

Abstract

Acute myeloid leukemia (AML) is an example of a myeloid malignancy characterized by a dynamic and aggressive clinical course. The disease affects all age groups, however the median age at diagnosis is approximately 65 years. AML is a heterogeneous and aggressive clonal disorder of hematopoietic progenitor cells. It is characterized by substantial diversity in chromosomal abnormalities, gene mutations, and alterations in the expression of numerous genes. Over the past decade, significant progress has been made in understanding the pathogenesis of AML. Importantly, up to 86% of AML cases are associated with the presence of at least two mutations, and additional genetic alterations may emerge during disease progression and treatment.

The high genetic heterogeneity of AML necessitates the use of high-throughput diagnostic methods that enable the detection of multiple mutations within a single assay. Next-generation sequencing (NGS) represents a major innovation in genomic analysis. NGS technologies allow for the identification of a wide spectrum of genetic alterations, including single-nucleotide variants, insertions and deletions, as well as gene fusions. The widespread application of NGS in AML diagnostics and research has been made possible by the introduction of commercial panels that enable the simultaneous analysis of multiple genes relevant to myeloid malignancies. This approach allows for the determination of an individual patient's mutational profile, thereby accelerating the diagnostic process and facilitating earlier implementation of personalized therapy.

The aim of this study was to analyze the mutational profile of 109 patients diagnosed with acute myeloid leukemia who were hospitalized at the Holy Cross Cancer Center in Kielce between 2020 and 2023.

The study material consisted of DNA and RNA isolated from bone marrow and/or peripheral blood samples. Mutational profiling was performed using NGS based on Ion Torrent technology. Genetic material amplification and library preparation were carried out using the Oncomine™ Myeloid Research Assay (Thermo Fisher Scientific), which enables the analysis of 40 genes as well as a panel of 29 fusion driver genes (a total of 687 fusion isoforms) associated with AML, myelodysplastic syndromes (MDS), and myeloproliferative neoplasms (MPN).

The obtained results confirmed the high genetic heterogeneity of the AML patient cohort treated at the Holy Cross Cancer Center in Kielce. Molecular alterations were identified in 95 patients (87.16%). Mutations were detected in classical AML genetic markers, including *FLT3*

(ITD and TKD), *CEBPA*, *NPM1*, *TP53*, *RUNX1*, *ASXL1*, *IDH1*, and *IDH2*, as well as in gene fusions such as *RUNX1::RUNX1T1*, *CBFB::MYH11*, and *PML::RARA*. The genes most frequently harboring mutations were *FLT3* (25%) and *NPM1* (17%), which are characteristic of AML and have significant clinical relevance. Additionally, mutations were identified in genes that are not routinely analyzed in standard AML diagnostics, including *JAK2*, *CALR*, *BCOR*, and *MPL*. These alterations occurred less frequently and have unclear prognostic significance, indicating the need for further investigation. Furthermore, data analysis revealed patterns of co-occurrence and mutual exclusivity of mutations, suggesting the existence of specific interactions among genes involved in AML development and their participation in distinct molecular pathways. The conducted genetic analyses may provide a basis for the implementation of targeted therapies in patients harboring mutations in specific genes, such as *FLT3*, *IDH1*, and *IDH2*.

Incorporating a broader range of genes into diagnostic testing may contribute to the development of more effective therapeutic strategies. The obtained results support the validity of using high-throughput parallel sequencing methods in the diagnosis of acute myeloid leukemia.

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