Prognostic Relevance of DNMT3A, FLT3 and NPM1 Mutations in Syrian Acute Myeloid Leukemia Patients

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Abstract

Objective: Among all types of hematological neoplasms, acute myeloid leukemia (AML) has the highest death rate. Recently, cytogenetic and molecular genetics are crucial in the management, as a consequence of their effect on AML pathogenesis, classification, risk-stratification, prognosis and treatment. Methods: 100 Syrian adults with Normal Karyotype (NK) newly diagnosed AML patients were included in this study, all cases confirmed histologically and immunohistochemically. Patients were divided into six subgroups using flow cytometry and cytological results. Polymerase chain reaction (PCR) was performed on exon 11-12 for FMS-like tyrosine kinase-3 internal tandem duplication (FLT3-ITD), exon 12 for Nucleophosmin1 (NPM1), and exon 23 for DNA methyltransferase 3A (DNMT3A) using target primers, the electropherograms were analyzed for gene mutations by comparing with the reference DNA sequence. Data were compared and aligned with different sequences using the NCBI BLAST Assembled Genomes tool. Results: FLT3-ITD, NPM1 and DNMT3A were detected in 24%, 22% and 4% patients respectively. M2 subtype had the most frequent incidence of diagnosis in AML. FLT3-ITD mutation patients had the highest mean of death cases, while the DNMT3A mutation patients had the lowest. On the other hand, the highest mean of remission was in patients with NPM1 mutation and the lowest in the carriers of the FLT3-ITD mutation. It was observed that the mean relapsed patients with FLT3-ITD and DNMT3A mutation was 3.4 and 2 months respectively, with no significant differences between (FLT3-ITD and DNMT3A) carriers and non-carriers relapsed. On the contrary, the mean relapsed for NPM1 mutation carriers was 2.4 months with significant statistical differences. The mean survival time for patients with FLT3-ITD and NPM1 mutation was 5.9 months and 5.85 months respectively, with significant correlation. Between it was 5.88 months in DNMT3A patients with no significant differences. Finally, It was noted that the mean event free survival (EFS) of FLT3-ITD mutation patients was 4.818 months and the mean EFS of NPM1 mutation patients was 4.805 months, with significant statistical differences (p<0.05) between the mutation patients and non-mutated patients regarding to EFS. While this mean was not statistically significant in patients carrying DNMT3A mutation. Conclusion: Patients with FLT3-ITD and NPM1 mutations have the worst prognosis, where the presence of those mutations was significantly related to overall survival (OS) and EFS. Our study reflects that DNMT3A was not an extremely bad prognostic effect as an independent factor. We can declare according to this study that genetic mutation and variants detection could easily be incorporated into the regimen evaluation of AML patients.

Keywords: Acute myeloid leukemia (AML)- cytogenetic- prognostic factors- DNMT3A, FLT-ITD

