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ORIGINAL ARTICLE

Characteristics and Outcome of FLT3-ITD-Positive Acute Myeloid Leukemia

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SUMMARY

Backgrounds: **AML patients with FLT3-ITD mutation experience a poor prognosis. Our study evaluated the clinical characteristics, remission, relapse, and clinical outcomes of these patients. We also assessed the effectiveness of allogeneic hematopoietic stem cell transplantation (allo-HSCT) and sorafenib in treating AML patients with FLT3-ITD mutation.**
Methods: **Fifty-five newly diagnosed AML patients with FLT3-ITD mutation in our center were retrospectively enrolled between January 2018 and June 2023. Multiple fusion genes and gene mutations were identified for the diagnosis of AML. Survival curves were calculated by employing the Kaplan-Meier method, and the differences between them were evaluated by using the log-rank (Mantel-Cox) test.**
Results: **Twenty-seven patients underwent allo-HSCT. The allo-HSCT group had a significantly extended follow-up period compared to the non-HSCT group ($p < 0.001$). Mutations in both NPM1 and FLT3-ITD were present in 18 out of the 55 patients (32.7%). Among them, eleven patients were given sorafenib plus chemotherapy induction therapy, and forty-four received mono-chemotherapy. The HSCT group had a higher overall survival (OS) rate than the non-HSCT group ($p < 0.001$), and a higher relapse-free survival (RFS) rate as well ($p = 0.0017$). No statistically significant difference in OS and RFS was observed when compared with sorafenib plus chemotherapy and mono-chemotherapy ($p > 0.05$). FLT3-ITD-positive patients with and without NPM1 mutation did not experience a significant difference in OS and RFS rates ($p > 0.05$).**
Conclusions: **Allo-HSCT immediately following complete remission could improve outcomes for young adults diagnosed with FLT3-ITD-positive AML. However, we found no statistical difference in the overall response rate (ORR) and clinical outcome between sorafenib combined with chemotherapy and chemotherapy alone.**
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INTRODUCTION

Acute myeloid leukemia (AML) is a diverse disease, both clinically and genetically, marked by the clonal expansion of abnormal hematopoietic progenitor cells [1]. Research has shown that FLT3, a critical receptor tyrosine kinase in cell signaling, can cause abnormal cell proliferation and contribute to tumor formation [2]. This process is particularly relevant to the onset and progres-

sion of acute myeloid leukemia (AML). FLT3 mutation is one of the most common genetic alterations detected in AML. FLT3 internal tandem duplication (ITD) mutations occur in approximately 25% of patients with newly diagnosed AML. AML patients with FLT3-ITD mutation experience shorter periods of remission and face a higher likelihood of relapse compared to those with FLT3 wild-type [3]. Despite these findings, there remains considerable heterogeneity among patients with FLT3-ITD-mutated AML. In addition to the inherent features of internal tandem duplication (ITD), including the quantity, size, insertion location, and allele ratio (AR), other genetic abnormalities alongside FLT3-ITD also impact patient prognosis. In 2017, the European Leukemia Network (ELN) recommended that patients with FLT3-ITD+ AML utilize the FLT3-ITD allele ratio (AR) and the NPM1 mutation status for risk stratification [4]. However, the prognosis of AML with FLT3-ITD in combination with other gene mutations or fusion genes is unknown.

Various FLT3 inhibitors, including sorafenib, have been widely used in patients with AML harboring the FLT3-ITD mutation in recent years. A previous study showed a 17% increase in early mortality in older patients, when sorafenib was combined with intensive chemotherapy [5]. For younger patients, event-free survival (EFS) was improved by shortening sorafenib treatment and adding daunorubicin [6]. Several clinical trial studies have shown that sorafenib maintenance therapy can improve the prognosis of AML patients with FLT3-ITD mutation undergoing allo-HSCT [7,8]. Although these findings are broadly positive, sorafenib combined with intensive chemotherapy as a first-line treatment for patients with FLT3-ITD AML before HSCT remains controversial [9]. To date, the benefit of sorafenib in the treatment of FLT3-ITD-positive AML is unclear.

