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Mutation profile of the patients diagnosed with myeloid neoplasia tested with next generation sequencing and clinical implications

Yasa Gul Mutlu[a,*], Berrin Balik Aydin[a], Akif Ayaz[b], Fatma Seca[c], Omur Gokmen Sevindik[a]

[a] *Istanbul Medipol University, Medical School, Department of Hematology, Istanbul, Turkey*
[b] *Istanbul Medipol University, Medical School, Department of Medical Genetics, Istanbul, Turkey*
[c] *Istanbul Medipol University, Department of Medical Genetics, Genetic Disease Assesment Center, Istanbul, Turkey*

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Abstract

Aim: To analyze the distribution of gene mutations in myeloid neoplasias, based on next generating sequencing technology (NGS) and evaluate their clinical implications and impact on the risk stratification.

Materials and Methods: 67 bone marrow samples which belong to 48 different patients who were diagnosed with myeloid neoplasia and tested with 30 gene myeloid panel by NGS in our center were evaluated retrospectively. Distribution of genomic alterations and clinical implications were compared in different groups.

Results: Samples were separated into different groups according to the diagnostic categories. Most common diagnosis was acute myeloid leukemia (AML) with the rate of 58.3%. FLT3 mutation was the most common mutation in AML and whole population. After the incorporation of the NGS results into the prognostic classification in newly diagnosed AML group, 47.1% of the patients were up-staged or down-staged according to the European Leukemia Network (ELN) risk stratification system.

Conclusion: Analyzing the mutation profiles with NGS in myeloid neoplasias has an important and remarkable effect on diagnosis and management of this group of diseases.

Introduction

Myeloid neoplasms are a group of clinically heterogeneous diseases that include acute myeloid leukemia (AML), myelodysplastic syndromes (MDS) and myeloproliferative neoplasms (MPN) which originate from hematopoietic stem cells (HSCs). Current management of myeloid malignancies has been rapidly evolving. The combination of cytogenetics, reverse transcriptase polymerase chain reaction (RT-PCR) based molecular techniques and Sanger sequencing has allowed for detailed classification and prognostic assessment of myeloid neoplasms [1]. NGS technology which is inspired from Sanger sequencing can provide to perform genomic testing in an easy and comprehensive way [2].

AML with recurrent genetic abnormalities which is an entity of 2016 World Health Organization (WHO) classification of myeloid neoplasms is an example of genetic assessment importance for diagnostic approach [3]. Besides, the ELN 2017 recommendations emphasizes the importance of molecular genetic testing assessment in the risk stratification of AML [4]. Mutations in NPM1, CEBPA and RUNX1 genes should also be screened because they define disease categories, as well as, mutations in FLT3 together with data on the mutant to wild type allelic ratio also should be checked for their prognostic impact according to the ELN risk classification [5] and TP53, ASXL1 should be screened at the time of diagnosis because they have been also associated with poor prognosis [6]. Molecular testing by RT-PCR can be a fast and useful approach for detecting these mutations but this technique could not allow to measure variant allele frequencies (VAF) of the mutations which is very important for FLT3 mutations with rather high or low allelic ratio.

The emergence of NGS technique has expanded the genetic landscape of MPNs. Driver mutations like JAK2, CALR, MPL are essential for diagnosis of MPNs. On the other hand, aforementioned driver mutations which are specific for MPNs, somatic mutations in genes that regulate DNA methylation, histone modification, mRNA splicing, tran-

*Corresponding author:
Email address: yasagulmutlu@hotmail.com (Yasa Gul Mutlu)