Introduction

Acute Myeloid Leukemia is a genetically diverse clonal cancer that arises from clonal hematopoietic stem cells, characterized by chromosomal abnormalities, recurrent gene mutations, epigenetic chromatin alterations, and microRNA deregulations. Among the numerous variables that have an impact in therapy effectiveness for AML patients, genomic heterogeneity, patient individual variability, and gene alterations are a few key roadblocks. An tremendous diversity of genetic mutations has been uncovered due to the use of advanced molecular devices (Döhner et al., 2017). FLT3, NPM1, and DNMT3A are examples of such genes, patients with AML have been documented to have and incorporated into the risk categories proposed by the European Leukemia Net (ELN).
A recurrent chromosomal translocation occurs in 30-40% of AML cases, while the remaining 60-70% have a normal karyotype. Because the genetic basis of AML in patients with a normal karyotype is still unknown, researchers have focused on finding particular gene alterations. Gene mutations have been found in 85% of AML cases with a normal karyotype, with FLT3 and NPM1 alterations being the most common (Moarini and Papaemmanuil, 2017). One of the most common genetic lesions seen in AML is NPM1 mutations, they found in 45-64% of instances of AML with a normal karyotype. Other human neoplasms consistently demonstrate nucleus-restricted NPM1 expression, indicating that NPM1 mutations are AML-specific. NPM1 and other recurrent genetic abnormalities are mutually exclusive. NPM1 mutations are typically heterozygous with the leukemic cells retaining a wild-type allele. They are most commonly seen in exon 12 of the NPM1 gene (Sami et al., 2020; Takahashi, 2011). NPM1 mutations have been linked to particular pathologic and clinical characteristics in AML patients with French-American-British (FAB) categories: M4 and M5. Multilineage involvement (myeloid, monocytic, erythroid, and megakaryocytic but not lymphoid), higher blast and platelet counts, a higher frequency of extramedullary involvement in the form of gingival hyperplasia, lymphadenopathy, and a female preponderance are other features of NPM1 mutated AML (Bhattacharyya et al., 2018; Bertoli et al., 2020). NPM1 analysis has substantial clinical consequences, since patients with normal karyotype AML who have NPM1 mutations had a higher complete remission rate after induction therapy than those who do not have NPM1 mutations (Grafone et al., 2012). FLT3 is a type III receptors tyrosine kinase involved in the differentiation and proliferation of hematopoietic stem cells. In most cases of AML, FLT3 is expressed on both progenitor and blast cells. FLT3 mutations can be divided into two major types: (FLT3-ITD) internal tandem duplication mutations within the juxtamembrane domain (JMD) (involving exon 14 and sometimes part of exon 15), which represents the most common type of FLT3 mutation, found in about 25% of all AML patients, and (FLT3-TKD) point mutations or deletion in the tyrosine kinase domain which affecting codons (835 or 836) occurring in approximately 7-10% of all cases with prognostic value uncertain (Schnittger et al., 2012). It was noticed that the occurrence of FLT3-ITD increases with age, where a study indicated that the incidence was lowest in pediatric AML patients and highest in elderly AML patients (Faiz and Rashid, 2019), besides, the mutation was linked to weak emergence and a poor prognosis (Sheikhi et al., 2017). AML patients with FLT3-ITD mutation who had a poor prognosis were always with a lower survival rate, indicating that cytogenetic is a crucial prognostic factor in AML (Ait boujima et al., 2021). FLT3 inhibitors have been licensed by the US Food and Drug Administration (FDA) and have demonstrated to increase clinical response in AML patients when used alone or in combination with chemotherapy (Schnittger et al., 2012). As a result, the European Leukemia Network recommends that FLT3 mutational test results should be available within 72 hours in all AML patients so that treatment options can be made based on the patient’s mutation status (Kennedy and Smith, 2020). DNMT3A alterations were identified at various frequencies in multiple myeloid and lymphoid neoplasms, and are often associated with poor prognosis. DNMT3A is an important factor not only for the establishment, but also for maintenance of cellular methylation patterns. Researchers have declared that DNMT3A is often mutated in AML patients 18-23%. Most of the variants are located at R882 in exon 23 (Small, 2006). DNMT3A R882 mutation disturbs the normal ligation of methyltransferase protein subunits, creating a dominant negative influence on DNMT3A protein function, according to the findings. As an inferior prognostic marker for AML patients, DNMT3A has a unique function in hematopoiesis and AML pathogenesis. The prevalence of other non-R882 variants, including DNMT3A frameshift mutations, is substantially less common, and their prognostic relevance is not completely understood (Small, 2006; Choi et al., 2018).
Flow Cytometry

CD45, HLA-DR, CD34, CD25, FMC7, CD10, CD19, CD20, CD22, CD7, CD5, CD2, CD3, CD6, CD16, TDT, CD14, CD64, CD33, CD13, CD117 (c-kit), CD15, CD11b, CD11c, CD41, CD61. Cyto MPO and glicophorin A were studied using flow cytometry FACScalibur (Human genetic laboratory, atomic energy commission, Syria). The Cell Quest software suite was used to conduct the analysis. We divided patients into six subgroups using flow cytometry and cytological results, according to the FAB classification.

DNA Extraction

Genomic DNA was isolated from PB or BM samples from AML patients using the QIAamp DNA Blood Mini kit (Qiagen, Germany) according to the manufacturers instructions and was stored at -20°C. The total DNA of each sample was measured by using a spectrophotometer followed by quantity ultraviolet light absorbance.

