Evaluating the clinical significance of FLT3 mutation status in Syrian newly diagnosed acute myeloid leukemia patients with normal karyotype

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ABSTRACT

The FMS-like tyrosine kinase-3 internal tandem duplication (FLT3-ITD) is one of the most prevalent mutations, affecting between 20 and 30 percent of cases in patients with acute myeloid leukemia (AML). The Patients with a FLT3-ITD mutation have a poor prognosis. In the present study, we investigated the FLT3 (ITD-TKD) mutations in 100 newly adult Syrian patients with AML-Normal karyotype (NK). Our results revealed that prevalence of FLT3-ITD mutation was 24%. Interestingly, 20 patients had a typical duplication mutation and four patients had different mutations. From those four mentioned patients, two of them carried a 39 base pair (bp) duplication in different location: (c.1838_1877dup39, p.591–603dup) and (c.1836_1874 dup 39, p.591–603dup), the third patient, showed FLT3-ITD duplication and a newly insertion together, this insertion was not demonstrated before: (c.1842_1865dup24, c.1865_1866insGAA). Finally, the fourth patient exhibited a duplication of 21bp (c.1855_1875dup21, p.597–603dup). In addition, statistically significant differences were observed for the relation between the presence of FLT3-ITD mutation and lactate dehydrogenase (LDH) level, overall survival (OS), relapse, and event free survival (EFS). We demonstrated that our patients with FLT3-ITD mutation had a poor prognosis. Also, the frequency of FLT3-TKD mutation was low 2% and no compound between the two mutations was found, as individuals showed to carry the two mutations were not detected. These findings are likely useful for a better understanding of molecular leukemogenetic steps in AML-NK patients and may be beneficial for clinical relevance for risk grouping, study design and choice of therapy in Syrian population.

1. Introduction

Acute myeloid leukemia (AML) is a type of cancer that affects proliferating, clonally aberrant, or weakly differentiated hematopoietic cells. It is recognized by clonal evolution and genetic heterogeneity [1]. Cytogenetic profiles are virtually a predictive indicator of clinical outcomes in AML, which allow the stratification of patients conforming to risk and could guide therapeutic decisions [2]. AML represents from 15 to 20% of acute leukemia cases in younger, and 80% in adults. AML is the predominant form of leukemia in neonatal and adult periods, but represents a cramped fraction of cases during infancy and adolescence [3]. The approach that genetic and molecular abnormalities describe unique subtypes of leukemia with significant clinical and prognostic features has led to an overall modification of direction in the classification of AML, from a neat morphologic classification to a more genetic and molecular based classification, as in the most up-to-date World Health Organization classification (WHO) [3]. It is an extended, intricate process where cooperation of different genetic alterations are required to obtain full-blown leukemia [4]. The genetic alterations responsible for AML include mutations in genes like as FMS-like tyrosine kinase 3 (FLT3), KIT Proto-Oncogene (KIT), and Rat Sarcoma (RAS), which activate signal transduction pathways to increase the proliferative and survival potential of hematopoietic progenitors [5]. The FLT3 gene is a class III, trans-membrane ligand-activated receptor tyrosine kinase (RTK), which is typically articulated by hematopoietic or progenitor cells and is essential for controlling the growth and differentiation of both myeloid and lymphoid lineage [6]. Through sundry signaling pathways, including: RAS, PI3K (phosphatidylinositol 3-kinase), and STAT5 (Signal Transducer And Activator Of Transcription 5A), the
extracellular FLT3 ligand binds and triggers FLT3, promoting and enhancing cell survival, proliferation, and transformation [6]. Mutations of FLT3 are reported in loosely 30% of a newly diagnosed AML cases and betide as internal tandem duplication (ITD) in approximately 25% of cases or less frequently as point mutations in the tyrosine kinase domain (TKD) in 7–10% [7]. In-frame duplications of FLT3-ITD mutations range in size from three nucleotides up to 1,000, and thus are situated in the juxtamembrane domain (JMD) of the auto inhibitory receptor. FLT3-JMD restrains the activity of the receptor in WT-FLT3 (wild type), therefore, ITDs cuts off a certain inhibitory consequence from occurring, which activates the component [8]. Point mutations in the activation loop of the receptor called FLT3-TKDs, this type of mutations, level off the active kinase structure and abidance, moreover, it could be lead to promote constitutive kinase operationalization [9]. The adequacy of FLT3-TKD mutations for prognosis is equally clear and could even change based on the existence of co-occurring mutations and chromosomal customization [10]. Presently, FLT3-TKD mutation existence does not weak formal acute myeloid leukemia risk appraisal. Both FLT3-ITD and FLT3-TKD mutations constitutively actuate FLT3 kinase activity, with the result that proliferation and survival of AML [11]. Various studies have pointed out that FLT3-ITD mutations were linked to a decreased overall survival rate and a higher probability of relapse in acute myeloid leukemia. Likewise, it has been established that the low or missing expression in the allele of the gene FLT3 was linked to unfavorable and adverse results [12]. The aim of this study is to assess the prognostic relevance of activating FLT3 mutations in adult AML patients. Our primary objective was to determine whether the presence of a FLT3-ITD mutation defines a group with poor prognosis within the heterogeneous group of AML patients with normal karyotype. We have very little data on genetic alteration in Syrian population and prognostic significance, so we present the mutational spectrum of the FLT3 gene in Syrian newly diagnosed AML patients.