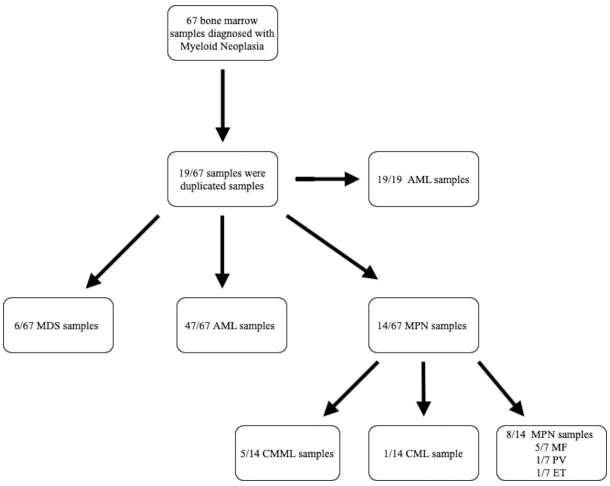

Figure 1. Flow chart of the patients who are classified according to the diagnosis

scription and signal transduction have been shown to play important roles in disease progression [7]. CALR is very well known mutation in MPNs which is related with a favorable and prolonged overall survival and five more genes ASXL1, EZH2, SRSF2, IDH1 and IDH2 called as high molecular risk (HMR) group genes are included in prognostic scoring systems such as MIPSS-70 and MIPSS-70 plus for myelofibrosis (MF) patients [8]. According to the MIPSS-70 and MIPSS-70 plus scoring systems, patients in high risk group has an obviously inferior 5-year overall survival comparing to low or intermediate risk patients [8].

Last but not least, in MDS NGS could identify any molecular mutations in nearly 90% of the patients [9]. MDS with ring sideroblasts which is a favorable subcategory of MDS was defined as >5% ring sideroblasts with harboring SF3B1 mutation in the most recent revision of the WHO classification. Despite the prognostic scoring systems are still depends on the patients' peripheral blood parameters, bone marrow blasts and cytogenetics like IPSS-R which is the gold standard prognostic scoring system in MDS, molecular data were successfully incorporated into the scoring systems in studies [10, 11]. The integration of molecular markers into the IPSS-R system is already in preparation.

Whereas Sanger sequencing allows to analyze only a few short fragments of up to 1,000 base pairs in length, NGS could analyze simultaneous sequencing of millions of DNA fragments [12].

In this study we aimed to analyzing the distribution/frequency of gene mutations in myeloid neoplasias, based on NGS and evaluate the clinical implications, correlation with conventional and low-resolution genetic techniques and their impact on risk stratifications.

Materials and Methods

In this study we aimed to investigate the patients who were diagnosed with Myeloid neoplasias AML, MDS, MPNs between 2019-2020 in our center and bone marrow samples which had been evaluated with a 30 gene Myeloid neoplasia panel by NGS (Figure 1).

All the samples were bone marrow materials. Patients older than 18-year-old were included in the study. Demographical data like age and gender were recorded. Patients were separated according to their presentation and disease remission status as, de-novo disease, relapse and complete remission. Samples which belongs to patients' first material were recorded as "primary case" and recurrent samples regarding to first case were accepted as "duplicated cases". Patient's peripheral blood parameters as white blood count, hemoglobin value and platelet number were recorded, and bone marrow blast count at the time of NGS also were recorded. Patients' cytogenetic and FISH results which were evaluated from bone marrow samples were noted.

Presences of mutations were grouped according to the mechanistic approach. Methylation mutations were DNMT3A, TET2, IDH1/2 and WT1. Chromatin modification mutations were ASXL1 and EZH2. Spliceosome mutations were SF3B, SRSF2 and ZRSR2. Transcription factors mutations were RUNX1, ETV6 and CEBPA. Cytokine receptor and tyrosine kinases mutations were FLT3, KIT, JAK2, CALR and CSF3R. Ras signaling mutations were PTPN11, NRAS, KRAS, and CBL. Check point and cell cycle mutation was TP53. NPM1 mutation was in the other group of mutations.