Identification of Gene Mutations

PCR analysis were performed on exon 11 and 12 for FLT3-ITD, exon 12 for NPM1, and exon 23 for DNMT3A. The sequences of primers used in PCR were all described (table 1). PCR cycle was run according to MyTaq HS polymerase condition (Bioline, London, UK), with an initial denaturation step at 95°C for one minute, followed by 35 cycles at 95°C for 15 seconds, 58°C for 15 seconds, 72°C for one minute on a thermal cycler (Eppendorf Mastercycler, USA). The PCR products were purified using GeneJET PCR Purification Kits (Thermo scientific, USA), and were labeled with 0.5 μl of BigDye Terminator Version 3.1 (Applied Biosystems, Foster City, California, USA) in a final volume of 10 μl of the sequencing reaction. Sequencing was conducted in both directions, while only reverse sequence was performed for NPM1 by using the primers as PCR. Sequencing reactions were purified using the Ethanol/Ethylene di amine tetra acetic acid (EDTA)/Sodium acetate precipitation method were purified using the NCBI BLAST Assembled Genomes tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Criteria of Response and Survival Definitions

Complete remission (CR) required bone marrow blasts of <5.0%, absence of blasts with Auer rods, absence of extra medullary disease, absolute neutrophil count of >1.0x109/L, and platelet count of >100x109/L with independance from red cell transfusions. Relapse was defined by bone marrow blasts of ≥5.0%, reappearance of blasts in the blood, or development of extra medullary disease. OS was defined as the time from diagnosis to death, and EFS as the time from diagnosis to an event (either failure to achieve remission, death in first CR, or relapse), with patients not achieving remission being counted as having an event on day one.

Results

We identified 100 newly diagnosed AML patients. All patients were with normal conventional cytogenetics. The median age was 43.47 years (range, 17–86 years). FLT3-ITD, DNMT3A and NPM1 were detected in 24%, 4% and 22 % patients, respectively. Patient’s data were taken in terms of: gender, age, smoking, alcohol, diagnosis according to FAB, leukocyte count, hemoglobin, presence of NPM1, FLT3-ITD, DNMT3A mutation as shown in the (Table 2). It was noted that subtype M2 had the highest rate of diagnosis in acute myeloid leukemia, and the other subtypes were something similar (Figure 1).

Table 1. Sequences of Primers Used in Polymerase Chain Reaction (PCR)

| Gene   | Primer       | Sequences (5'-3')                     |
|--------|--------------|---------------------------------------|
| FLT3-ITD | Forward primer | 5'-GCAAATTAGGTATGAAAGCCAGC-3'         |
|         | Reverse primer | 5'-CCTTTAGCATTGTTAGCGCAACCATC-3'     |
| NPM1    | Forward primer | 5'-TTAATCGTCTGTTGGTAGATGAA-3'        |
|         | Reverse primer | 5'-CAAAGCTATTGCGACATTATC-3'          |
| DNMT3A  | Forward primer | 5'-TCCTGCTGTTGTTGAGGCGAC-3'         |
|         | Reverse primer | 5'-ATGAGTCCAAACTTCTC-3'             |

FLT3-ITD, FMS-like tyrosine kinase-3 internal tandem duplication; NPM1, Nucleophosmin1; DNMT3A, DNA methyl transferase 3A.
after induction chemotherapy. The highest mean of relapse cases was in FLT3-ITD mutation patients 28.08% and the lowest was in DNMT3A mutation carriers 19.75%, and since (p >0.05), there are no statistically significant differences in the mean of relapse ranks. The results of the Kruskal Wallis test comparing the average ranks of relapses in the three mutations shown in (Figure 2). The FLT3-ITD mutation patients had the highest mean of death cases, while the DNMT3A mutation patients had the lowest, but this difference was not statistically significant. On the other hand, the highest mean of remission was in patients with NPM1 mutation and the lowest in the carriers of the FLT3-ITD mutation. It was observed that the mean relapsed patients with FLT3-ITD mutation was 3.4 months with a confidence interval of 95% (5.4-1.4) months, with no significant differences (p >0.05) between FLT3-ITD carriers and non-carriers: (Log rank=0.016, p-value=0.900), Figure 3 (A). The same result was identified with DNMT3A mutation, when no relation was found between the patients with mutation and non- holder, in terms of relapse: the mean of relapse was two months, Figure 3 (C). On the contrary, It was observed that the mean relapsed for NPM1 mutation carriers is 2.4 months with a confidence interval of 95% (3.8-0.9) months, with significant statistical differences (p <0.05) between the mean of relapse of mutation carriers and non-carriers, Figure 3 (B).

