Araştırma Makalesi
Research Article

Investigating FLT3 mutations in acute myeloid leukemia: A Single-Center Real-World data study on patient outcomes and treatment strategies

AKUT MİYELOİD LÖSEMİDE FLT3 MUTASYONLARININ ARAŞTIRILMASI: HASTA SONUÇLARI VE TEDAVİ STRATEJİLERİ ÜZERİNE TEK MERKEZLİ GERÇEK YAŞAM VERİSİ

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ABSTRACT

Acute Myeloid Leukemia (AML) is a complex hematological malignancy with considerable genetic heterogeneity. Fms-like tyrosine kinase 3 (FLT3) mutations are associated with poor prognosis and occur in nearly 30% of AML cases. This study delves into the prevalence of FLT3 mutations, their impact on clinical outcomes, and the efficacy of various treatment approaches in a cohort of AML patients.

Materials and Methods: We examined 157 de novo AML patients aged 20-95 years, screening for FLT3-ITD and FLT3-TKD mutations. We tailored chemotherapy based on age, ECOG performance status, and FLT3 mutation presence. IBM SPSS Statistics for Windows 26.0 was used for statistical analyses.

Results: Our research revealed that 27.3% of patients harbored FLT3 mutations, with 65% FLT3-ITD and 35% FLT3-TKD mutations. Those with FLT3 mutations exhibited higher mortality rates compared to patients without mutations. Age, FLT3 mutation status, and relapsed/refractory disease emerged as independent risk factors for mortality. Patients treated with midostaurin faced a lower mortality risk than those administered sorafenib.

Conclusion: This study underscores the significance of FLT3 mutations in AML, their influence on clinical outcomes, and the advantages of targeted therapies. Our findings stress the urgency for further investigation aimed at enhancing the prognosis for AML patients with FLT3 mutations.

Keywords: Flt3 mutations, acut myeloid leukemia, real world data.

ÖZ

Amaç: Akut Miyeloid Lösemi (AML), önemli ölçüde genetik heterojeniteye sahip karmaşık bir hematolojik malignitedir.

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Fms benzeri tirozin kinaz 3 (FLT3) mutasyonları, kötü prognoz ile ilişkilidir ve AML vakalarının yaklaşık %30'unda görülür. Bu çalışma, bir AML hasta kohortunda FLT3 mutasyonlarının prevalansını, bunların klinik sonuçlar üzerindeki etkilerini ve çeşitli tedavi yaklaşımlarının etkinliğini araştırmaktadır.

Gereç ve Yöntem: Yaşları 20-95 arası 157 akut promiyelositik lösemi dışı de novo AML tanılı hasta FLT3-ITD ve FLT3-TKD mutasyonları açısından taranarak incelendi. Kemoterapiyi yaşa, ECOG performans durumuna ve FLT3 mutasyon varlığına göre uyarlandı. Araştırmamız hastaların %27,3'ünde FLT3 mutasyonu, %65'inde FLT3-ITD ve %35'inde FLT3-TKD mutasyonu olduğunu ortaya koydu. FLT3 mutasyonları olanlar, mutasyonları olmayan hastalara kıyasla daha yüksek ölüm oranları sergiledi. Yaş, FLT3 mutasyon durumu ve tekrarlayan/dirençli hastalık, mortalite için bağımsız risk faktörleri olarak ortaya çıktı. Midostaurin ile tedavi edilen hastalar, sorafenib uygulananlara göre daha düşük bir ölüm riskine sahipti.

Sonuç: Bu çalışma, AML'deki FLT3 mutasyonlarının önemini, bunların klinik sonuçlar üzerindeki etkilerini ve hedefe yönelik tedavilerin avantajlarını vurgulamaktadır. Bulgularımız, FLT3 mutasyonları olan AML hastalarının prognozunu güçlendirmeyi amaçlayan daha ileri araştırmaların aciliyetini vurgulamaktadır.

