ABSTRACT

Cytogenetic and molecular genetic analyses are necessary for precise assessment of diagnosis, prognosis and treatment of patients with AML. The karyotypic analysis allows the distribution of patients into the basic risk groups, while the methods of molecular biology offer further possibilities to stratify patients within particular risk subgroups. Moreover, using quantitative PCR, they enable to follow the course of minimal residual disease (MRD) and foresee the eventual relapse of the disease.

The aim of this thesis was to analyse the prognostic impact of new molecular markers in patients with AML, particularly in those with favourable (acute promyelocytic leukemia (APL), CBF-AML) and intermediate (influence of *FLT3* mutations and others) cytogenetic profiles.

The presence of fusion genes *PML/RARα*, *AML1/ETO* and *CBFβ/MYH11* was tested by qualitative PCR. Patients harbouring fusion genes *AML1/ETO* or *CBFβ/MYH11* (CBF-AML) were further analysed using either sequencing or restriction digest analysis, for the presence of *C-KIT*, *K-RAS*, *N-RAS* and *FLT3* mutations. Patients with intermediate cytogenetic risk were tested for presence of internal tandem duplications of *FLT3* (*FLT3*/ITD), mutations in tyrosine kinase domain of *FLT3* (*FLT3*/TKD), *DNMT3A* and *ASXL1* mutations. Cases with a complex karyotype were screened for *TP53* mutations. Real time PCR was used for monitoring of MRD. The impact of these aberrations on disease progression and prognosis in particular risk groups of patients was analysed.

Out of 654 patients, in 141 (21.6%) one of the prognostically favourable fusion genes were detected: *PML/RARα* (92 patients), *AML1/ETO* (27) and *CBFβ/MYH11* (22). Patients carrying the fusion genes *PML/RARα* or *AML1/ETO* had a lower risk of relapse and almost 70% of them were alive 3 years after the diagnosis of AML. The chance to reach CR in cases with *PML/RARα* was diminished by the presence of *FLT3*/ITD. Patients harbouring *CBFβ/MYH11* fusion had a higher incidence of relapse and overall survival (OS) of these patients was around 60%. The relapse rate in patients with CBF-AML was increased by *C-KIT*, *K-RAS* and *FLT3*/TKD mutations, as well as by the persisting positivity of MRD.

According to the results of karyotypic analysis, 394 (60.2%) patients were included within the intermediate cytogenetic risk group. Both *FLT3* (*FLT3*/ITD, *FLT3*/TKD) and *DNMT3A* mutations had a strong adverse impact on the relapse rate in these patients. Those carrying *FLT3*/ITD had a much shorter OS (3 years after the diagnosis, only 17% of patients were alive), regardless of the ITD length and insertion site. *ASXL1* mutations had no impact on prognosis of AML.

119 patients (18.2%) were assigned into the unfavorable risk group. In 60 of them, complex karyotypic changes were shown. OS after 3 years within this group was below 10%.

This study confirmed the unfavorable prognostic impact of *FLT3*/ITD (but no additional impact of its length, insertion site and mutated allele burden was demonstrated) and *DNMT3A* mutations. The adverse impact of *C-KIT*, *FLT3*/TKD and *K-RAS* mutations on the relapse rate of CBF-AML patients was demonstrated. The methods for MRD monitoring were developed for cases with the most frequent types of *DNMT3A* mutations.

Kewords: acute myeloid leukemia – acute promyelocytic leukemia – core binding factor AML – *FLT3*/ITD – *FLT3*/TKD – *DNMT3A* – *ASXL1* – mutation – prognosis.