We conducted a retrospective analysis of patients with newly diagnosed AML that had FLT3-ITD mutation, regardless of the presence of additional mutations or fusion genes. Our study evaluated the clinical characteristics, remission, relapse, and clinical outcomes of these patients. We also assessed the effectiveness of hematopoietic stem cell transplantation (HSCT) and sorafenib in treating AML patients with FLT3-ITD mutation.

MATERIALS AND METHODS

Patients

Fifty-five newly diagnosed AML patients with FLT3-ITD mutation in our center were retrospectively enrolled from January 2018 to June 2023. Patients with acute promyelocytic leukemia were excluded. The study was reviewed and approved by the hospital´s Ethics Committee, and written informed consent was obtained from all patients. All patients were diagnosed according to the WHO classification criteria, including morphology, immunology, cytogenetics, and molecular biology (MICM). The next-generation sequencing (NGS) method was used for gene detection, and the AR value of FLT3-ITD was calculated. The R-banding method analyzed the karyotype of bone marrow fluid and described it according to the International System for Human Cytogenetics Nomenclature (ISCN 2013). The responses to induction therapy, complete remission (CR), relapse, overall survival (OS), and relapse-free survival (RFS) were analyzed.

Molecular and cytogenetic analysis

AML was determined by using stained bone marrow aspiration/biopsies following ELN criteria [10]. The International System for Human Cytogenetic Nomenclature was used to describe the cytological results [11]. Multiple fusion genes and gene mutations were identified for the diagnosis of AML, including AML1-ETO, CBFB/MYH11, MLL/AF6, RNUX1, NRAS, TET2, FLT3-ITD, NPM1, CEBPA, C-kit, and DNMT3A. Multiplex nested RT-PCR detected the fusion gene. The nested RT-PCR detection method and reaction system were utilized according to the previous reports [12,13]. High-throughput sequencing technology was used to detect AML-related mutated genes.

Treatment regimens

All 55 patients received IA (daunorubicin + cytarabine), DA (daunorubicin + cytarabine), or decitabine + CAG (clarithromycin + cytarabine + G-CSF) 3+7 chemotherapy regimens. Out of the 55 patients with FLT3-ITD mutations, eleven received induction therapy with sorafenib combined with the "3+7" chemotherapy regimen.

Transplant protocols

Twenty-seven patients underwent allogeneic hematopoietic stem cell transplantation after complete remission with induction chemotherapy. Twenty-two cases of related haploidentical peripheral blood stem cell transplantation were performed. Two patients underwent unrelated, entirely identical peripheral blood stem cell transplantation. Two patients underwent unrelated haploidentical peripheral blood stem cell transplantation. Only one patient underwent homogeneous peripheral blood stem cell transplantation. The regimen of busulfan (Bu)/cyclophosphamide (Cy) combined with cytarabine was used for conditioning chemotherapy.

Evaluation

CR is defined as < 5% of blasts in BM. Hematologic recovery is measured by absolute neutrophil (> 1 x 10^9/L) and platelet (> 100 x 10^9/L) counts in peripheral blood. Clinical recurrence after CR is defined by the presence of ≥ 5% blasts in the bone marrow, the reappearance of leukemic blasts in the peripheral blood, or the development of extramedullary disease. OS is the time from initial diagnosis to death, last follow-up, or loss to follow-up. The RFS period is from initial diagnosis to relapse, death, or last follow-up. Overall survival was our primary outcome, and RFS was our secondary outcome.

Table 1. The characteristics of the study population.