Anahtar Kelimeler: Flt3 mutasyonları, akut myeloid lösemi, gerçek yaşam verisi

Acute Myeloid Leukemia (AML) is a hematological malignancy marked by the clonal expansion of aberrantly differentiated myeloid progenitor cells in the bone marrow (1). AML exhibits considerable heterogeneity, with multiple genetic alterations driving its pathogenesis and contributing to a wide range of clinical outcomes (2). Fms-like tyrosine kinase 3 (FLT3) is a receptor tyrosine kinase expressed on hematopoietic stem cells, playing a pivotal role in cell proliferation, differentiation, and survival (3). FLT3 mutations, among the most prevalent genetic abnormalities in AML, occur in approximately 30% of cases and correlate with poor prognosis (4,5). The two primary FLT3 mutations include internal tandem duplications (FLT3-ITD) and tyrosine kinase domain (FLT3-TKD) mutations (6). FLT3-ITD mutations are more frequent, accounting for 20-25% of AML cases, while FLT3-TKD mutations occur in 5-10% of cases. AML patients with FLT3 mutations face higher relapse rates and lower overall survival compared to those without FLT3 mutations. FLT3-ITD mutations, in particular, are linked to poor prognosis due to their high allelic burden and frequent co-occurrence with other high-risk genetic abnormalities (6). The prognostic impact of FLT3-TKD mutations remains less clear, with conflicting results reported in the literature. However, recent studies suggest that they may also be associated with adverse outcomes (7). The poor prognosis linked to FLT3 mutations has spurred the development of targeted therapies, such as tyrosine kinase inhibitors (TKIs) like midostaurin and sorafenib, which have shown potential in improving clinical outcomes for AML patients with FLT3 mutations (8).

The FDA approved midostaurin, in combination with chemotherapy, for the treatment of newly diagnosed adult FLT3-mutated AML patients based on the RATIFY trial's results (5). Other novel FLT3 inhibitors, such as quizartinib and gilteritinib, have also been evaluated in clinical trials and demonstrated potential efficacy in treating FLT3-mutated AML patients (9,10).

This study investigates the prevalence of FLT3 mutations in AML patients, their impact on clinical outcomes, and the efficacy of first-generation FLT3 inhibitors.

MATERIALS AND METHOD

A total of 157 patients with de novo non-acute promyelocytic leukemia (APL) AML, aged between 20 and 95, without severe organ dysfunction, and with an ECOG score of <2, were screened for FLT3-ITD and FLT3-TKD mutations. Written informed consent was obtained from the patients before chemotherapy and FLT3 inhibitor treatment.

Genomic DNA (gDNA) isolation from samples was performed using the Qiagen "QIAamp DNA Blood" kit. Cell lysis was carried out using 200 µl of the sample with proteinase K and lysis buffer solution. Following a series of washing steps, genomic DNA materials were collected by elution through spin columns provided in the kit. The quality control of isolated genomic DNAs was performed by spectrophotometric measurement using a Nanodrop device (Thermo Fisher Scientific, USA). FLT3 gene exons were amplified from samples with sufficient concentration and purity by PCR method (polymerase chain reaction) using primer pairs specifically designed for exon-intron junctions.

The PCR reaction included 5 µl reaction buffer, 1 µl dNTP, 2 µl forward primer, 2 µl reverse primer, 25 ng sample gDNA, 0.5 µl Phire Hot Start II DNA Polymerase, and distilled water to reach a final volume of 25 µl. Amplification was performed in a thermal cycler (Thermo Fisher Scientific, USA) using the program outlined in Table 1.

Table 1. A sample PCR protocol for Phire II enzyme system.

Temperature	Duration	Cycle
98 °C	1 min	1
98 °C	20 sec	
60 °C	20 sec	35
72 °C	20 sec	
72 °C	1 min	1
4 °C	indefinite	-

Size and quality controls of PCR products were performed by electrophoresis on a prepared 2% agarose gel containing EtBr. The PCR step was repeated for samples with insufficient quality for the sequence. Samples with adequate quality and quantity were purified using magnetic beads. Concentrations of purified amplicons were measured on a fluorometric measurement instrument (Qubit 2, Thermo Fisher Scientific, USA).

Oligonucleotides containing sample-specific adapter and index sequences were added to the ends of amplicons by ligation using the Nextera XT DNA Library Preparation Kit (Illumina, USA). Next-generation sequencing was performed using the MiSeq system (Illumina, USA) with 150 base pairs and paired-end reads.