Genomic DNA was isolated from bone marrow aspirate samples according to the manufacturer's instructions. High-throughput sequencing data obtained using the Sofia DDM library kit and MiSeq ((Illumina, San Diego, CA) platform was analyzed with the Sofia bioinformatics program. The following genes are included in this panel: CEBPA, CSF3R, FLT3, MPL, U2AF1, CBL, NRAS, ABL1, JAK2, HRAS, SF3B1, ZRSR2, NPM1, IDH1, DNMT3A, IDH2, TET2, BRAF, PTPN11, RUNX1, ETV6, ASXL1, SETBP1, WT1, KIT, SRSF2, KRAS, CALR, TP53 and EZH2. The minimal depth of coverage was 1000x. Read pairs were aligned to Refseq hg19 by Burrows–Wheeler Aligner. The obtained variants were classified according to the criteria recommended by the Association for Molecular Pathology, American Society of Clinical Oncology and College of American Pathologists [13].

This study was apporoved by local ethical committee of Istanbul Medipol University with the approval id of E-10840098-772.02-2892

Statistical Analyses

All statistical analysis was performed using SAS 9.4 software.

The sample size were detected via a two tailed power analysis using SAS Power Analysis package with an 80% power at a 0.05 significance level according to the data obtained from previous reports. Characterization of the study population was conducted using descriptive statistics including means and standard deviations of the mean if the variable has a normal distribution or median (range), otherwise. Discrete variables were expressed as frequency (percent). Differences between the frequencies of mutational parameters among different diagnostic groups were evaluated with Chi-Square test or Fisher's Exact test accord-

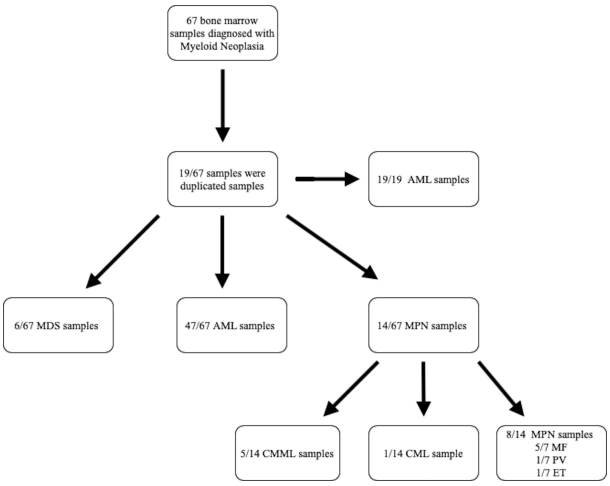

Figure 2. Caption

ingly. Survival analysis was performed by using Kaplan Meier survival estimates between two groups.

A p value less than 0.05 was considered statistically significant.

Results

Totally 67 bone marrow samples which belongs to 48 different patient which were diagnosed with myeloid neoplasia had included in the study. Patients were separated into two different groups according to disease subtypes. There were 34 patients in AML/MDS group and 14 patients in CML/CMML/MPN group. The median age was 55.3 (18-86) year-old in all patient groups. The median age was similar for both AML/MDS group and CML/CMML/MPN group and it was 56-year-old. 43.8% of patients were female. Most common diagnosis was AML, it was 58.3% of all patients, the other diagnosis were 12.5% MDS, 10.4% CMML, 16.7% MPNs and 2.1% CML. Patients' demographics and characteristics among AML/MDS AND CMML/MPN groups are detailed in Table 1.

Patients who are at their initial diagnosis were the greater part of the patients and it was 55.2% of all patient groups. 31.3 % of the patients were at their first remission and the rest 13.4% had relapsed refractory disease with regard to the disease status at the time of sampling for NGS.

Most commonly observed mutations were the ones involved in methylation machinery and cytokine receptor/tyrosine kinase pathways with a frequency of 32.8% and 35.8 %. FLT3 mutation was the most common mutation in all samples. DNMT3A, NPM1, ASXL1 and TET2 mutations were the other most common mutations in all patients. FLT3 was again the most common mutation in patients who were diagnosed with AML. DNMT3A and ASXL1 is the most common mutations in MDS and CMML patients. Mutation frequencies of groups were detailed in Figure 2-3-4-5.

