent cytotoxicity (CDC), antibody-dependent cellular phagocytosis (ADCP), PCD, modulation of enzymatic activity and immunomodulatory activity. It promotes antitumor immune responses rather than targeting the cancer directly. It is a safe and effective agent in treating multiple myeloma.

ADCC and ADCP are induced by mAb binding to activating FcγRs on immune effector cells, for example, NK cells and to Fc domains. Fc–FcγR interactions enhance the agonistic activity of mAb or induce PCD. PCD induced by agonistic mAb targeting the death receptors is enhanced by cross-linking via binding to FcγRs.

The own experience of the research team, both clinical and laboratory, encourages the investigation of the topic. Our preliminary results conducted on the T-ALL cell lines showed that daratumumab alone does not affect survival of the investigated cell lines and most probably crosslinking of daratumumab is required to achieve cell death induction by daratumumab.

The proposed study will be conducted on T-ALL cell line and on T-ALL blastic cells isolated from the bone marrow obtained at the time of diagnosis from patients diagnosed with T-ALL.

We plan to perform the viability and proliferation tests of cells, the analysis of apoptotic pathways in T-ALL cells stimulated with daratumumab in the presence or absence rabbit anti-human IgG F(ab')2 fragment.

We also plan to co-culture T-ALL cells with NK cells isolated from healthy patients in the presence or absence of the effective concentration of daratumumab and presence or absence of rabbit anti-human IgG F(ab')2 fragment.

The general aim of the study highlights the importance of immunotherapy in T-ALL. The study will indicate the efficacy and importance of immunotherapy in T-ALL and show the possible enhancing mechanisms to augment the mechanisms of action of daratumumab. Furthermore, data on the mechanisms of action of daratumumab is determined basing on its effect on multiple myeloma cells and we still need data on AL cells.