Raw data obtained in FASTq format as a result of the sequence were aligned to the Human Genome 19 (hg19) using the "BWA" algorithm and analyzed with the Integrated Genomics Viewer (IGV, version 2.8.9, Broad Institute). The effects of the detected variants were investigated by searching the "Ensemble" and "gnomAD" databases.

Statistical Analysis

Statistical analyses of the collected data were performed using IBM SPSS Statistics for Windows 26.0 (IBM Corp., Armonk, NY, USA). The normality of the data distribution was assessed using the Kolmogorov-Smirnov test. Numerical variables with a normal distribution were expressed as mean ± standard deviation, while those with a non-normal distribution were expressed as median (min-max). Categorical variables were reported as numbers and percentages.

For comparisons between the two groups, the Student's t-test or the Mann-Whitney U test were utilized

based on the normality of the data distribution. The chi-square and Fisher's exact chi-square tests were employed for comparing categorical variables. To identify potential independent predictors of mortality, multivariable Cox logistic regression analyses were conducted. Kaplan-Meier analysis was used to create survival plots. A p-value of <0.05 was considered statistically significant.

RESULTS

The study population consisted of 157 patients, 63 females and 94 males (mean age: 52.9±16.4 years). FLT3 mutation was detected in 27.3% (n: 43) of the patients. Demographic and clinical characteristics of the patients are shown in Table 2. Age and sex distribution was similar in patients with and without FLT3 mutation. Mortality was higher in patients with FLT3 mutation compared to non-mutated patients (p<0. 05). Relapsed refractory disease was present in 86.6% of the patients. Allogeneic bone marrow transplantation was performed in 44% of the patients.

Table 2. Demographic and clinical characteristicss have been shown in Table 1.

Table 2. Demographic and clinical characteristics

Variables	Total n=157	FLT3 mutation		p
		No n=114	Yes n=43	
Age, year	52.9±16.4	53±16.9	52.6±15.4	0.72
Sex, n(%)				
Female	63(40.1)	43(37.7)	20(47.4)	0.341
Male	94(59.9)	71(62.3)	23(52.6)	
FLT3 Mutation, n(%)				
No	114(72.7)	114(100.0)	-	
Yes	43(27.3)	-	43(100.0)	
CNS Involvement				
No	148(94.2)	111(97.4)	37(86)	0.62
Yes	9(5.8)	3(2.6)	6(14)	
Mortality, n(%)				
Alive	52(33.6)	43(36.9)	9(23.7)	0.005*
Exitus	105(66.4)	71(63.1)	34(76.3)	
Treatment, n(%)				
Chemo	114(72.6)	114(100.0)	0(0)	
Midostaurin+ Chemo	32(20.3)	-	32(74.4)	<0.001*
Sorafenib+ Chemo	11(7.1)	-	11(25.6)	
Relapsed-Refractory Disease, n(%)				
No	21(13.4)	18(15.8)	3(7.5)	0.04*
Yes	136(86.6)	96(84.2)	40(92.5)	
Allogeneic-HSCT, n(%)				
No	88(56)	59(51.7)	29(67.4)	0.11
Yes	69(44)	55(48.3)	14(32.6)	

Categorical variables were shown as numbers (%). Numerical variables showing normal distribution were shown as mean±SD, and numerical variables with non-normal distribution were shown as median (min-max).

*p< 0. 05 indicates statistical significance.

Of the FLT3 mutated patients, 65% (n: 28) had FLT3-ITD mutation and 35% (n: 15) had FLT3-TKD mutation (Table 3). Mortality rate was higher in patients with FLT3-TKD mutation (86.7%) than in patients with FLT-ITD mutation (75%) (p<0. 05).