Patients were evaluated with the conventional metaphase cytogenetic and FISH results. Even in patients who had a normal karyotype and FISH result, there were 72 different mutations which were detected by NGS. Mechanistic

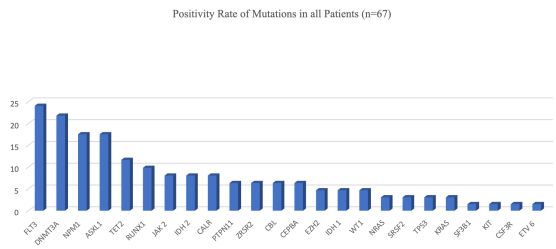

Figure 3. Positivity rate of mutations in all patients (n=67)

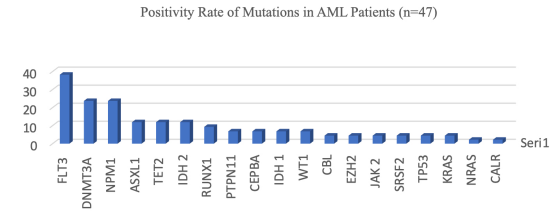

Figure 4. Positivity rate of mutations in AML patients (n=47)

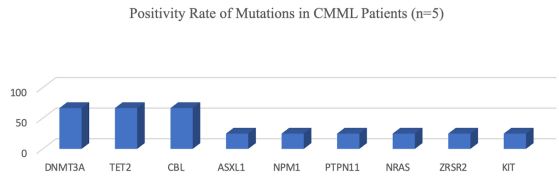

Figure 5. Positivity rate of mutations in CMML patients (n=5)

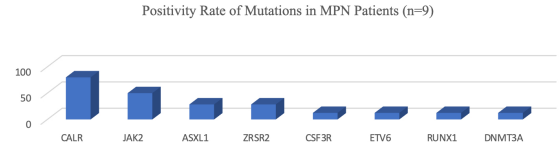

Figure 6. Positivity rate of mutations in MPN patients (n=9)

Table 1. Demographics and Patient Characteristics among AML/MDS and CMML/MPN Groups

	All Patients (n=48)	AML/MDS (n=34)	CML/CMML/MPN (n=14)
Age at Diagnosis (median, yrs)	55.3 (18-86)	56.5 (31-76)	56 (18-86)
Gender (F/M %)	43.8 / 56.3	50 / 50	28.6 / 71.4
Diagnosis (%)			
AML	58.3		
MDS	12.5		
CMML	10.4		
MPN(s) (PV/ET/Myelofibrosis)	16.7(2.1 / 2.1 / 12.5)		
CML	2.1		
Overall Survival (Median, 95% CI, months)	54 (3.02-104.98)	54 (10.19-97.81)	Not Achieved
OS in AML Patients		14.3 (0.1-45.98)	Not Applicable
OS in MDS Patients		Not Achieved	Not Applicable

Table 2. Mechanistic Distribution of NGS Abnormalities among Conventional Cytogenetic and FISH Findings

Number of different mutations detected by NGS			
Conventional metaphase cytogenetics			
Normal karyotype	FISH	Normal	72
		Inv 16	2
		Monozomy 8	2
		Del 7q	2
Complex karyotype	FISH	Normal	1
		Trisomy 8	2
Ph chr.	FISH	Normal	2
Unknown	FISH	Normal	7
Del 3q	FISH	Normal	1
Monozmy 18	FISH	Normal	2
Trisomy 18	FISH	Trisomy 8	2
Trisomy 10	FISH	Normal	0

distribution of NGS abnormalities among conventional cytogenetic and FISH findings were detailed in Table 2.

17 newly diagnosed AML patients were categorized according to the their ELN risk groups, favorable, intermediate and poor. Most of the newly diagnosed patients were in poor group with the rate of 52.9%. The other 29.4% of the patients were in intermediate group and the remaining 17.6% of the patients were in favorable group. 47.6% of the patients had an upstaging or downstaging of ELN risk classification with the addition of NGS results. Effect of NGS findings on ELN risk stratification of newly diagnosed AML patients were detailed in Table 3.

