DNA of the cells of many cancers (including the leukemias) characterized in global DNA hypomethylation. This suggests that DNA hypomethylation is responsible for the genomic instability and malignant transformation. Because despite many reports about the participation of active DNA demethylation in the development of acute leukemia so far they have no application in clinical practice, we decided to answer the following questions:

- Are the products of epigenetic processes such as active demethylation and deamination of DNA, measured in DNA of peripheral blood cells, plasma, or urine may in the future become a biomarker of acute leukemias development, which will allow the prediction and monitoring of the treatment of aforementioned diseases?
- Is it possible to track abnormalities in the functioning of metabolic pathways involved in epigenetic processes in individuals suffering from acute leukemia and in those who have had chemotherapy.
- Is it possible to do so by analyzing changes in the concentrations of specific products of these pathways at the level of cellular DNA, and their concentration in plasma and urine?
- Whether the treatment with demethylating agents result in molecular changes in metabolic pathways engaged in epigenetic processes?
- Finally, whether the mutations in the genes encoding the various elements of the investigated metabolic pathways, translate to the changes in concentration of their products?

In order to answer the aforementioned questions we are going to measure and compare the concentration of products of metabolic pathways involved in the epigenetic processes such as deamination and active demethylation in patients suffering from acute lymphocytic leukemia (ALL), acute myelogenous leukemia (AML), in patients with myelodysplastic syndrome (MDS) and in patients with MDS-based ALL, as well as in the control group. Those measurements will be done at two time points, before treatment and during the period of remission. Obtained measurements will be compared with the results of genes involved in the investigated processes expression, as well as with the status of their mutations.

In patients in which a significant percentage of the leukemic clone cells will be present in peripheral blood, we will make an attempt to isolate them, and subsequently we will identify demethylated and deaminated nitrogen bases in their genetic material. Moreover, by using the scoring methods and classification trees we will make an attempt to select a biomarker or combination of biomarkers that will be characterized by the highest diagnostic and predictive value, that in the future may be a base for the construction of simple diagnostic test, which would come into routine clinical practice on a permanent basis.

In order to determine the abovementioned modifications in DNA and urine will use a highly advanced technique, namely automatic online two-dimensional ultra-high performance liquid chromatography with tandem mass spectrometry (2D-UPLC/MS/MS), in the case of determinations carried out in the urine, we will use the techniques of gas and liquid chromatography combined with two-dimensional mass spectrometry. For the analysis of genes involved in epigenetic processes expression, we intend to use the qRT-PCR technique by using the LightCycler 480 - one of the most advanced devices of this type on the market. We will also use it to evaluate the presence of point mutations by innovative HRM-PCR technique.

The proposed project will allow more comprehensive understanding of epigenetic processes, especially recently known processes active DNA demethylation. It is particularly important in the context of the pathogenesis and therapy of acute leukemias, since abnormalities in epigenetic regulation of genes responsible for cells proliferation and differentiation are underlying the development of these diseases. We are hoping that our findings will support the hypothesis that global determination of epigenetic modifications might be a useful diagnostic tool that would allow to predict and monitor the process of disease treatment, as well as to estimate the risk of its recurrence. Those results might also constitute the background for the development of cheap, simple diagnostic tests, that will examine the concentration of selected biomarkers with the highest predictive value, which ultimately would enter into the routine clinical practice. Furthermore, this study might contribute in introduction of personalized therapeutic approaches, which would be based on the application of the most effective chemotherapeutic agents for the treatment of each individual patient.