Table 3. Correlation between FLT3 mutation and survival

FLT3 mutation subtype, n(%)	Total n=43	Alive n=9	Exitus n=34	p
FLT-ITD	28	7(25%)	21(75%)	
FLT3-TKD	15	2(13.3%)	13(86.7%)	<0.002*

Increasing age (HR: 1. 01; p<0.001), FLT3 mutation (HR: 1.86; p=0.006), and having r/r disease (HR: 19.4; p=0.001) were associated with higher mortality risk. Patients receiving midostaurin had a lower risk of mortality than patients receiving sorafenib(HR: 0.69; p=0.01). The mortality risk was low in those who had allogeneic-HSCT (HR:0.2; p<0.001) (Table 4).

Table 4. Multivariate analysis for overall survival.

Variables	Survival		Univariable Cox Regression		
	Alive n=52	Exitus n=105	HR	95% CI	p
Age, year	44.8.±14.1	57.6±15.2	1.01	1-1.03	<0.001*
Sex, n(%)					
Female	24(48.0)	39(37.1)	ref		
Male	28(56.0)	66(62.9)	1.2	0.8-1.83	0.2
FLT3 Mutation, n(%)					
No	43(82.6)	71(67.6)	ref		
Yes	9(17.4)	34(32.4)	1.86	1.2-2.8	0.006*
CNS Involvement, n(%)					
No	50(96.2)	98(92.8)	ref		
Yes	2(3.8)	7(7.2)	1.9	0.9-2.2	0.8
Treatment, n(%)					
Sorafenib+ Chemo	2(4.0)	9(5.1)	ref		
Midostaurin+ Chemo	7(14.0)	25(24.2)	0.69	0.7-0.96	0.01*
Relapsed-Refractory Disease, n(%)					
No	18(34.6)	3(2.8)	ref		
Yes	34(65.4)	102(97.2)	19.4	3.29-169.95	0.001*
Allogeneic-HSCT, n(%)					
No	7(10.0)	81(75.8)	ref		
Yes	45(90.0)	24(24.2)	0.2	0.12-0.32	<0.001*

Abbreviations: HR: Hazard ratio, CI: Confidence interval

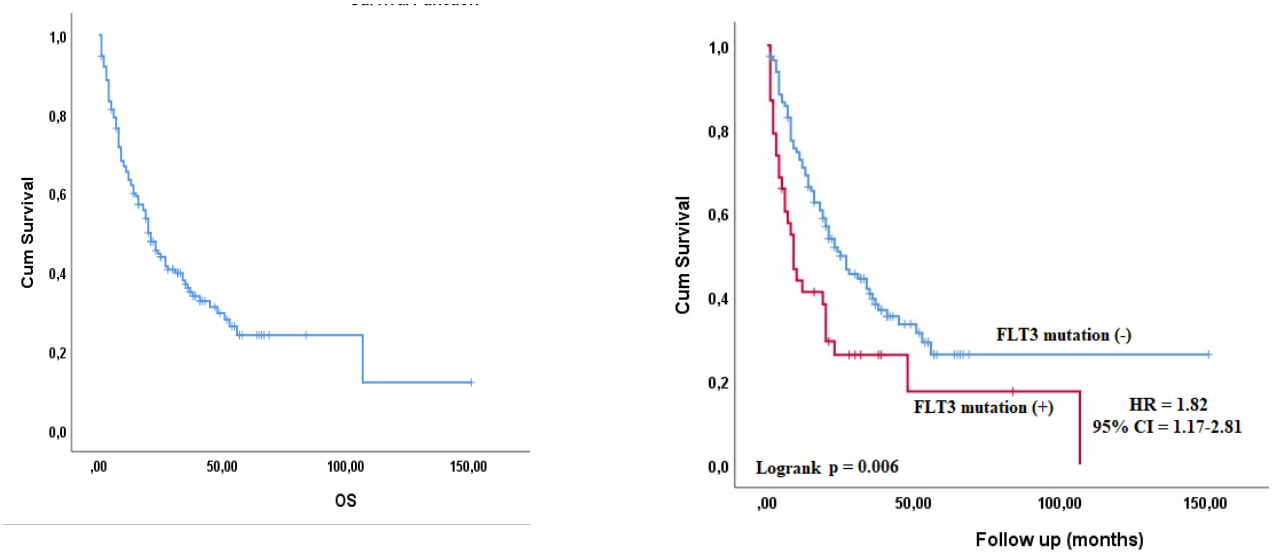
Potential risk factors associated with mortality risk were included in the regression model and it was found that presence of r/r disease (HR: 13.2; p=0.005), bone marrow blast level (HR: 1.2; p<0.001) and LDH level (HR: 1.1; p<0.001) were independent risk factors for mortality (Table 5). Mortality risk was lower in those with allogeneic-HSCT (HR: 0.2; p <0.001).