Table 3. Effect of NGS Findings on ELN Risk Stratification of Newly Diagnosed AML Patients

	Newly diagnosed AML patients (n=17)
Regarding newly diagnosed AML, distrubiton of ELN risk groupsFavorable/Intermadiate/ Poor	17.6/ 29.4/ 52.9
Regarding newly diagnosed AMLUpstaging or downsstaging potential of NGS on ELN risk stratificationYes/No	47.1/ 52.9

Table 4. Comparison of mutation profiling between AML/MDS versus CMML/MPNs

	AML/MDS	CMML/MPNs	p
FLT3	24.5	0	0.033
DNMT3a	17	21.4	0.482
NPM1	17	7.1	0.329
ASXL1	13.2	21.4	0.346
TET2	9.4	14.3	0.453
RUNX1	9.4	7.1	0.633
JAK2	3.8	21.4	0.046
IDH2	9.4	0	0.576
CALR	1.9	28.6	0.006
PTPN11	5.7	7.1	0.838

The median overall survival was not achieved in AML patients who were classified as having a favorable disease according to the ELN risk stratification. In patients who were classified as having an intermediate or adverse risk AML the median OS was calculated as 14 and 12 respectively (p=0.251). Regarding the presence of different mechanistic mutation profiles, having a check-point mutation was associated with a significantly poor outcome. The median OS was 54 months who lacked check-point mutation and 6 months who harbored (p=0.028). A similar but vice-versa effect was relevant for the patients who harbored spliceosome mutations. Patients with spliceosome mutations had a significantly longer OS when compared with the ones who lack those mutations (p=0.44). It was striking that none of the patients who had check-point or spliceosome mutations, as they had a direct impact on survival, harbored any chromosomal aberrations with both conventional cytogenetics and FISH testing. The comparison of the mutation frequency between AML/MDS and CMML/MPNs group are detailed in Table 4.

There was a significant difference in the frequency of FLT3, JAK2 and CALR mutations between these two groups. FLT3 mutation was significantly had higher positivity rate in AML/MDS group (24.5% versus 0% and p = 0.033). Besides, JAK 2 and CALR mutations were significantly

had higher positivity rate in CMML/MPNs groups (21.4% and 28.6% p = 0.046 versus p = 0.006).

Discussion

Genomic profiling by using NGS are becoming backbone technic of diagnosis and management of myeloid neoplasias. ELN risk stratification which is a standard of care prognostic classification in AML is highlighting the importance of the mutations. There are several studies which investigated the gene mutations in patients with AML and their impact on prognosis [14, 15]. In our study majority of the patients were diagnosed with AML, even do our serials is not as large as the previous published studies, it is the first study which evaluates the genomic myeloid profiling with NGS technique in Turkey.

Currently there are several different NGS panels which are commercially available and covering varying number of genes between 20 to 410 [16]. Our panel includes 30 most frequent genes in myeloid neoplasia. National Compherensive Cancer Network (NCCN) and ELN guidelines recommends testing minimum 6 to 9 genes including NPM1, CEPBA, RUNX1, FLT3, TP53, ASXL1, IDH1, IDH2, KIT for AML. Rests of the mutations in our panel are also able to screen for myeloid malignancies other than AML. In daily practice at-least these genes should be screened in order to make sure an appropriate approach to myeloid neoplasias. This 30 gene panel was used in this purpose in our center. Myeloid panels could be expanded for the investigational studies.

Myeloid malignancies especially AML has a wide genetic heterogeneity and a very large genomic landscape. Papaemmanuil et al had reported 5234 driver mutations across 76 genes or genomic regions which were identified in 86 % of the 1540 patients who were diagnosed with AML. Most common mutation was FLT3 mutation and NPM1 mutation was the second one. Another large cohort which included 453 newly diagnosed AML patients was reported from China. NPM mutation was the most common mutation with the rate of 12.3 % [13]. In our study FLT3 was the most common mutation in AML group and DNMT3A and NPM1 were the following commonest ones.