Variables	Total (n = 55)	Non-HCST (n = 28)	HCST (n = 27)	p-value
Gender, n (%)				0.793
Female	28 (51.9)	15 (53.6)	13 (50)	
Male	26 (48.1)	13 (46.4)	13 (50)	
Age, n (%)				0.022
< 60	44 (80.0)	19 (67.9)	25 (92.6)	
≥ 60	11 (20.0)	9 (32.1)	2 (7.4)	
WBC, mean ± SD	61.7 ± 93.2	64.1 ± 107.5	59.1 ± 76.9	0.846
HB, mean ± SD	87.2 ± 21.7	88.2 ± 20.4	86.1 ± 23.4	0.731
PLT, mean ± SD	61.7 ± 50.8	56.4 ± 56.9	67.5 ± 43.7	0.424
LDH, mean ± SD	578.6 ± 652.1	607.1 ± 664.2	548.9 ± 651.1	0.748
ALB, mean ± SD	1,330.0 ± 9,378.7	2,570.4 ± 13,140.2	42.0 ± 4.7	0.331
Myeloblast, mean ± SD	68.3 ± 22.3	70.4 ± 22.7	66.2 ± 22.2	0.492
FAB types, n (%)				0.544
M1	7 (12.7)	5 (17.9)	2 (7.4)	
M2	22 (40.0)	9 (32.1)	13 (48.1)	
M4	2 (3.6)	1 (3.6)	1 (3.7)	
M5	24 (43.6)	13 (46.4)	11 (40.7)	
Cytogenetics, no. (%)				0.198
Normal karyotype	41 (74.5)	19 (67.9)	22 (81.5)	
Abnormal karyotype	13 (23.6)	9 (32.1)	4 (14.8)	
NA	1 (1.8)	0 (0)	1 (3.7)	
FLT3 mutations n (%)				0.485
Low ratio	41 (74.5)	22 (78.6)	19 (70.4)	
High ratio	14 (25.5)	6 (21.4)	8 (29.6)	
Follow-up time	32.0 (15.5, 47.5)	16.0 (11.8, 25.0)	45.0 (32.0, 51.5)	< 0.001

Statistical analysis

The chi-squared test was used to compare categorical variables, and the Wilcoxon rank-sum test was used to determine the median difference for continuous variables. The Kaplan-Meier method was used to assess patient survival patterns, and the log-rank (Mantel-Cox) test was used to determine the differences. SPSS 24.0 was utilized for the statistical analysis. A statistically significant difference is defined as $p < 0.05$.

RESULTS

Population characteristics

The study included 55 AML patients who were found to have an FLT3-ITD mutation. There were 44 patients (80%) who were 60 years old or older, with a median age of 49 (18 - 69 years). Twenty-seven patients underwent allo-HSCT. The summary of the patient characteristics grouped by HSCT can be found in Table 1. The proportion of the HSCT group under 60 years old was higher than that in the non-HSCT group ($p = 0.022$). The HSCT group had a significantly extended follow-up period than the non-HSCT group ($p < 0.001$). There was no significant difference in the remaining clinical features, as shown in Table 1.

Fusion genes and gene mutations

Table 2 summarizes the statuses of fusion genes and gene mutations. Mutations in both NPM1 and FLT3-ITD were present in 18 out of the 55 patients (32.7%). The FLT3-ITD mutation and other gene mutations, in addition to NPM1, were observed as follows: DNMT3A (8/55, 14.5%), CEBPA (7/55, 12.7%), CBFB/MYH11 (6/55, 10.9%), IDH1 (6/55, 10.9%), KMT2A (5/55, 9.1%), NRAS (5/55. 9.1%), AML/ETO (4/55, 7.3%), IDH2 (4/55, 7.3%), RUNX1 (3/55, 5.5%), C-Kit (3/55, 5.5%), TET2 (3/55, 5.5%), TP53 (3/55, 5.5%), JAK2 (2/55, 3.6%), U2AF1 (2/55, 3.6%), MLL/ELL (1/55, 1.8%), and GATA2 (1/55, 1.9%) mutations. There was no statistically significant difference in the distribution of concomitant fusion genes and gene mutations be

Table 2. Distribution of concomitant gene mutations in FLT3-ITD-positive AML patients in the allo-HSCT group and non-HSCT group.