2. Materials and methods

2.1. Subjects

One hundred newly diagnosed patients with AML between November 2018 and August 2021 were included in this study. This study consist of 51 male and 49 female, all patients were adults, the mean age was (43.48 ± 18.178). The diagnosis of AML done based upon complete blood count test, blood smear, cytogenetics and flow cytometers. All Cases confirmed histologically and immunohistochemically with no prior treatment. According to conventional cyogenetic and molecular cyogenetic the AML selected patients for molecular analysis showed a normal karyotype. The classification of AML Types made according to French-American-British (FAB) classification from M1 to M6. We evaluated LDH levels for the patients using (DxC 700 AU, Beckman) Chemistry analyzer, and the chemical reagent for this blood test was: (Chongqing Zhongyuan Biological Co.). Normal value range according to the standard is 80–285 units per liter for both male and female above the age of 18. Signed written informed consent was obtained from each patient (100 patients) or family members before take the sample. Peripheral blood or bone marrow aspirates were collected for all patients. The Syrian Ministry of Education’s Ethics Committee gave its permission to such a work.

2.2. Treatment protocol

Chemotherapy for both induction phase and consolidation phase was given to all AML patients. Doxorubicin (50 mg/m²) for three days and Cytarabine (200 mg/m²) for seven days, after that a high-dosage of Cytarabine based consolidation phase (3 mg/m²)/12 h for three days, the last repeated twice to three times, make up the course of treatment.

Figure 1. Sanger sequencing confirmation of a (c.1842_1865dup) and (c.1865_1866insGAA) mutation in FLT-ITD in patient 10.
2.3. Cytogenetic and molecular cytogenetic analyses

The analysis of chromosomes utilizing GTG-banding was done on bone marrow (BM) samples before treatment according to standard protocols [13]. Fluorescence in situ hybridization (FISH) utilizing certain probes to identify translocations t (8; 21), t (15; 17), inv (16) or t (16; 16), t (12; 21), t (9; 11), t (6; 9) and deletion del (13q), were done using a conventional procedure to rule out patients who had chromosomal abnormalities, as originally described [13].

2.4. DNA extraction

Using the QIAamp DNA Blood Mini kit (Qiagen, Germany), genomic DNA was extracted from peripheral blood (PB) or bone marrow (BM) samples for the patients in accordance with the manufacturer guidelines and was kept at −20 °C. Each sample's DNA content was determined using a spectrophotometer, and then the quantity of UV light absorbance was measured.