Treatment choices in leukemias especially in AML have been dramatically changed in recent years. Advances in the understanding of the complex mechanism of AML leukemogenesis have led to the development of novel therapeutic approaches in this field. FLT3 mutation which is the most common genetic mutation in AML is one of the most important targetable gene. Midostaurin and Gliteritinib are the agents which has a high potential of targeting activated FLT3 and approved by US Food and Drug Administration (FDA) [17]. We have achieved prolonged overall survival rates with this new therapeutic era. Another important mutation in AML is IDH1/2 which is also targetable genes with two novel agents. Ivosidenib and Enasidenib are targeting mutated IDH1/2 and offer a reduced toxicity and prolonged overall survival [18]. Detecting these mutations with NGS is becoming an important and remarkable diagnostic test with the advent of new targeted therapies. Unfortunately, we have no data on the effect of targeted therapy choices in these mutations which are also a limitation of the study as the access to these novel agents is restricted in our country.

Many of these recurrently mutated genes have also been shown to be excellent biomarkers for minimal residual disease (MRD) monitoring for assessing treatment response and predicting future relapse especially in AML patients. MRD positivity at various timepoints after treatment predicts a worse outcome. NGS would able to screen and detect one leukemic cell in 106 hematopoietic stem-cells.

One of the limitations of NGS is its inability to distinguish between germline and somatic origins of current mutations [19]. In our serial we had a patient who was diagnosed with CML harboring Ph chromosome positivity. This patient had concomitant CSF3R mutation which is not possible to explain with CML in NGS results. According to the results obtained from fibroblast culture of patient's skin biopsy this concomitant mutation was confirmed to be a germline mutation and had no impact on patient's diagnosis and management.

Another important aspect of our study is re-classification of the newly diagnosed AML patients. 47.1% of the patients had an upstaging or downstaging effect on ELN risk category after the incorporation of NGS data. Even there were a limited number of newly diagnosed AML patients in our study, considering the importance of ELN risk stratification in management of the patients and its definitive role on determining allogeneic transplantation is a viable consolidative option or not, this data served for a certain potential to impact the management of these patients.

Leukemic transformation is one of the most important and unwanted complication in MPNs. There are several genes that could be correlated with increased risk of leukemic transformation [20-22]. ASXL1, TET2, SRSF2, RUNX, TP53 and EZH1 are the genes which have been reported and also take a part in our myeloid NGS panel.

As a conclusion, genomic myeloid panels using NGS technique are "essential" for an accurate diagnosis, risk assessment and management of the patients with myeloid neoplasias, especially in the novel era of targeted therapies.

References

1. Shumilov E, Flach J, Kohlmann A, Banz Y, Bonadies N, Fiedler M, et al. Current status and trends in the diagnostics of AML and MDS. Blood Rev. 2018;32(6):508-19.
2. Aguilera-Diaz A, Vazquez I, Ariceta B, Manu A, Blasco-Iturri Z, Palomino-Echeverria S, et al. Assessment of the clinical utility of four NGS panels in myeloid malignancies. Suggestions for NGS panel choice or design. PLoS One. 2020;15(1):e0227986.
3. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391-405.
4. Dohner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Buchner T, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood. 2017;129(4):424-47.
5. Gale RE, Green C, Allen C, Mead AJ, Burnett AK, Hills RK, et al. The impact of FLT3 internal tandem duplication mutant level, number, size, and interaction with NPM1 mutations in a large cohort of young adult patients with acute myeloid leukemia. Blood. 2008;111(5):2776-84.
6. Devillier R, Mansat-De Mas V, Gelsi-Boyer V, Demur C, Murati A, Corre J, et al. Role of ASXL1 and TP53 mutations in the molecular classification and prognosis of acute myeloid leukemias with myelodysplasia-related changes. Oncotarget. 2015;6(10):8388-96.

