Chronic lymphocytic leukemia (CLL) is a one of hematopoietic diseases in which mature lymphocytes accumulate in the blood and bone marrow, and as the disease progresses also in the lymph nodes, spleen and liver**.** CLL is the most commonly diagnosed type of leukemia in people over 65 years of age, being a 30-40% of all leukemia diagnosed in Western Europe and the United States of America.

The clinical course of CLL is characterized by high heterogeneity, including cases requiring treatment at the time of diagnosis, delayed treatment as well as never requiring treatment. Patients' survival varies from months in cases of rapid progression to over a dozen years in relatively mild cases and low clinical advancement. That reflexes CLL's diversity of genetic and epigenetic changes, which are involved in CLL pathogenesis. Despite recent advances in the development of therapeutic options, CLL remains largely incurable.

Research, which try to identify new targeted therapies for patients with CLL aims at personalizing treatment that will improve both the effectiveness and tolerance of therapy. The rapid progress in next generation sequencing (NGS) have become possible to identify a number of mutations with clinical implications and to discover of new driver mutations. It is desirable that development of new personalized therapies should be based on the existence of individual mutations or differential gene expression. One example is a *NOTCH1* mutation, which occur in ~ 10% CLL and is associated with an unfavorable clinical outcome. Recently, anti-leukemic activity of bepridil, the calcium channel blocker associated with inhibition of the NOTCH pathway was reported for CLL patients. Furthermore the same biological consequences like activating gene mutation could have overexpression of certain gene in particular signaling pathway. The next therapeutic target of CLL therapy is *Exportin1* (*XPO1*), overexpression, deregulation, or dysfunction of which has been reported in various types of cancer. The *XPO1* antagonist – selinexor is now being investigated in clinical trials in CLL. Although *XPO1* mutation is rare (<4%) in patients with CLL, the gene overexpression in greater number of patients might provide rationale for selinexor therapy in CLL.

Another promising target in treatment of CLL is *Enhancer of zeste homolog 2* (*EZH2*), which has a critical role in multiple biological processes *via* epigenetic regulation of gene transcription. In CLL, *EZH2* mutations were found in individual patients, however, *EZH2* overexpression was significantly correlated with CLL poor prognosis. That suggest, that high level of gene expression might provide rationale for targeted therapy. In addition, *EZH2* pharmacological first-in-class inhibitor (tazemetostat, EPZ-6438) is currently validated in clinical trials for other types of B-cell malignancies (DLBCL and FL). Interestingly in FL and DLBCL patients without *EZH2* mutation 30% overall response rates were observed, what might be related with overexpression of *EZH2*.

The above mentioned examples emphasize the importance of assessing the sensitivity of CLL cells to available inhibitors. According to current knowledge, no studies have yet been conducted to determine the biological consequences of overexpression without mutation as compared to overexpression with mutation. Therefore, this project includes determining the mutations profile for 50 patients with CLL using NGS and functional studies, which will determine the biological consequences of both mutations and overexpression of *EZH2* in patients with CLL. Moreover, the sensitivity of cells with a mutation and overexpression of *EZH2* to tazemetostat will be determined. The results will be compared with the clinical parameters of the patients to perform the correlation analysis. An analysis of the results with known molecular prognostic factors *(TP53, SF3B1, BIRC3, MYD88, NOTCH1, ATM)* will also be carried out.

The main aim of this study is to confirm, that inhibition of EZH2 signaling pathways might be effectively used in CLL depending on molecular mutations and/or gene expression. The results of presented project might provide a rationale for the new clinical trials to identify the best therapeutic option for certain CLL patients, which could considerably improve the results of treatment in CLL.