Table 5. Risk factors which are strong predictors of mortality

Variables	Multivariable Cox regression		
	HR	95% CI	p
Relapsed-Refractory Disease	13.2	2.2-108	0.005*
Allogeneic-HSCT	0.2	0.13-0.3	<0.001*
Bone Marrow Blast Level	1.2	1.03-1.26	<0.001*
LDH Level	1.1	1.06-1.14	<0.001*

Survival of the patients was analyzed according to total survival and mutation status. Median follow-up and overall survival were lower in patients with FLT mutations. The cumulative mortality rate in all patients was 66.9%, median follow-up of 35 (range, 0.7-67.9) months and the median overall survival (OS) was 19.1 months (Figure 1). Presence of FLT3 mutation (HR:1.82; p=0.007) increased the risk of mortality.

Figure 1. Total survival rates and survival by mutation status



DISCUSSION

In our single-center study, we scrutinized the characteristics and outcomes of 157 AML patients, delving into the incidence of FLT3 mutations, mutation types, and their influence on clinical outcomes. Our findings revealed that 27.3% of patients had FLT3 mutations, a figure in line with the previously documented incidence in AML patients [4]. Interestingly, this percentage is somewhat lower than the incidence noted in Western countries and more akin to that observed in Asian countries (11,12). Such a difference could stem from variations in study populations and geographic distribution.

Examining our cohort, we found that 65% of FLT3-mutated patients presented with FLT3-ITD mutations, while 35% exhibited FLT3-TKD mutations. These proportions correspond with reported frequencies in existing literature, where FLT3-ITD mutations occur in approximately 20-25% of AML cases and FLT3-TKD mutations in around 5-10% of cases (6). In our investigation, we discovered that FLT3 mutations correlated with elevated mortality rates, echoing prior studies that show AML patients with FLT3 mutations experience higher relapse rates and diminished overall survival compared to those without FLT3 mutations (4). Moreover, our findings revealed that patients with FLT3-TKD mutations faced a heightened mortality rate (86.7%) compared to those with FLT3-ITD mutations (75%). This outcome aligns with recent studies proposing that FLT3-TKD mutations could also be linked to adverse consequences (6).

In light of the significant impact FLT3 mutations have on AML patients and the emergence of targeted therapies, this study aims to investigate the incidence of FLT3 mutations, clinical outcomes, and treatment strategies in a cohort of AML patients. Through this investigation, we hope to contribute to the current understanding of the role of FLT3 mutations in AML and explore the potential benefits of targeted therapies for this patient population.

Conclusion

The increased mortality risk factors identified in our research, such as; advancing age, FLT3 mutation, and the presence of relapsed/refractory (r/r) disease, correspond with recognized risk factors in existing literature (4). Additionally, our study uncovered that patients treated with midostaurin faced a reduced mortality risk compared to those receiving sorafenib, highlighting the potential advantages of targeted therapies for FLT3-mutated AML patients (5,8).

To conclude, our single-center investigation offers insightful real-world data concerning the prevalence, attributes, and consequences of FLT3 mutations in AML patients within our geographic area. Our findings reveal that FLT3 mutations correlate with increased mortality rates, with FLT3-TKD mutations exhibiting a higher mortality rate compared to FLT3-ITD mutations. These results underscore the potential advantages of targeted treatments, such as midostaurin, and the significant impact allogeneic HSCT has on enhancing the prognosis of AML patients with FLT3 mutations.

It is crucial to recognize our study's limitations, which encompass its single-center design that may not be applicable to other populations or geographic regions. Additionally, our research concentrated on a select number of clinical aspects and did not explore the extensive genetic landscape of AML, known for its high heterogeneity and potential to influence FLT3-mutated patient outcomes. Moreover, the sample size in our study may restrict the ability to discern smaller outcome differences among various patient subgroups.

