Uracil in DNA and aberrant DNA methylation/hydroxymethylation as a new biomarkers of multiple myeloma development? A search for a possible link between genetic/epigenetic DNA modifications and multiple myeloma evolution.

The DNA of all living cells undergoes continuous structural and chemical alteration, which may be derived from exogenous sources, or endogenous, metabolic pathways, such as cellular respiration, replication and DNA demethylation. It has been estimated that approximately 70,000 DNA lesions may be generated per day in a single cell, and this has been linked to a wide variety of diseases, including cancer.

DNA methylation is involved in a range of diverse biological processes, including gene expression which, in turn, has a profound impact on cellular identity and organismal fate. Cytosine methylation in cellular DNA is dynamic and linked with a family of ten-eleven translocation proteins (TET 1, 2 & 3 enzymes) which are responsible for the process of active DNA demethylation. TETs can catalyze oxidation of 5-methylcytosine (5-mCyt) to form 5- hydroxymethylcytosine (5-hmCyt) and the oxidation reaction can proceed further to generate 5-formylcytosine (5- fCyt) and 5-carboxycytosine (5-caCyt).

Our proposal is aimed to determine a role of the above mentioned modifications in Multiple Myeloma (MM) development. MM is the second most common hematological malignancy, which results in over 100,000 deaths/year, worldwide. MM is a progressive disease almost always preceded by an asymptomatic stage - monoclonal gammopathy of undetermined significance (MGUS). **MGUS is common in older age group where is present in 3% of the population over 50 years, increasing to almost 9%** of over 85 years old , and progresses to multiple myeloma at a rate of ∼1% per year. MGUS is often followed by an intermediate stage -smoldering multiple myeloma (SMM), prior to symptomatic fully developed multiple myeloma (FMM) and plasma cell leukemia (PCL).

MM cells originate from hematopoietic stem cells (HSC) through B-cells toward plasmocytes (PC). Differentiation of B cells towards PC during maturation involves diversification of their *Ig* genes (*V(D)J* segment) by somatic hypermutation (SHM) and class switch recombination (CSR). CSR involves joining of DNA double strand breaks (DSB) which, in turn, are initiated by activation-induced cytosine deaminase (AID), which triggers the process of enzymatic deamination of cytosine to uracil in Ig locus in B cell lymphocytes. Notably, aberrant AID expression, can lead to off-target activity of the enzyme and genome-wide mutations in other than Ig genes and/or in other than lymphoid cells. This, in turn, may contribute to harmful genetic changes resulting in malignant transformation. Only recently the role of AID (and APOBEC) in MM development was recognized.

We hypothesize that: i/ deregulation of DNA methylation/demethylation pathways may be linked with variation of PC differentiation states and aberrant expression of AID what should result in genome wide significant changes in; a) the level of uracil in DNA and b) active demethylation products (5-hmCyt, 5-fCyt, 5-caCyt and 5-hmUra); These events may be a meaningful factors/biomarkers of MM development.

We recently developed a rapid, highly-sensitive and specific isotope-dilution automated two-dimensional ultraperformance liquid chromatography tandem mass spectrometry (2D-UPLC-MS/MS) method that isspecifically tailored to analyze global levels of 5-methyl-2`-deoxycytidine (5-mdC) and their derivatives, 5-methyl-2`-deoxyuridine (5-hmdU), 2`-deoxyuridine (dU) and 8-oxo-2`-deoxyguanosine (8-oxodG), simultaneously.

The main objectives are to analyse major hallmarks of MM development:·

1. **genome instabilit**y – reflected in: **i/** dU presence in DNA and **ii/** mutations of the certain genes involved in MM pathogenesis; (e.g. *TET*s, *RAS*), to find out possible C to T transition characteristic for cytosine deamination to mutational motif known to be the AID target;
2. **epigenetic defects** - mirrored in: **i/** changes of the whole spectrum of DNA epigenetic modifications and **ii/** aberrant methylation pattern of promoters of the key genes involved in MM development;
3. **dysregulation of DNA repair** – *via* expression of mRNA/proteins of key repair genes and urinary exertion rates of the repair products.

Therefore, they may deliver prognostic factors/biomarkers which may be helpful to work out risk stratification and to predict survival.

Thus, our analyses address one of the key, unanswered question - what is original-initial source of genome instability/ epigenetic changes - the hallmarks of MM development.

The drugs used during MM chemotherapy induce oxidative stress. To clarify whether oxidative stress/DNA damage is linked with development of the resistance to chemotherapy we will analyse widely used biomarker of oxidative stress/DNA damage, namely 8-oxodG, in cellular DNA and urine.