2.5. FLT3-ITD mutation analysis

The FLT3-ITD mutation of Exons 11 and 12 were amplified utilizing the following forward and reverse primers respectively: (5’-GCAATT-TAGGTATGAAAGCCAGC-3’), (5’-CTTTCAGCATTTTGACGGCAACC-3’). An amount of 200 ng of genomic DNA, 10xPCR buffer (100 mM Tris-HCl, pH 8.8, 500 mM KCl), 2 mM MgCl2, 200 μM dNTPs, 10 pM of either primer, and 1 U of Taq DNA polymerase were used in the procedure of preparing Polymerase Chain Reaction (PCR) and the program was

Figure 2. Sanger sequencing confirmation of the mutations: (c.1838_1877dup), (c.1836_1874dup) and (c.1855_1875dup) in FLT-ITD in patients 13, 44 and 20 respectively.

Table 1. List of causative variants in FLT3-ITD mutations in the four mentioned patients.

| Patient number | Genomic position (GRCh37) | Nucleotidic alteration | Aminoacidsic alteration | Coding Impact | Variant type | ACMG Classification |
|----------------|---------------------------|-----------------------|-------------------------|---------------|--------------|-------------------|
| P10            | g. 28608101               | c.1842_1865dup        | p.Val615_Gly622dup      | In frame      | Insertion    | Pathogenic        |
|                | g. 28608101               | c.1865_1866insGAA     | p.Lys623dup             | In frame      | Insertion    | Likely Pathogenic |
| P13            | g. 28608089               | c.1836_1877dup        | p.Asn626LysfsTer21      | In frame      | frameshift   | Pathogenic        |
| P20            | g. 28608091               | c.1855_1875dup        | p.Gly619_Met625dup      | In frame      | Insertion    | Pathogenic        |
| P44            | g.28608092                | c.1836_1874dup        | p.V624_M625insGKNGMCQMFLQHFSIGKSLKCTYSPFVFAGKVLGSAGAFK           | In frame      | Insertion    | Likely Pathogenic |
The four new FLT3-ITD (+) Yes −1855.5833 378.02893 0.000 −2632.6329 −1078.5337 FLT3-ITD(+) Yes 1855.5833 378.02893 0.000 1078.5337 2632.6329 FLT3-ITD(−) No −32.80667 274.25101 0.906 −596.5377 530.9244
curve (ROC curve) was used to determine the effectiveness of white blood cells (WBC) and hemoglobin (HG) test in determining the existence of mutation, the multivariate statistical analyze was used to identify the critical factors that influence the likelihood of mortality, as overall survival and event free survival. \( P < 0.05 \) was considered statistically significant.

### 2.8. Criteria of response and Survival definitions

Complete remission (CR) needed absolute neutrophil counts of \( >1.0 \times 10^9/L \), platelet counts of \( >100 \times 10^9/L \), independence from red cell transfusions, bone marrow blasts of 5.0%, absence of blasts with Auer rods, lack of extra medullary diseases. Relapse was characterized by BM blasts more or equal to 5.0%, blasts reappeared in blood, or extra medullary disease developing. Overall survival (OS) was defined as the interval between the time of diagnosis and the date of the last follow-up visit, and event-free survival (EFS) as the interval between a diagnosis and an event (such as relapse, death in the first CR, or failure to reach remission), with patients not attaining remission being counted as having an event on day one.

