Multiple myeloma (MM) is a lymphoid malignancy affecting around 5.5/100 000 people in Europe, mainly elderly patients - median age at onset equals 67 years old. MM is derived from terminally differentiated lymphocytes B that, in most of the cases, produce characteristic monoclonal protein, which is either a complete immunoglobulin or a free light chain of an immunoglobulin. Symptoms of the disease are caused by malignant cells hyperplasia in bone marrow and by high serum concentration of the monoclonal protein and consist mainly of: lytic bone lesions that causes pathological fractures, anemia, immunodeficiency and renal failure.

There has been a huge breakthrough in multiple myeloma therapy in the last 15 years. It is connected with introduction of many novel drugs, containing as different classes of medications as proteasome inhibitors (PIs), immunomodulatory agents, histone deacetylase inhibitors or monoclonal antibodies. The advances in therapy allowed to prolong patients median overall survival time, from the moment of diagnosis, from previous 3 to current 6 years. Unfortunately, even such a huge progress couldn't manage to make multiple myeloma curable.

MM is incurable despite the fact that most of the patients responds to the first line treatment, implemented after diagnosing of symptomatic multiple myeloma, with a significant decrease in malignant cells burden and achieve the state of remission. However, after different period of time, attributed to the disease's heterogeneity, an inevitable relapse occurs. After several lines of treatment, the relapsed disease becomes refractory to every available drugs and patient dies in the state of active disease. It is the main reason why studies on acquired drug-resistance appear to be crucial in terms of further progression of myeloma therapy. Despite many efforts, we still lack in comprehensive description of this process that could lead us to breaking the resistance.

One of the most important class of the new drugs, present in almost every regimen of the therapy, are proteasome inhibitors - bortezomib and carfilzomib. Proteasome is an enzymatic aggregate that is responsible for cleavage of specifically tagged proteins that were previously directed to degradation pathway. Drug-induced proteasome inhibition causes accumulation of defective proteins, leading to the induction of apoptotic mechanisms and finally death of a cell. Introduction of the PIs into myeloma treatment regimens, significantly improved patients' prognosis. However, these drugs are also subjects of acquired resistance.

The aim of the study is investigating the mechanisms responsible for acquired resistance to carfilzomib and bortezomib. Toward this goal, proteasome inhibitor-resistant cell lines will be generated in vitro by our team from multiple myeloma cell lines. It will be achieved by long-term incubation of the cells with increasing concentration of the drugs. After establishing the resistance, a comparative proteomic (proteome is a „genome of proteins") analysis will be performed between resistant and sensitive cells and between bortezomib-resistant and carfilzomib-resistant cell lines. The analysis will be made with highly effective proteomics, which is a technique that allows to globally assess all of the proteins present in a cell. What makes this approach unique in comparison to more popular methods, like for example gene expression analysis using microarrays, is the fact that as a result of proteomic study we obtain a protein profile of a cell, which has a decisive role in the cells' biology. While, due to such mechanisms as for example post-translational modification of the proteins, in the case of genomic studies we cannot establish such a direct connection of its results with the cells' physiology.

As a result of comparative proteomic profiling of the resistant and sensitive cells a group of differentially accumulated proteins will be obtained. These results will be subsequently verified using Western blot technique, which allows to detect previously known proteins.

In the next step we will validate, if the in-vitro observed effects have their counterpart in changes that occur in real-life setting - in myeloma cells obtained from patients. To do so, the same Western blot technique will be used on purified plasmocytes from the patients bone marrow samples. The patients will be divided into two groups: those with newly diagnosed multiple myeloma that responded well to bortezomib-based therapy (PI sensitive group) and the PI resistant group - with progressive disease despite treatment with bortezomib and/or carfilzomib. Moreover, the concentration of the proteins-biomarkers will be measured in these two groups also in serum samples.

If the validation studies on both, cell lines and patients' plasmocytes, prove the proteomic results, additional functional studies will be performed. The goal of this studies will be to check if knocking out the genes corresponding with identified proteins will lead to increasing cells' sensitivity to proteasome inhibitors.

The results of this study should importantly contribute to better understanding of the mechanisms responsible for acquired PI-resistance. Establishing of the biomarkers will pave the road to defining new therapeutical targets for potential adjuvant drugs that could break the myeloma cells' resistance to therapy. While identification of these proteins in serum samples will enable its further, prospective evaluation as a prognostic factor that describes patients' response to treatment with bortezomib or carfilzomib. This would lead to efficient individualization of a therapy.