7. Zuo Z, Li S, Xu J, You MJ, Khoury JD, Yin CC. Philadelphia-Negative Myeloproliferative Neoplasms: Laboratory Workup in the Era of Next-Generation Sequencing. Curr Hematol Malig Rep. 2019;14(5):376-85.
8. Guglielmelli P, Lasho TL, Rotunno G, Mudireddy M, Mannarelli C, Nicolosi M, et al. MIPSS70: Mutation-Enhanced International Prognostic Score System for Transplantation-Age Patients With Primary Myelofibrosis. J Clin Oncol. 2018;36(4):310-8.
9. Papaemmanuil E, Gerstung M, Malcovati L, Tauro S, Gundem G, Van Loo P, et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. Blood. 2013;122(22):3616-27; quiz 99.
10. Greenberg PL, Tuechler H, Schanz J, Sanz G, Garcia-Manero G, Sole F, et al. Revised international prognostic scoring system for myelodysplastic syndromes. Blood. 2012;120(12):2454-65.
11. Nazha A, Narkhede M, Radivoyevitch T, Seastone DJ, Patel BJ, Gerds AT, et al. Incorporation of molecular data into the Revised International Prognostic Scoring System in treated patients with myelodysplastic syndromes. Leukemia. 2016;30(11):2214-20.
12. Kamps R, Brandao RD, Bosch BJ, Paulussen AD, Xanthoulea S, Blok MJ, et al. Next-Generation Sequencing in Oncology: Genetic Diagnosis, Risk Prediction and Cancer Classification. Int J Mol Sci. 2017;18(2).
13. Li MM, Datto M, Duncavage EJ, Kulkarni S, Lindeman NI, Roy S, et al. Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. J Mol Diagn. 2017;19(1):4-23.
14. Zhao CX, Wang JM, Li JM, Zou SH, Chen FY, Liang AB, et al. [Using next generation sequencing technology to analyze gene mutations in patients with acute myeloid leukemia and the impact on prognosis]. Zhonghua Yi Xue Za Zhi. 2019;99(40):3145-51.
15. Sasaki K, Kanagal-Shamanna R, Montalban-Bravo G, Assi R, Jabbour E, Ravandi F, et al. Impact of the variant allele frequency of ASXL1, DNMT3A, JAK2, TET2, TP53, and NPM1 on the outcomes of patients with newly diagnosed acute myeloid leukemia. Cancer. 2020;126(4):765-74.
16. Leisch M, Jansko B, Zaborsky N, Greil R, Pleyer L. Next Generation Sequencing in AML-On the Way to Becoming a New Standard for Treatment Initiation and/or Modulation? Cancers (Basel). 2019;11(2).
17. Stone RM, Mandrekar SJ, Sanford BL, Laumann K, Geyer S, Bloomfield CD, et al. Midostaurin plus Chemotherapy for Acute Myeloid Leukemia with a FLT3 Mutation. N Engl J Med. 2017;377(5):454-64.
18. DiNardo CD, Stein EM, de Botton S, Roboz GJ, Altman JK, Mims AS, et al. Durable Remissions with Ivosidenib in IDH1-Mutated Relapsed or Refractory AML. N Engl J Med. 2018;378(25):2386-98.
19. Bacher U, Shumilov E, Flach J, Porret N, Joncourt R, Wiedemann G, et al. Challenges in the introduction of next-generation sequencing (NGS) for diagnostics of myeloid malignancies into clinical routine use. Blood Cancer J. 2018;8(11):113.
20. McNamara CJ, Panzarella T, Kennedy JA, Arruda A, Claudio JO, Daher-Reyes G, et al. The mutational landscape of accelerated- and blast-phase myeloproliferative neoplasms impacts patient outcomes. Blood Adv. 2018;2(20):2658-71.
21. Zhang SJ, Rampal R, Manshouri T, Patel J, Mensah N, Kayserian A, et al. Genetic analysis of patients with leukemic transformation of myeloproliferative neoplasms shows recurrent SRSF2 mutations that are associated with adverse outcome. Blood. 2012;119(19):4480-5.
22. Elliott MA, Pardanani A, Hanson CA, Lasho TL, Finke CM, Belachew AA, et al. ASXL1 mutations are frequent and prognostically detrimental in CSF3R-mutated chronic neutrophilic leukemia. Am J Hematol. 2015;90(7):653-6.