### 3. Results

A total of one-hundred newly adult patients diagnosed AML. 51 male 51% and 49 female 49% were included in this study, the mean age was \( (43.48 \pm 18.178) \) years. When we studied the presence of FLT3 mutations, we found that the frequency rate of FLT3 mutations (FLT3-ITD, FLT3-TKD) mutations was 26% of the 100 AML patients, we identified FLT3-ITD mutation in 24 patients 24%, and FLT3-TKD in only two patients 2%. Furthermore, in 20 out 24 cases FLT3-ITD mutations were positive in a typical state (two bands, fragments size were 329 + 400 bp) 83.3%. Nevertheless, four patients: (P10, P13, P20, P44) were showed atypical FLT3-ITD mutation (fragment size was ~400 bp), the sequence analysis of those four mentioned patients revealed different duplication as showed in Figures 1 and 2. Interestingly, patient 10 carried a 24 bp duplication and had a newly insertion mutation, this insertion was not demonstrated before: (c.1842_1865dup), (c.1865_1866insGAA) Figure 1. This patient was female with 20 years old, the AML subtype was M1. Patient 13 was male, 58 years old, with AML-M2. The mutation of this patient was a 39 bp duplication in loci: (c.1838_1877dup, p.591–603), YVDFREYEYDLKW(13AA). Patient 44 (male, 68 years old, AML-M2) had a 39 bp duplication in loci: (1836–1874dup, p.591–603) YVDFREYEYDLKW (13 AA). Lastly, the patient 20 (male, 48 years old, AML-M2) had a 21 bp duplication (1855–1875dup 21, p.597–603dup YEYDLKW (7AA) as shown in Figure 2, these four patients received the typical treatment, they failed in achieving a complete remission, and the overall survival rate for them was obviously short between 0.37 and 1.66 months. The list of causative variants in FLT3-ITD mutations in the four mentioned patients are presented in Table 1. FLT3 protein is composed of 993 amino acids, with three main sections relating to the membrane,
extracellular, and intracellular domains. The range of affected amino acids in those four mentioned patients, expanded from position 622 to 626, all the mutations affected the functioning of the kinase domain 1 in exon 15 as shown in Figure 3.

Regarding to FAB subtypes of AML-FLT3 mutations, it was found that the AML-M2 is the most frequent subtypes in this cohort, followed by AML- M3 subtype, no statistical significance was observed (P = 0.233) according to Pearson Chi-Square. Regarding to the study of the relationship between prognostic factors such as age, WBC count, blasts percentage, gender, HB and platelets, in our patients, there were no noticeable changes between FLT3-ITD mutation and these biological markers as shown in Table 2. For gender, FLT3-ITD mutation were found more frequently in female 51% than male patients 49%, but this difference was not significant (P = 0.242). The same results were for age; no correlation was found. The correlation between FLT3-ITD mutation and complete remission (CR) in our AML patients was studied. Among all participants which they underwent conventional chemotherapy 3 complete remission rate was lower in patients harboring FLT3-ITD mutation than FLT3-ITD-mutation, with asymptotic significance (0.370) and was no significant (P > 0.052).

An ANOVA test was conducted to compare the values of LDH levels between three groups: the patients carrying the FLT3-ITD mutations, those who did not carry the mutation, and the four patients who carried the new FLT3-ITD mutations, LDH levels were noticeably higher among the last group. The results referred that the value of the Sig is less than 5%. Therefore, we conducted the multiple comparisons test, and we note from its results that there were a statistically significant difference between the three previously mentioned groups of patients in LDH levels as shown in Table 3.

When we evaluate the occurrence between OS rate and FLT3-ITD mutation in AML patients, we found a statistically significant difference for the relation between the presence of FLT3-ITD mutation and OS rate (P-value = 0.000), Kaplan-Meier curve portrayed that when patients with FLT3-ITD mutations had a mean survival time of 9.39 months (95% CI 3.4–15.3 months) as shown in Figure 4, with significant differences between the mean survival of FLT3-ITD positive and those negative to the mutation (Log-rank = 34.257, P = 0.000). Multivariate Cox model analysis reflect that patients with positive FLT3-ITD mutation were 5.7 times more likely to death than negative ones (P = 0.000; HR = 5.716; 95% CI 2.962–11.032) (Figure 5). Patients with FLT3-ITD mutation demonstrated a higher relapse rate (30%) than those without FLT3-ITD mutation (17.7%) among all patients who reached CR, nonetheless, that failed to showed statistical importance as P = 0.81. In multivariate analysis, the presence of the mutation was the most significant prognostic factor for both OS and CR rate (P = 0.0287). Additionally, when we analyzed the relation of EFS for patients with FLT3-ITD mutation, we noted that the probability of survival after half a month is 0.97 for those who died after 0.5 months. The probability of survival for those who died after 28 months is nil, with significant differences (P < 0.05) between the mean EFS of holders and non-FLT3-ITD mutation holders, the mean EFS of FLT3-ITD mutation carriers is 1.839 months (95% CI 0.821–2.857 months), Figure 6 illustrates this, with, (Log rank = 41.490, P = 0.00). The event which indicates to failure in achieving remission or death or relapse, was estimated by Cox regression between the two groups (positive and negative FLT3-ITD mutation patients), and the results analysis that positive patients are endanger to the events 5.5 folds more than the another group, as shown in Figure 7 (P = 0.000; HR = 5.549; 95% CI 3.093–9.954).

