Abstract:

Myelodysplastic syndromes (MDS) are a set of severe hematological diseases characterized by ineffective clonal hematopoiesis in the bone marrow, leading to cytopenia in the peripheral blood, the development of transfusion dependence and a high risk of progression to acute myeloid leukemia (AML). The disease is caused by genetic and epigenetic changes leading to the development of pathological stem cells that are unable to mature sufficiently in the bone marrow into blood elements. These changes vary widely between patients, which is reflected in different clinical manifestations, response to treatment, overall survival and, last but not least, this heterogeneity represents a challenge for the study of this disease. The present dissertation is aimed at studying the pathophysiological manifestations and consequences of selected genetic alterations, especially somatic mutations of key genes and other functional units of the genome, in relation to the clinical course of MDS and transformation to AML. Therapy of high-risk MDS is currently based on hypomethylating drugs including 5-azacytidine (AZA). Treatment leads to prolongation of disease progression to AML, but this fate is irreversible for the vast majority of patients whose prognosis becomes hopeless at this point. Results of genetic analysis by next-generation sequencing (NGS) of serial samples before and during AZA treatment in 38 patients (achieving a median overall survival of 24 months with 60% of patients achieving clinical response) yielded the identification of 116 somatic pathogenic variants with allelic frequency (VAF) >5%. We found that almost half of the variants were stable, while the remaining variants were highly dynamic. Patients with a significant decrease in allelic load on AZA treatment achieved clinical response. A similar analysis was performed in an academic randomized trial that compared the effect of adding granulocyte growth factor (G-CSF) to standard AZA therapy (EudraCT#: 2013-001639-38). We observed the capture of 140 pathogenic mutations in 70 enrolled patients. Of the variants detected, mutations in the DNMT3A (p=0.0131), ETV6 (p=0.0012), EHZ2 (p=0.0044) and SF3B1 (p=0.0005) genes negatively and positively affected overall survival, respectively. To better study AZA resistance, we developed an AZA resistance model from cell lines derived from AML patients that preceded MDS, the stability of which was verified by transplantation into immunocompromised NSGS mouse strain. By examining mRNA expression and DNA variants of the AZA-resistant phenotype, we observed deregulation of several pathways related to oncogenesis, including phosphatidylinositol-3 kinase (PI3K) signaling. We further showed that these pathways can be modulated by specific inhibitors that, while blocking the proliferation of AZA-resistant cells, are unable to increase their sensitivity to AZA. The data

from this work reveal a set of molecular mechanisms that can be targeted by specific inhibitors to expand therapeutic options during AZA treatment progression.

Keywords:

MDS, AML, AZA, NGS, therapeutic resistance