Variables	Total (n = 55)	Non-HCST (n = 28)	HCST (n = 27)	p-value
NPM1, n (%)	18 (32.7)	8 (28.6)	10 (37)	0.504
DNMT3A, n (%)	8 (14.5)	3 (10.7)	5 (18.5)	0.469
CEBPA, n (%)	7 (12.7)	4 (14.3)	3 (11.1)	1
IDH1, n (%)	6 (10.9)	4 (14.3)	2 (7.4)	0.669
CBFB/MYH11, n (%)	6 (10.9)	2 (7.1)	4 (14.8)	0.422
KMT2A, n (%)	5 (9.1)	3 (10.7)	2 (7.4)	1
AML/ETO, n (%)	4 (7.3)	4 (14.3)	0 (0)	0.111
IDH2, n (%)	4 (7.3)	2 (7.1)	2 (7.4)	1
NRAS, n (%)	5 (9.1)	1 (3.6)	4 (14.8)	0.193
RUNX1, n (%)	3 (5.5)	2 (7.1)	1 (3.7)	1
C-Kit, n (%)	3 (5.5)	3 (10.7)	0 (0)	0.236
TET2, n (%)	3 (5.5)	0 (0)	3 (11.1)	0.111
JAK2, n (%)	2 (3.6)	0 (0)	2 (7.4)	0.236
U2AF1, n (%)	2 (3.6)	1 (3.6)	1 (3.7)	1
TP53, n (%)	3 (5.5)	3 (10.7)	0 (0)	0.236
GATA2, n (%)	1 (1.9)	0 (0)	1 (3.8)	0.481
MLL/ELL, n (%)	1 (1.8)	0 (0)	1 (3.7)	0.491
ASXL2, n (%)	1 (1.8)	1 (3.6)	0 (0)	1

Table 3. Treatment response between the allo-HSCT group and non-HSCT group in FLT3-ITD-positive patients with AML.

Variables	Total (n = 55)	Non-HSCT (n = 28)	HSCT (n = 27)	p-value
Therapy regimens				0.08
Mono-chemotherapy, no. (%)	44 (80.0)	25 (89.3)	19 (70.4)	
Sorafenib + chemotherapy, no. (%)	11 (20.0)	3 (10.7)	8 (29.6)	
Treatment response				0.471
NR, no. (%)	5 (9.1)	4 (14.3)	1 (3.7)	
PR, no. (%)	16 (29.1)	8 (28.6)	8 (29.6)	
CR, no. (%)	34 (61.8)	16 (57.1)	18 (66.7)	
ORR, no. (%)	50(90.9)	24(85.7)	26(96.3)	
Relapse, no. (%)	25 (45.5)	20 (71.4)	5 (18.5)	< 0.001

NR - no response, PR - partial remission, CR - complete remission, ORR - overall response rate, FLT3-ITD - FMS-like tyrosine kinase 3-internal tandem duplication mutation.

tween the HSCT and non-HSCT groups ($p > 0.05$).

Treatment response

All patients were given the '3+7' induction chemotherapy regimen. No significant difference was observed in the frequency of CR between the non-HSCT and HSCT groups (57.1% vs. 66.7%; $p = 0.47$, as shown in Table 3). The overall response rate (ORR) for the HSCT group was 85.7%, while the HSCT group had an ORR of 96.3%. The non-HSCT group had a significantly higher recurrence rate than the HSCT group (71.4% vs. 18.5%; $p < 0.001$). Out of the 55 individuals with FLT3-ITD mutations, 11 were given sorafenib and chemotherapy induction therapy, and 44 received monochemotherapy. Eight patients were able to receive consolidation therapy with sorafenib after being given

Table 4. Treatment response between the mono-chemotherapy and sorafenib plus chemotherapy group in FLT3-ITD-positive patients with AML.