The mean overall survival time for patients with FLT3-ITD mutation was 5.9 months, with a 95% confidence interval (CI between: 3.7-8.2) months, with significant differences (p<0.05) between the overall survival of non-FLT3-ITD and exist FLT3-ITD patients as shown in Figure 4 (A), (Log rank=24.459, p=0). The mean overall survival time for NPM1 mutation carriers was 5.85 months, with a 95% confidence interval (CI between: 3.7-7.9) months, with significant differences (p<0.05) between the survival of non-NPM1 and NPM1 patients holder as shown in Figure 4 (B). where (Log rank=19.337, p-value=0). The mean overall survival time for patients with a DNMT3A mutation was 5.88

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**Table 2. Pathological Features of Syrian (CN) AML Patients with NPM1, FLT3-ITD and DNMT3A Mutations**

|                   | FLT3-ITD | NPM1 | DNMT3A | With mutation | without mutation |
|-------------------|----------|------|--------|---------------|------------------|
| Total number      | 24       | 22   | 4      | 41            | 59               |
| Mean age in years (range) | 42.3 (17-64) | 44.9 (18-81) | 39.3 (18-65) | 43.3 (18-71) | 43.6 (18-86) |
| Number of patients <60 years | 21       | 17   | 0      | 34            | 44               |
| Gender (M/F)      | 10/22    | 13/9 | 02/22  | 20/21         | 31/28            |
| Smoking (yes/no)  | 17/7     | 14/8 | 4/0    | 29/12         | 37/22            |
| Alcohol (yes/no)  | 03/21/22 | 05/17/22 | 0/4   | 8/33          | 8/51             |
| Mean WBC (range)  | 30.4 (1.3-100) | 53 (0.80-300) | 23.5 (1.3-46.1) | 41.2 (0.8-300) | 23.6 (0.2-165) |
| Mean Hb (range)   | 8 (2.4-12.5) | 8.3 (3-16.7) | 6.9 (6-7.9) | 8.1 (2.4-16.7) | 8.6 (3.5-14) |
| FAB subtypes      |          |      |        |               |                  |
| M1                | 4        | 2    | 1      | 5             | 10               |
| M2                | 9        | 6    | 3      | 15            | 17               |
| M3                | 2        | 4    | 0      | 5             | 13               |
| M4                | 4        | 6    | 0      | 9             | 11               |
| M5                | 5        | 4    | 0      | 7             | 8                |

FAB, French-American-British classifications; Hb, hemoglobin; WBC, White blood cells

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![Figure 1. Samples Diagnosis According to FAB Classification](image-url)
months, with a 95% confidence interval (CI between: 0.8–10.9) months, with no significant differences (p>0.05) between the mean survival for DNMT3A mutation patients and those who do not have it due to the smallness of the sample as shown in Figure 4 (C), where (Log rank=0.708, p-value=0.4). Finally, it was noted that the mean survival time for patients carrying the mutation of any kind is 6.46 months with a confidence interval of 95% (4.7-8.3) months, with significant differences (p <0.05) between the mean survival of the carriers of any kind of mutation and non-carriers. When using the cox regression method to determine the most important variables in the incidence of death, it was noted that the variables of age (β₁=0.026, p-value=0.01) and the presence of the mutation (β₂=2.201, p-value=0) are the most important variables in the incidence of death. Therefore The estimated model, which measures the relationship between the incidence of death, the variables of age and the presence of a mutation:

\[ h = 0.026 \times \text{age} + 2.201 \times \text{mutation} \]

And that 98.99% where (R² = 0.989) of the changes affecting the incidence of death are due to the variables of age and the presence of a mutation.

Regarding to the relation between EFS and the target mutations, our results demonstrate that the mean EFS of FLT3-ITD mutation patients is 4.818 months with a confidence interval of 95% (2.9-6.7) months, while the mean EFS of NPM1 mutation patients is 4.805 months and a confidence interval of 95% (1-2.8) months, with significant statistical differences (p<0.05) between the mean EFS of FLT3-ITD and NPM1 mutation patients and non-mutated patients (p<0.05), While this mean was not statistically significant (5.875 months) in patients carrying DNMT3A mutation (Figure 5).

