

## The role of 5-hydroxymethyluracil in chronic lymphocytic leukemia development

### **Project objective:**

The objective of this project is to clarify the function of 5-hydroxymethyluracil (hmU), one of the most enigmatic modifications in human genetic material. We propose that hmU is an epigenetic modification that regulates gene expression, and as such, contributes to development of chronic lymphocytic leukemia (CLL). In particular, we plan to develop new research tool to hmU profiling and determine how hmU impacts development of chronic *lymphocytic* leukaemia as well as whether hmU can serve as a biomarker for CLL disease progression.

### **Study description:**

We will perform a number of analyses and experiments on clinical material from patients suffering from CLL and in custom designed cellular models, using the most advanced research technology, including two-dimensional, high performance liquid chromatography with tandem mass detection (2D-UPLC-MS/MS), direct and indirect flow cytometry, genome sequencing. The planned research will allow: i) development of additional tools enabling the detection and quantification of hmU not only at the genome level, but also at the level of individual cells (single-cell-resolution analysis) and in particular DNA regions (MoDIP-MS and ChIP-MS techniques); ii) define the role of hmU in chronic lymphocytic leukemia. In particular, the proposed project will allow us to define the specific genotype and phenotype of the CLL cells containing enormous amount of hmU. Another important aspect of the study is to determine whether the level of hmU, and its distribution in the genome, characterizes a subgroup of patients with premalignant monoclonal B cell lymphocytosis (MBL) with greatest risk of developing full blown CLL.

### Rationale and motivation:

5-hydroxymethyluracil is one of the most enigmatic modifications in human DNA. Its sources, genomic location, as well as mechanisms of generation and removal are still poorly defined. Research to date indicate that hmU has all the features required of an epigenetic mark. Still, the role and participation in specific processes such as regulation of gene expression or chromatin remodeling remain largely unexplored partly due to lack of suitable model system and precise methodology. Our preliminary results indicate an unprecedented, approximately 10-fold, increase in the level of hmU in patients with chronic lymphocytic leukemia (which accounts for about 30% of all leukemias). Notably, high hmU levels appear to be a particular characteristic for CLL as we did not observe significant changes in hmU levels in other hematological malignancies. This indicates a potential involvement of hmU in CLL development.

### **Expected outcomes:**

The proposed research will allow us to determine the function of hmU in the human genome and its role in the development of chronic lymphocytic leukemia. This project will also enable the establishment of international cooperation with a leading research center. In the long term, the project has potential to improve management of patients at risk of developing CLL by offering the newest diagnostic tools that are not routinely used in clinical practice, such as: screening for mutational status, clonality assessment, and extended phenotyping.