Treatment response (%)	Total (n = 55)	Mono-chemotherapy (n = 44)	Sorafenib plus chemotherapy (n = 11)	p-value
NR, no. (%)	5 (9.1)	5 (11.4)	0 (0)	0.571
PR, no. (%)	16 (29.1)	13 (29.5)	3 (27.3)	1
CR, no. (%)	34 (61.8)	26 (59.1)	8 (72.7)	0.502
ORR, no. (%)	50 (90.9)	39(88.6)	11(100)	0.571
Relapse, no. (%)	25 (45.5)	22 (50)	3 (27.3)	0.176

NR - no response, PR - partial remission, CR - complete remission, OR - overall response.

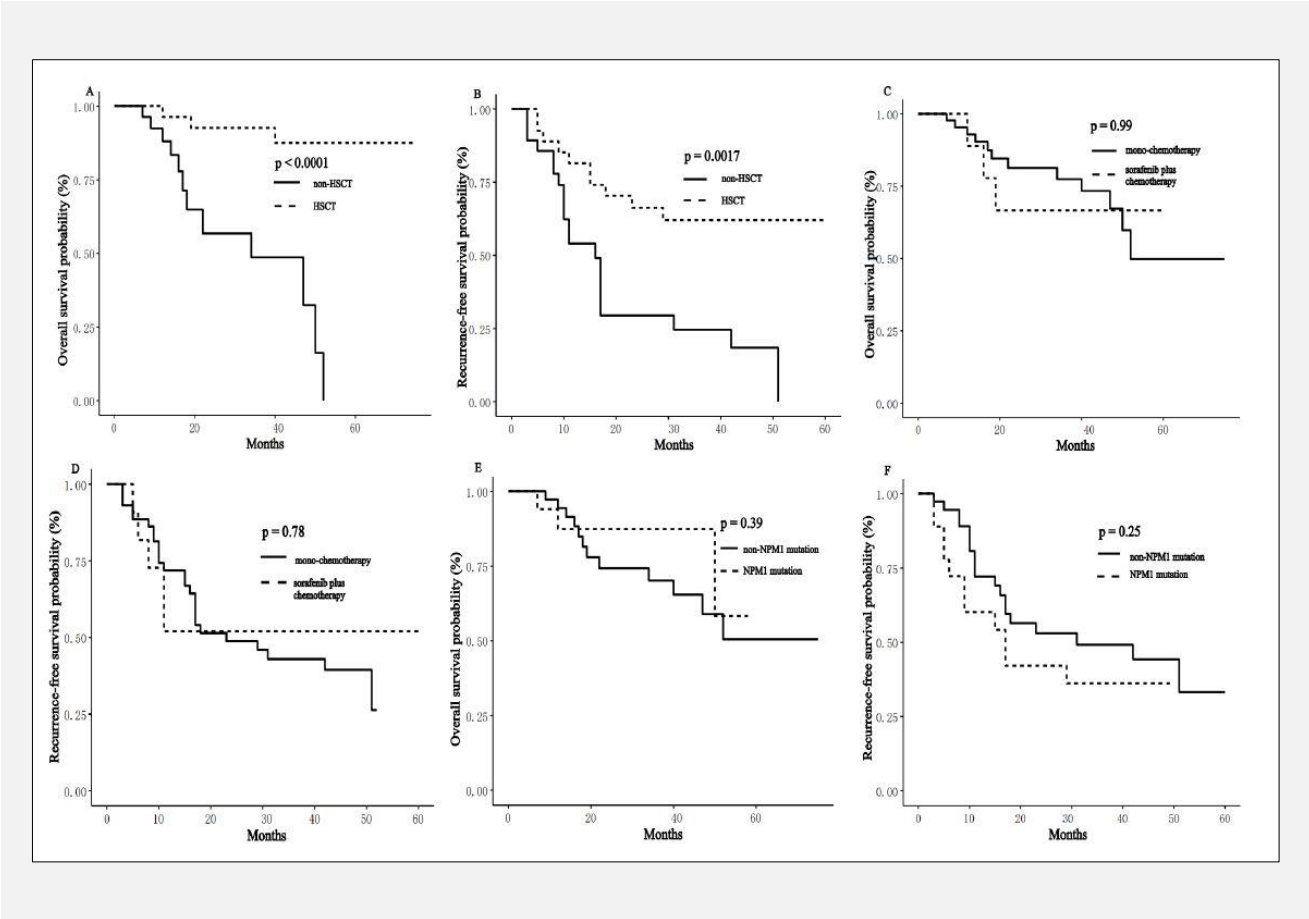

Figure 1. Comparison of OS and RFS in patients with FLT3-ITD-positive AML.

(A) OS and (B) RFS in allo-HSCT and non-HSCT groups. (C) OS and (D) RFS in the mono-chemotherapy and sorafenib plus chemotherapy groups. (E) OS and (F) RFS in NPM1 mutation and non-NPM1 mutation groups.

HSCT. Three of the 28 patients without allo-HSCT were treated with chemotherapy and sorafenib. There was no significant difference in CR and recurrence rates in patients with FLT-ITD mutations between sorafenib plus chemotherapy and chemotherapy alone (Table 4).

Survival analysis

Our study had a median follow-up period of 32 months, ranging from 15.5 to 47.5 months. By the end of the follow-up period, 40 patients had survived, while 15 had, unfortunately, passed away. One patient relapsed five

months after receiving haploidentical HSCT and died 20 months later. The HSCT group had a higher OS rate than the non-HSCT group ($p < 0.001$) and a higher RFS rate as well ($p = 0.0017$). No statistically significant difference in OS and RFS was observed, when combined with sorafenib and mono-chemotherapy ($p > 0.05$). FLT3-ITD-positive patients with and without NPM1 mutation did not experience a significant difference in OS and RFS rates ($p > 0.05$). The survival curve is shown in Figure 1.

DISCUSSION