**Discussion**

Many populations of AML have been examined for gene alterations, however in our community, this is the first study, to our knowledge, detected these three
gene mutations in adults, normal karyotype patients in our cohort. In each community, the frequency of gene mutations varied. All cytogenetically normal acute myeloid leukemia patients were sequenced for hotspot mutation exons in FLT3, NPM1, and DNMT3A genes. We have explored the incidence and type of FLT3 mutations. We observed that the JMD mutations (exons 14 and 15) were the most common type of alteration (24%) compared to TKD mutations at exon 20 (2%). When comparing the frequency of mutations in only normal karyotype of AML patients. Reports from Western countries have shown that the prevalence of FLT3-ITD mutations are found in approximately 25% of newly diagnosed AML cases (Daver et al., 2019), this percentage was similar to our study, between Ahmad et al., (2010) reported 19.1% of isolated ITD mutation, which was lower than our study. A decreased incidence of ITD was observed in the Korean populations 13% (Bang et al., 2008). NPM1 mutation was observed in 22% of CN-AML patients in the current study, a lower frequency than those of many past studies from Western and Asia (Schlenk et al., 2008; Park et al., 2012). In addition, recurrence of NPM1 mutation in this study moreover lower than those of CN-AML in a ponder of Central Thailand where the frequency was 38.1% (Boonthimat et al., 2008). The contrasts in these change frequencies may be due to numerous diverse components counting the hereditary foundations of each populace, geographic locale and environmental components (Wangkumhang et al., 2013). DNMT3A is an enzyme that catalyzes the exchange of methyl groups to particular CpG structures in DNA, called DNA methylation, which controls gene expression by selecting proteins included in gene suppression or by inhibiting the binding of translation factor(s) to DNA. Transformations in DNMT3A quality change the gene’s typical work and play an critical part in AML prognosis (Ley et al., 2010). DNMT3A mutation was found in 4% of CN-AML but other studies detailed the frequency run of 13.3-34.2% in CN-AML (Marcucci et al., 2012; Ahmad et al., 2014). In expansion, the recurrence of DNMT3Amut in CN-AML from Central

Figure 4. Kaplan-Meier Overall Survival of Patients with CN-AML, (A): OS of FLT3-ITD patients, (B): OS of NPM1 patients, (C): OS of DNMT3A patients

Figure 5. Kaplan-Meier Event Free Survival of Patients with CN-AML, (A): EFS of FLT3-ITD patients, (B): EFS of NPM1 patients, (C): EFS of DNMT3A patients
In AML, relapse remains the principal cause of treatment failure for the majority of patients. Therefore the ability to identify those patients with a high risk of relapse would represent a valuable advance, which may lead to the introduction of alternative forms of therapy in this group of patients. The first deliberate of the ELN genetic categories was to standardize announcing of hereditary abnormalities especially for correlations with clinical characteristics and result, so we focused in this study on the correlation between the genetic mutations and clinical features, and what was clearly obvious that patients with DNMT3A mutations had different results than patients with FLT3-ITD and NPM1 mutations. Different studies shown that DNMT3A mutations reflects independent prognostic factor of shorter OS in patients with de novo AML (Hou et al., 2014). Moreover, other study (Shirarov et al., 2013) also illustrated that DNMT3A mutations were related with more regrettable result counting essentially shorter OS and EFS and that is totally different from previous study (Jain et al., 2014).

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establishing the truth of our findings. On the other hand, numerous studies showed the prevalence and prognosis of these mutations in AML, but this was the first report of the prognostic significance of these mutations in Syria. The present study revealed the effects of these mutations in single terms of OS, EFS and relapse. We demonstrate that mutations in NPM1, FLT3-ITD, and DNMT3A are among the most important predictors for outcome. Our results further emphasize the value of comprehensive molecular genetic screening for the dissection of this heterogeneous patient subgroup, which may ultimately lead to improved risk stratification.

Author Contribution Statement

Authors: Yahia Moualla (MY), Faten Moassass (MF), Bassel Al-Halabi (HB), Walid Al-achkar (AW), Michael Georgeos (GM), Haissam Yazigi (YH), Atieh Khamous (KA). MF, KA and AW performed banding cytogenetics and molecular genetics, MY and HB performed the molecular genetic analysis, MY, YH, and GM provided the clinical data and the chemotherapy plan. MY, MF, and AW, drafted the paper. All authors worked on the final version of the paper. All authors read and approved the final manuscript.

Data availability statement

The data that support the findings of this study are available from the corresponding author (Yahia M. Moualla), upon reasonable request. All relevant material is included in this publication.