Despite these limitations; our study contributes to the growing body of literature on FLT3 mutations in AML, particularly in the context of real-world data and geographic differences. Further multi-center, large-scale studies across diverse populations and regions are needed to better understand the factors that influence the incidence and outcomes of FLT3 mutations in AML patients. Continued research in this area, along with the development of novel targeted therapies and combination treatment strategies, is crucial to improving the prognosis and management of patients with FLT3-mutated AML.

Conflict of İnterest

All authors have no conflicts of interest to disclose

REFERENCES

1. Döhner, H., Weisdorf, D. J., & Bloomfield, C. D. (2015). Acute myeloid leukemia. New England Journal of Medicine, 373(12), 1136-1152.
2. Papaemmanuil, E., Gerstung, M., Bullinger, L., Gaidzik, V. I., Paschka, P., Roberts, N. D., ... & Campbell, P. J. (2016). Genomic classification and prognosis in acute myeloid leukemia. New England Journal of Medicine, 374(23), 2209-2221.
3. Stirewalt, D. L., & Radich, J. P. (2003). The role of FLT3 in haematopoietic malignancies. Nature Reviews Cancer, 3(9), 650-665.
4. Döhner, H., Estey, E., Grimwade, D., Amadori, S., Appelbaum, F. R., Büchner, T., ... & Larson, R. A. (2017). Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood, 129(4), 424-447.
5. Stone, R. M., Mandrekar, S. J., Sanford, B. L., Laumann, K., Geyer, S., Bloomfield, C. D., ... & Döhner, K. (2017). Midostaurin plus chemotherapy for acute myeloid leukemia with a FLT3 mutation. New England Journal of Medicine, 377(5), 454-464.
6. Daver, N., Schlenk, R. F., Russell, N. H., & Levis, M. J. (2019). Targeting FLT3 mutations in AML: review of current knowledge and evidence. Leukemia, 33(2), 299-312.
7. Alvarado, Y., Kantarjian, H. M., Luthra, R., Ravandi, F., Borthakur, G., Garcia-Manero, G., ... & Cortes, J. E. (2018). Treatment with FLT3 inhibitor in patients with FLT3-mutated acute myeloid leukemia is associated with development of secondary FLT3-tyrosine kinase domain mutations. Cancer, 120(14), 2142-2149.
8. Burchert, A., Bug, G., Fritz, L. V., Finke, J., Stelljes, M., Röllig, C., ... & Wagner, E. M. (2019). Sorafenib Maintenance After Allogeneic Hematopoietic Stem Cell Transplantation for Acute Myeloid Leukemia With FLT3–Internal Tandem Duplication Mutation (SORMAIN). Journal of Clinical Oncology, 37(26), 2329-2339.
9. Cortes, J. E., Khaled, S., Martinelli, G., Perl, A. E., Ganguly, S., Russell, N., ... & Bixby, D. (2019). Quizartinib versus salvage chemotherapy in relapsed or refractory FLT3-ITD acute myeloid leukaemia (QuANTUM-R): a multicentre, randomised, controlled, open-label, phase 3 trial. The Lancet Oncology, 20(7), 984-997.
10. Perl, A. E., Martinelli, G., Cortes, J. E., Neubauer, A., Berman, E., Paolini, S., ... & Levis, M. J. (2019). Gilteritinib or chemotherapy for relapsed or refractory FLT3-mutated AML. Blood, 134(6), 981-988
11. Chen, W., Xie, H., Wang, H., Chen, L., Sun, Y., Chen, Z., ... & Huang, X. (2015). Prognostic significance of KIT mutations in core-binding factor acute myeloid leukemia: a systematic review and meta-analysis. PloS one, 10(3), e0121218.
12. Yalniz, F. F., Abou Dalle, I., Kantarjian, H., Borthakur, G., Kadia, T., Patel, K., ... & Ravandi, F. (2018). Prognostic significance of baseline FLT3-ITD mutant allele level in acute myeloid leukemia treated with intensive chemotherapy with/without sorafenib. American Journal of Hematology, 93(2), 164-171.