4. Discussion

In this study, we investigated the frequency of FLT3-ITD and FLT3-TKD mutations in our AML patients. In Syria, since the FLT3 mutational profile is not typically carried out in hospitals, the statistics as well as findings about the frequency, heterogeneity, and impact of FLT3 mutations on clinical evolution are very poor. The incidence of the FLT3-ITD mutation was 24%; twenty patients carried the typical FLT3-ITD mutations, and four patients showed different duplications, between the FLT3-TKD mutation was detected only in two patients 2%. Both FLT3-ITD and FLT3-TKD changes constitutively initiate FLT3 kinase activity resulting the endurance of AML. The above uncontrolled activation impedes natural formation of blood cells, plus affords in the development of leukemia. As well as FLT3-ITD which happens as a duplicated arrangement in the juxtamembrane, also FLT3-TKD of the FLT3 receptor and distinct in location and length [15]. The prevalence of FLT3-ITD mutation on a global scale is between 25% and 30% [16, 17] and ~7% for FLT3-TKD point mutation of the activation loop domain

| OS | SE | Wald | Sig. | HR | 95.0% CI |
|----|----|------|------|----|---------|
| .335 | 27.005 | .000 | 5.716 | 2.962 | 11.032 |

OS: overall survival, SE: standard error, Sig: significance, HR: hazard ratio, CI: confidence interval.

Figure 5. Cox regression analysis of cumulative (Cum) survival and cumulative Hazard curves which illustrate the effect of FLT3-ITD on overall survival of AML patients.
Our results for FLT3-ITD were approximately similar to the global percentage 24%, whereas the FLT3-TKD was only 2% among our patients and this percentage was lower [19].

According to several studies, the incidence of FLT3-ITD mutations was different, some of studies were reported high prevalence for this mutation, whereas was lower in others studies [8]. The biology of AMLs is very diverse and varies in different populations, therefore, the variations in these findings could also be attributed to sample sizes, geographic location, and racial composition of the people under study. In addition, the results of the nucleotide sequence study of the participants showed the different duplication of FLT3-ITD in the JMD as heterozygous mutations in four patients, the first: (c.1855_1875dup), p.597–603dup, YEYDLKW (7AA), the second: (c.1838_1877dup), p.591–603dup, YVDFREYEYDLKW (13AA), the third: (c.1836_1874dup), p.591–603dup, YVDFREYEYDLKW (13 AA). Remarkably, the fourth patient exhibited a novel FT3L3-ITD frameshift mutation, whereas: duplicated 24 bp (c.1842_1865dup), p.593–600: DFREYEYD (8AA) and insertion: GAA (c.1865_1866insGAA) were detected. To our knowledge, this mutation was not observed before (COSMIC database for somatic samples from hematopoietic and lymphoid tissue). According to structural studies, which demonstrated that the only 15 base pair ITD insertions in JMD can disrupt the FLT3 kinase auto-inhibition conformation, thus promoting ligand-independent FLT3 receptor dimerization, and leading to constitutive auto phosphorylation and activation of downstream survival and proliferation signaling [20, 21]. Also, there have also been reports of duplicated intron 11 and exon 12 sequences in mutant protein products [22].