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Declaration

I confirm that this work is a part of an approved PhD thesis which was approved by university board’s decision No.2979 of 07/08/2018, and this work is an original and has not been published elsewhere, nor is it currently under consideration for publication elsewhere.

Ethical approval statement

The work was approved by the Ethics Committee in Syrian Ministry of Higher Education and written informed consent was obtained from all the participants according to the Declaration of Helsinki.

Conflict of Interests

The authors declare that they have no competing interests.

References

Ahmad F, Mandava S, Das BR (2010). Analysis of FLT3-ITD and FLT3-Asp835 mutations in de novo acute myeloid leukemia: evaluation of incidence, distribution pattern, correlation with cytogenetics and characterization of internal tandem duplication from Indian population. Cancer Invest, 28, 63-73.

Ahmad F, Mohota R, Sanap S, Mandava S, Das BR (2014). Molecular evaluation of DNMT3A and IDH1/2 gene mutation: frequency, distribution pattern and associations with additional molecular markers in normal karyotype Indian acute myeloid leukemia patients. Asian Pac J Cancer Prev, 15, 1247-53.

Ait boujmia O, kaloutoum, Lamchabab M, Hda N, Quesara A (2021). Characteristics and Survival of 927 Moroccan Adults with Acute Myeloid Leukemia: Monocentric Experience. Asian Pac J Cancer Prev, 6, 5-3.

Bang S-M, Ahn JY, Park J, et al (2008). Low frequency and variability of FLT3 mutations in Korean patients with acute myeloid leukemia. J Korean Med Sci, 23, 833-7.

Bertoli S, Dumas P-Y, Béard E, et al (2020). Outcome of Relapsed or Refractory FLT3-Mutated Acute Myeloid Leukemia before Second-Generation FLT3 Tyrosine Kinase Inhibitors: A Toulouse–Bordeaux DATAML Registry Study. Cancers, 12, 773.

Bhattacharyya J, Nath S, Saikia KK, et al (2018). Prevalence and clinical significance of FLT3 and NPM1 mutations in acute myeloid leukaemia patients of Assam, India. Indian J Hematol Blood Transfus, 34, 32-42.

Boonthimat C, Thongnoppakunn W, Auewarakul CU (2008). Nucleophosmin mutation in Southeast Asian acute myeloid leukaemia: eight novel variants, FLT3 coexistence and prognostic impact of NPM1/FLT3 mutations. Haematologica, 93, 1565-9.

Choi E-J, Lee J-H, Lee J-H, et al (2018). Comparison of anthracyclines used for induction chemotherapy in patients with FLT3-ITD-mutated acute myeloid leukemia. Leuk Res, 68, 51-6.

Daver N, Schlenken RF, Russell NH, Levis MJ (2019). Targeting FLT3 mutations in AML: review of current knowledge and evidence. Leukemia, 33, 299-312.

Dönherr H, Estey E, Grimmwade D, et al (2017). Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood, 129, 424-47.

Faiz, M, Rashid F (2019). Molecular Study of FLT3 gene mutations in Acute Myeloid Leukemia from Pakistan: Correlation with clinicopathological parameters. Asian Pac J Cancer B, 4, 81-4.

Gaidzik VI, Schlenken RF, Paschka P, et al (2013). Clinical impact of DNMT3A mutations in younger adult patients with acute myeloid leukemia: results of the AML Study Group (AMLSG). Blood, 121, 4769-77.

Grafone T, Palmisano M, Nicci C, Storti S (2012). An overview on the role of FLT3-tyrosine kinase receptor in acute myeloid leukemia: biology and treatment. Oncol Rev, 6.

Hou H-A, Kuo Y-Y, Liu C-Y, et al (2012). DNMT3A mutations in acute myeloid leukemia: stability during disease evolution and clinical implications. Blood, 119, 559-68.

Hou H, Lin C, Chou W, et al (2014). Integration of cytogenetic and molecular alterations in risk stratification of 318 patients with de novo non-M3 acute myeloid leukemia. Leukemia, 28, 50-8.

Jain P, Kantarjian H, Patel K, et al (2014). Mutated NPM1 in patients with acute myeloid leukemia in remission and relapse. Leuk Lymphoma, 55, 1337-44.

Jang W, Park J, Kwon A, et al (2019). CDKN2B downregulation and other genetic characteristics in T-acute lymphoblastic leukemia. Exp Mol Med, 51, 1-15.

