ABSTRACT

Acute myeloid leukemia (AML) is a genetically and clinically heterogeneous disease characterized by an absence of normal hematopoiesis, proliferation and accumulation of immature blasts in the bone marrow and peripheral blood. AML is an aggressive, rapidly progressive disease that prevents the immune system from forming an effective response to leukemia cells. In the majority of AML patients eligible for chemotherapy based on a comprehensive clinical evaluation, treatment with the 7-day cytarabine infusion and the 3-day daunorubicin infusion ("7 + 3") has not changed in the past 50 years.

Checkpoint inhibitors (ICI) represent a breakthrough in the field of oncology. In hematological neoplasms, the use of ICI in therapies is limited. In acute myeloid leukemia, in the available pilot studies, ICIs have shown promising results when combined with hypomethylating agents in patients with relapsed / refractory AML or in relapsed AML after allogeneic haematopoietic stem cell transplantation. It should be emphasized that the potential of checkpoint inhibitors requires further research to be widely used in AML therapy. Moreover, more studies underline the predictive and prognostic importance of immune checkpoints (PD-1, CTLA-4, PD-L1, B7-H3) already at the stage of the initial diagnosis of AML, as potential immunological biomarkers in predicting response to treatment and the impact on overall survival.

Therefore, the aim of this dissertation was: (1) To determine the predictive value of selected subpopulations of T lymphocytes in patients with AML, including PD-1, PD-L1, CTLA-4 and B7-H3 expression, together with the percentage of these lymphocytes in patients with AML undergoing standard chemotherapy. (2) Determination of the predictive value of PD-1, PD-L1, CTLA-4 and B7-H3 expression on the surface of CD33+ myeloblasts and the absolute number of these cells in AML patients undergoing standard chemotherapy. (3) Assessment of the obtained results in risk cytogenetic and molecular groups. In the *in vitro* studies: (4) Evaluation of the viability and proliferation of AML blasts in response to ICI in the presence or absence of the chemotherapeutic agent in AML therapy - cytarabine. (5) Assessment of viability, proliferation and changes in the activation status of T lymphocytes in response to ICI in the presence or absence of the chemotherapeutic agent - cytarabine.

Material for the studies involved peripheral blood collected for an anticoagulant (EDTA) from 72 patients with AML before the start of chemotherapeutic treatment. Using the multicolour flow cytometry technique, the expression level (MFI) of selected immunological check-points - PD-1, PD-L1, CTLA-4, B7-H3 - was determined on CD3+CD4+ T cells and CD33+ blasts as well as the percentage of CD3+CD4+ T cells and an absolute number of CD33+ blasts expressing the tested proteins. To evaluate changes in viability, the proliferation of blasts and lymphocytes and changes in the activation status of lymphocytes in the presence of the basic chemotherapeutic agent in AML therapy - cytarabine - and selected ICIs, the study was extended to cell culture studies with a subsequent cytometric evaluation of the cells. *In vitro* cell cultures with peripheral blood mononuclear cells (PBMC) in the presence of ICI, with/without the addition of cytarabine, were used from 16 patients with AML before treatment, including 8 persons in the CR and NR groups, respectively.

Based on the obtained results, it was shown: (1) Patients with AML who do not respond to treatment with standard chemotherapy (NR) show a higher percentage of T helper cells expressing PD-1 receptor and a higher PD-1 expression level on T helper cells, compared to patients responding to chemotherapy (CR). (2) Patients with AML not responding to treatment with standard chemotherapy (NR) show a higher absolute number of CD33+ blasts expressing B7-H3 and a higher

level of CTLA-4 receptor expression on CD33+ blasts compared to chemotherapy responders (CR). (3) AML patients responding to treatment with standard chemotherapy (CR) show a higher PD-1 expression level on CD33+ blasts compared to compared to patients not responding to chemotherapy (NR). (4) The assessment of the percentage of T helper lymphocytes and the number of CD33+ blasts expressing the B7-H3 receptor shows a negative prognostic potential for overall survival in AML patients. (5) The use of PD-1, PD-L1 or CTLA4 blocking antibodies in the presence or absence of cytarabine does not increase apoptosis of AML blasts in *in vitro* culture. (6) The use of PD-1 blocking antibodies in the presence of cytarabine in *in vitro* culture significantly inhibits the proliferation of blasts as compared to the culture with the chemotherapeutic agent alone. (7) The use of PD-L1 and PD-1 blocking antibodies in the *in vitro* culture of PBMC of AML patients in the presence of cytarabine leads to an increase in the activation of cytotoxic and helper lymphocytes. (8) The use of PD-1, PD-L1 and CTLA-4 blocking antibodies in the *in vitro* culture of PBMC of AML patients do not affect the number of regulatory T lymphocytes.

In conclusion, based on the performed research, the study showed that the immune checkpoints (PD-1, CTLA-4, PD-L1, B7-H3) are potential prognostic and predictive immunological biomarkers in AML.