

Chronic lymphocytic leukemia (CLL) is a lymphoproliferative disease characterized by the accumulation of mature B lymphocytes in the peripheral blood, bone marrow and lymphoid tissues, such as the spleen and lymph nodes. The heterogenous course of CLL is reflected in varied survival times, response to treatment, and the dynamics of disease progression. CLL represents the most common leukemia among adults in western countries. The CLL prevalence noticeably increases with age, with more than 70% being older than 60 years at diagnosis.

Recent progress in next-generation sequencing (NGS) has enabled characterization of the genetic aberrations associated with the development, progression, and treatment resistance in CLL patients. Furthermore, the identification of the impact of the microenvironment and of the signaling factors that play crucial role in leukemogenesis allowed the development of agents, which specifically target dysregulated pathways, such as B-receptor signaling and apoptosis machinery. The discovery of small molecule inhibitors, such as inhibitors of Bruton tyrosine kinase (BTK) and B-cell lymphoma 2 (BCL2) has changed the paradigm of treatment of relapsed disease and improved patients' survival. In the last decade, genomic and epigenomic studies have expanded the knowledge of the pathogenesis of CLL remarkably, unraveling a large number of novel alterations that might drive the evolution of the disease. Although the identification of different markers provided additional prognostic information, CLL remains an incurable disease, and its varied clinical course requires further research for new prognostic factors, especially those linked to pathomechanism.

Regarding the preliminary work, we assessed the expression of the predominant *R1* transcript of *NPM1* (*NPM1.R1*) in samples collected from 100 patients with CLL. NPM1 is multifunctional protein, and its activity is critical for normal cellular biology. The main function of NPM1 is to regulate the pre-RNA processing, which determines a proper proliferation and differentiation of cells. Our data indicate that a high level of expression of *NPM1.R1* is associated with poor prognosis in CLL patients.

Therefore, the key objective of this study is the investigation of the influence of the *NPM1.R1* expression on disease progression and treatment response in patients with CLL. Hence, we plan to use frozen biobanked samples collected from 100 patients with CLL to determine the prognostic significance of the *NPM1.R1* expression in enlarged cohort of CLL patients. Furthermore, 50 patients with CLL will be prospectively enrolled in the study. Collected samples will be used for the characterization of a gene expression profile of CLL cells with high or low *NPM1.R1* expression, as well as of cells with the silenced *NPM1* gene with the use of RNA-seq approach. Furthermore, we will identify cellular processes involved in response to selected targeted agents: venetoclax (inhibitor of BCL-2) and ibrutinib (inhibitor of BTK) interacted by silenced *NPM1* expression *in vitro* using Western Blot and flow cytometry analysis.

The results of presented project will determine whether the level of *NPM1.R1* expression is an independent prognostic factor for CLL patients and if the response to targeted therapies depends on *NPM1.R1* expression level. Furthermore, identification of novel NPM1-related therapeutic targets might provide a rationale for innovative treatment strategies.