Each cell of the human body contains the same genetic material, but in cells from various tissues it is expressed in a completely different way, which translates into a unique phenotype. Precise and dynamic regulation of the expression of individual genes underlies the plasticity of the human genome and enables rapid response to changing environmental conditions. Epigenetic processes, such as DNA methylation, and recently discovered active DNA demethylation, carried out by proteins from TET (Ten-Eleven-Translocation) family plays a crucial role in the regulation of gene expression. This newly identified group of enzymes, which include TET1, TET2 and TET3 proteins, is responsible for the enzymatic oxidation of 5-methylcytosine (product of DNA methylation) to 5-hydroxymethylcytosine as well as 5-hydroxymethylcytosine to 5-formylocytosine and 5-carboxycytosine, which are subsequently removed from DNA and replaced with unmodified cytosine. Moreover, it has been recently discovered that aforementioned proteins can also produce 5-hydroxymethyluracil from thymine - one of the most enigmatic DNA modifications, the origin of which is still not fully understood. It is worth mentioning that mutations in the genes encoding TET proteins are very often found in various types of cancer, in particular in haematological malignances, where disorders in active demethylation of DNA are one of the key factors in the process of carcinogenesis.

Despite the large interest in the scientific world, very little is known about the enzymatic activity of individual members of the TET protein family and their participation in the formation of epigenetic derivatives of 5-methylcytosine. Therefore, the objectives of our project are:

- to assess contribution of activity of each member of TET family in generation of 5-hydroxymethylcytosine, 5-formylcytosine, 5-carboxylcytosine and 5-hydroxymethyluracil;
- to identify proportion of 5-hmUra generated as a result of oxidation of thymine and deamination of 5-hmCyt;
- to determine if there are any differences in susceptibility of particular members of TET family to stimulatory effect of ascorbate

in order to do that, we will use the highly advanced genetic engineering techniques (including the novel CrispR/Cas9 technique) that will allow us to selectively silence the expression of individual TET proteins in human, established cell lines of normal and cancer cells, which will also be exposed to small molecule inhibitors and activators of the enzymatic activity of TET proteins. In order to determine the above-mentioned modifications in the DNA we will use a highly advanced research technique: two-dimensional ultra-performance liquid chromatography with tandem mass spectrometry (2D-UPLC/MS/MS), we will also determine the expression of individual TET genes using the RT-qPCR technique using LightCycler 480 and flow cytometry.

Our project will allow a better understanding of epigenetic processes, in particular the process of active DNA demethylation. Proposed study will also provide unique, highly accurate and reproducible data on the enzymatic activity of individual TET proteins and their contribution to the formation of epigenetic DNA modification patterns. This is particularly important in the context of the pathogenesis of many cancers and their treatment, as disorders in the epigenetic regulation of gene activity that determines cell proliferation and differentiation underlies the development of these disease entities.

The acquired data may in the future contribute to the development of fast and cheap diagnostic tests. Their use in routine clinical practice would allow for early detection of abnormalities in the functioning of TET proteins in cancer patients, without the necessity to analyze the profile of TETs mutations (eg. by DNA sequencing). It is also worth mentioning that data on TET protein dysfunction, reflected in the profile of individual epigenetic derivatives, can greatly facilitate the assessment of patient survival, help in the selection of the optimal therapeutic strategy, and enable the monitoring of the cancer treatment progress and the risk of its recurrence. What is more, these studies may be a contribution to the introduction of strictly personalized therapeutic strategies, based on the selection of the most effective chemotherapeutic agents for the treatment of an individual patient.