It has been noticed that in this four patients, the FLT3-ITD mutations affect the tyrosine kinase 1 domain. Compared to patients with FLT3-ITD mutations in JMD, those with TKD-ITDs have been linked to a poorer prognosis regarding to survival and treatment resistance [23]. Detecting ITD mutation heterogeneity in the clinical setting appears to be critical, as it might be a contributing factor causing diversity in therapy response in FLT3-ITD through AML patients. The location of the ITD in FLT3 affects tyrosine kinase inhibitor sensitivity [24]. However, from four patients, the mutations in three cases were detected on the β1 sheet of the first kinase domain, corresponding to amino acids 624–630, and one case was found in nucleotide binding loop (NBL). The FLT3-ITD with insertion site in the β2-sheet of the TKD1 step in phosphorylation of FLT3 and STAT5, indicating that non-JM domain FLT3-ITD mutations give constitutive activation of the receptor [25]. Three from the four mentioned variants showed up changes in the reading frame of transcript, when it did not occur in multiples of three nucleotides, this results could be affiliated with adverse outcome as some authors showed that in their study and revealed that increasing ITD size, and the transcript reading errors consider as bad prognosis [26]. Because there weren’t enough participants in our study, the previous analysis couldn’t be done, but that opened the door to investigate this factor in our cohort. During the assessment of CD34+ /CD38− fraction of the leukemic clone in the patients harboring FLT3-ITD mutation, it was clear that the mentioned cells enhanced with the character of leukemic stem cells (LSCs) with FLT3-ITD+, therefore, we can agree the studies that point out a functional correlation between FLT3-ITD and LSCs [27]. Additionally, complete

Figure 6. Kaplan–Meier curves of the effects of FLT3-ITDs on EFS of AML patients.
blood count (CBC) results of AML patients with the FLT3 mutation at diagnosis show higher WBC counts, lower platelet counts, and higher hemoglobin levels compared to those without the mutation [28]. In contrast, some studies found no correlation between FLT3 mutation and biological parameters [21]. In the present study, no noteworthy variations between FLT3 mutation patients in terms of age, sex, mean WBC count, Hg, platelets, and leukemic blast were found. It was frequent to evaluate LDH serum levels as an predictive factors in patients who suspect that they could have hematological malignancies like AML or myeloma. In individuals with AML, the level of LDH is inversely related to their medical prognosis. Previous research has linked a high LDH concentration to a perilous of deathblow in AML patients [29]. Increased LDH levels are a result of the tumor's elevated glycolytic activity and tumor necrosis caused by hypoxia, the latter of which is linked to a high tumor burden [30]. In our result, it was obvious that there was an inverse relationship between LDH serum levels and the OS time, so we can declare that LDH serum is an independent predictive marker for AML patients. This crucial result needs more investigation, taking in consideration the patient performance status at the time of enrollment to prove the link between LDH and early mortality. Several studies have discussed that the relationship between the presence of FLT3-ITD mutations and outcome (CR, relapse, overall survival, EFS), and discovered that FLT3-ITD is linked to a poor prognosis, including a higher chance of relapse, a shorter overall survival and worse performance status in AML patients [31, 32]. During this study, just 28% of the 100 patients who were given typical induction chemotherapy attained a complete remission. The CR rate was lower in patients with FLT3-ITD than non-existing FLT3-ITD with asymptotic significance and that was also no considerable (P > .05), so for the present study, there is no linkage between the two factors. Contrarily, a statistically significant difference in our patients for the relation between exist FLT3-ITD and OS was observed with a significance of (P = 0.000). The OS curve suggested a notable difference shorter OS in AML patients carrying the FLT3-ITD mutation comparing to those without it. In awe, the four AML patients with different duplications of FLT3-ITD had shorter survival rate, approximately one month after treatment. Moreover, when we studied the relapse rate in the patients who achieved CR and had FLT3-ITD mutation, we noticed that it was a little higher than patient with FLT3wt (relapse rate: 30% vs. 17.7%, P = .083). As well, Our study included the analog between EFS and OS for patients with FLT3-ITD mutation, EFS can be assessed much faster than OS. In addition, EFS is less affected by hematopoietic cell transplantation (HCT) or salvage therapy after relapse and takes into account the entire study population. When compared to individuals without FLT3-ITD mutations, it had been observed that patients with FLT3-ITD mutations exhibited shorter EFS rates, and a significant differences among the two groups had been notice. This findings supports previous research that showed EFS was one of the most critical factors in predicting FLT3-ITD mutations [33, 34]. According to Schnittger et al's study, the FLT3-ITD status alone is a reliable predictor of survival and event free survival in mutant AML. Therefore, the researchers assumed that FLT3-ITD cells confer a higher growth advantage compared to cells with WT-FLT3 or a heterozygous genotype. We can surmise EFS rates in patients with FLT3-ITD mutation consider as a crucial factors in assessing the prognostic status and treatment regime [35]. The biology of AMLs is very diverse and varies in different populations. Therefore, extensive mutation analysis is required to understand the pathogenesis of leukemia and the role that plays as a valuable prognostic marker in our patients. The FLT3-TKD mutations were appeared only in 2 patient, and it is not possible to know the role of this mutation in our AML patients.

