Hodgkin lymphoma (HL) is a cancer derived from germinal center B cells, developing in about 2-3 people per 100,000. They emerge during the B cell development where they obtain improper genetic alterations and, for not fully understood cause, survive instead of undergoing a typical in these circumstances programmed cell death – apoptosis, giving characteristic for this disease Hodgkin and Reed-Sternberg (HRS) cells. These cells differ in many ways from healthy B cells, showing signaling pathways disruptions. An important event during HRS cells creation are significant changes in DNA methylation profile, leading to tumor suppressors inactivation and deregulation of cell signaling pathways. As looking for these suppressors can lead to significant insights into cancer biology, we decided to research this topic.

Upon analysis of microarray-based expression and DNA methylation data, a *CXXC5* gene caught our attention. We identified its significant downregulation, which was accompanied by increased DNA methylation of its promoter region. These changes were observed only in samples derived from HL, in comparison to other B-cell lymphomas and non-cancer B-cells. These data suggest, that *CXXC5* may be a tumor suppressor gene, specific for HL, and its inactivation is caused by DNA hypermethylation. What is more interesting, the *CXXC5* gene is known for its broad epigenetic regulatory effect, based on its binding of unmethylated CpG islands and attracting further regulatory proteins. Because of this, its potential in shaping the cancer cells appears to be worth studying. Our hypothesis is that the suppression of *CXXC5* gene positively and significantly affects the cancer cells in HRS development and further survival.

In our project, we plan to find if its hypermethylation is indeed the mechanism behind this gene's silencing in HL. For this, we will perform a targeted DNA demethylation of this gene in Hodgkin lymphoma cell lines. The level of the decrease in DNA methylation will be estimated with bisulfite pyrosequencing and the effects – gene expression restoration – will be confirmed at the transcriptomic (RT-qPCR) and proteomic (Western blot) levels. Next, to investigate the impact of *CXXC5* on Hodgkin lymphoma cells' biology, we will induce a stable overexpression of CXXC5 protein in HL cell lines. We will investigate the implications of its presence in the cell, using proliferation, viability, and cell death tests. Our hypothesis is, the *CXXC5* expression induction will turn out to have an unfavorable effect on the cells, thus leading to the decrease in proliferation and viability, while an increase in apoptotic potential. Subsequently, we plan to expand our knowledge of the effects of CXXC5 presence on HL cell functioning. Because of the role of CXXC5 as a transcription factor, we want to identify which regions are targeted by the CXXC5 protein. To do so, we will perform ChIP-seq analysis. We think that the regions bound by the CXXC5 protein will be enriched with suppressor genes. For further evaluation of this, we will perform global DNA methylation analysis with the use of Oxford Nanopore Technology. Here, we also expect an enrichment of the same types of genes, with significant coverage between DNA methylation and CXXC5-ChIP-seq data. Colocation of these marks would be a strong indicator that, indeed, *CXXC5* is the leading agent, whose absence is related to methylation profile changes and thus Hodgkin lymphoma development or progression, and thus it may be a new potential tumor marker, used for HL diagnostics.