Hodgkin lymphoma (HL) is one of the most common neoplastic diseases in young adults (15-35 years old) and accounts for approx. 15% of all lymphoma cases in Poland. A common feature of HL is the presence of specific malignant cells named Hodgkin and Reed–Sternberg (HRS) cells in lymph node biopsies from HL patients. These cells are rare: usually constitute only about 1-5% of all cells in the lymphoid mass, which significantly limits the understanding of HL genetic background.

New possibilities for the genetic analysis of HL have appeared with introduction of liquid biopsy to the molecular studies and analysis based on cell-free DNA (cfDNA). Cell-free DNA are degraded DNA fragments (50 - 200 bp), released to the blood plasma upon cell death. Liquid biopsy consists of the following steps: 1) collecting of peripheral blood from the patient; 2) cfDNA isolation; 3) analysis of the tumor-derived cfDNA fraction, called circulating tumor DNA (ctDNA). It was previously shown, that plasma ctDNA reflects HRS cells genetics, which makes liquid biopsy a good technique for diagnostics and health monitoring of the patient.

Inactivation of the genes due to their hypermethylation, involving the addition of methyl groups to DNA, is extremely significant in HL development. Thus, the current project assumes the analysis of genomic DNA (gDNA) and cfDNA methylation level in samples derived from HL (cell lines, blood samples and nodal biopsies) and comparison of the obtained results with DNA methylation status of gDNA and cfDNA samples from healthy volunteers. The analysis will be performed using next generation sequencing (NGS) techniques which allow for fast and accurate determination of DNA sequence and identification of methylated nucleotides within the analyzed fragment of DNA. As a result of this part of the project, we plan to establish the panel of five genes - potential biomarkers of HL - hypermethylated in HL cells, but not methylated in non-lymphoma cells.

In the second part of our project implementation, we plan to re-analyse plasma cfDNA methylation profile of HL patients after therapy. After taking another blood sample from this group of patients cfDNA methylation level of selected potential biomarkers will be analyzed. We also plan to perform similar analysis, using blood samples collected from the newly diagnosed Hodgkin lymphoma patients. This will allow to estimate the utility of liquid biopsy as a tool to measure residual disease in HL, as well as to verify its usability for HL diagnosis.

To summarize, in this proposal we plan to establish an panel of genes - potential biomarkers of Hodgkin lymphoma and investigate the possibility of using a liquid biopsy as a tool for diagnosis and health monitoring of lymphoma patients.