In conclusion, we found that, the results confirmed the significant frequency of FLT3-ITD mutations in our adult AML-NK patients 24%, are consistent with published data. In addition, four patients had different duplications in FLT3-ITD and a new insertion. We demonstrated that the existence of FLT3-ITD mutation was substantially correlated with OS, relapse rate, and EFS. Finally FLT3-ITD mutation is important adverse prognostic factor, and it might be simply integrated into the standard evaluation of individuals with AML, and the variants of FLT3-ITD should be taken in consideration to know the prevalence of different types of those variants in our cohort and how could that affect the treatment, CR, Relapse and other aspects. To our knowledge, this is the first study to

| EFS | SE | Wald | Sig. | HR | 95.0% CI |
|-----|----|------|------|----|---------|
| .298 | 33.025 | .000 | 5.549 | 3.093 | 9.954 |

Figure 7. Cox regression analysis of cumulative (Cum) survival and cumulative Hazard curves which illustrate the effect of FLT3-ITD on event free survival of AML patients.
report the importance of FLT3 mutation screening and the correlation between the mutation and the context of AML in our cohort, in addition, our findings underscore the significance of thorough molecular genetic screening for the investigation of this heterogeneous patient group, which may ultimately lead to improved risk classification. However, our study has limitations due to its retrospective nature, small sample size, and absence of a controlled clinical trial setting.

Furthermore, the data offer an evaluation of the detrimental FLT3 mutations that predispose to AML in our area and their applicability in clinical settings.

5. Clinical practice point

Acute myeloid leukemia is one of the most widespread leukemia within adults, where ineffectual and dysfunctional erythropoiesis accompanied with BM failure are induced by clonal proliferation of young abnormal WBCs start to invade the BM and could reach the PB. Over through the past two decades, acute myeloid leukemia diagnosis, classification, and treatment plan have changed from being primarily based on histologic criteria to now being predominantly based on genetic, genomic, and molecular features. Cyto genetic analysis of AML has grown to be crucial for the diagnosis, categorization, prognostication, and treatment counseling of diseases. Patients with AML already routinely undergo molecular genetic testing for the genes DNMT3A, NPM1, and FLT3, and abnormalities in numerous other genes are becoming more significant. In addition to cytogenetic abnormalities, such as chromosomal deletions, duplications, or substitutions, the identification of specific molecular mutations that result in over or lower expression of one or more proteins also contributes to the understanding of the risk factor in AML. Scientific investigations on the genetics of AML have revealed developed tests according to the genetic and molecular vision which could assess and anticipate the drug of choice that may work best for the patient, therefore, estimating overall survival and prognosis.

Declarations

Author contribution statement

Yahia Moualla: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Faten Moassas: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Bassel AL Halabi, Michael Georgeos: Analyzed and interpreted the data.

Walid Al-achkar, Haissam Yazigi: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Haissam Yazigi: Conceived and designed the experiments; Analyzed and interpreted the data.

Atieh Khamis: Conceived and designed the experiments.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

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