Kennedy VE, Smith CC (2020). FLT3 Mutations in Acute Myeloid Leukemia: Key Concepts and Emerging Controversies. Front Oncol, 10, 2927.

Kim HJ, Ryu H, Choi H-K, et al (2021). Anti-leukemic Activity
of AUI2008 in FLT3-ITD-positive Acute Myeloid Leukemia. Anticancer Res, 41, 731-7.

Kottaridis PD, Gale RE, Frew ME, et al (2001). The presence of a FLT3 internal tandem duplication in patients with acute myeloid leukemia (AML) adds important prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy: analysis of 854 patients from the United Kingdom Medical Research Council AML 10 and 12 trials. Blood, 98, 1752-9.

Lenzi L, Lee-Jones L, Mostofa MA, et al (2020). Second Primary Malignancy after Acute Promyelocytic Leukemia: A Population-Based Study. Cancers, 12, 3610.

Ley TJ, Ding L, Walter MJ, et al (2010). DNMT3A mutations in acute myeloid leukemia. N Engl J Med, 363, 2424-33.

Marcucci G, Metzeler KH, Schwind S, et al (2012). Age-related prognostic impact of different types of DNMT3A mutations in adults with primary cytogenetically normal acute myeloid leukemia. J Clin Oncol, 30, 742-50.

Marková J, Michková P, Burčková K, et al (2012). Prognostic impact of DNMT3A mutations in patients with intermediate cytogenetic risk profile acute myeloid leukemia. Eur J Haematol, 88, 128-35.

Martelli MP, Pettirossi V, Thiede C, et al (2010). CD34+ cells from AML with mutated NPM1 harbor cytoplasmic mutated nucleophosmin and generate leukemia in immunocompromised mice. Blood, 116, 3907-22.

Moarri M, Papaemmanuil E (2017). Classification and risk assessment in AML: integrating cytogenetics and molecular profiling. Hematol Am Soc Hematol Educ Program, 2017, 37-44.

Park BG, Chi H-S, Park S-J, et al (2012). Clinical implications of non-A-type NPM1 and FLT3 mutations in patients with normal karyotype acute myeloid leukemia. Acta Haematol, 127, 63-71.

Park DJ, Kwon A, Cho B-S, et al (2020). Characteristics of DNMT3A mutations in acute myeloid leukemia. Blood Res, 55, 17-26.

Patel JP, Gönen M, Figueroa ME, et al (2012). Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. N Engl J Med, 366, 1079-89.

Patel SS, Kuo FC, Gibson CJ, et al (2018). High NPM1-mutant allele burden at diagnosis predicts unfavorable outcomes in de novo AML. Blood, 131, 2816-25.

Sami SA, Darwish NH, Barile AN, Mousa SA (2020). Current and future molecular targets for acute myeloid leukemia therapy. Curr Treat Options Oncol, 21, 1-16.

Schlenk RF, Döhner K, Krauter J, et al (2011). Prognostic impact of FLT3-ITD load in NPM1 mutated acute myeloid leukemia. Leukemia, 25, 1297-304.

Shivarov V, Gueorguieva R, Stoimenov A, Tiu R (2013). DNMT3A mutation is a poor prognosis biomarker in AML: results of a meta-analysis of 4500 AML patients. Leuk Res, 37, 1445-50.

Sirirat T, Chuncharunee S, Nipaluk P, et al (2017). Mutation analysis of isocitrate dehydrogenase (IDH1/2) and DNA methyltransferase 3A (DNMT3A) in Thai patients with newly diagnosed acute myeloid leukemia. Asian Pac J Cancer Prev, 18, 413-20.

Small D (2006). FLT3 mutations: biology and treatment. Hematol Am Soc Hematol Educ Program, 2006, 178-84.

Takahashi S (2011). Current findings for recurring mutations in acute myeloid leukemia. J Hematol Oncol, 4, 1-11.

Wang L, Xu W-L, Meng H-t, et al (2010). FLT3 and NPM1 mutations in Chinese patients with acute myeloid leukemia and normal cytogenetics. J Zhejiang Univ Sci B, 11, 762-70.

Wangkumphang P, Shaw PJ, Chaichoompu K, et al (2013). Insight into the peopling of Mainland Southeast Asia from Thai population genetic structure. PLoS One, 8, e79522.