AML is the most common type of acute leukemia in China. The incidence of AML increases with age. AML patients commonly have gene mutations such as FLT3, NPM1, and TP53. The FLT3 mutation in AML is harmful to recurrence and overall survival. Most patients diagnosed with FLT3-ITD in the present study had no fusion genes, as revealed by the analysis of concomitant genetic abnormalities. In the current study, FLT3-ITD-positive AML, the co-expression of NPM1, is the most frequently mutated gene with 32.7% mutations, followed by DNMT3A (14.5%), CEBPA (12.7%), IDH1 (10.9%), CBFB/MYH11 (10.9%), KMT2A (9.1%), NRAS (9.1%), AML/ETO (7.3%), IDH2 (7.3%), RUNX1 (5.5%), TP53 (5.5%), and ASXL1 (1.8%), which is consistent with a previous study. ASXL1, RUNX1, and TP53 mutations are negative factors, while NPM1, CEBPA, and CBFB-MYH11 mutations have been linked to a favorable prognosis [14].

This study confirms the benefits of allo-HSCT for eligible patients with AML and FLT3 mutations. The 3-year DFS rate following allogeneic HSCT was 60%, which is consistent with previous European Organization for Blood and Marrow Transplantation (EBMT) data [15]. The majority of the eligible patients underwent allo-HSCT soon after achieving CR with induction chemotherapy. Out of the 55 patients included in the study, 25 experienced relapses. Among those, five relapsed after allo-HSCT, which indicates the aggressive proliferation of FLT3-mutated AML cells. The constitutive activation of FLT3 is caused by FLT3-ITD mutations, which leads to aggressive cell proliferation.

We gained an additional understanding of the clinical and genetic prognostic factors in FLT3-ITD-associated AML. The impact of ITD-AR on prognosis was not considered until the inclusion of high FLT3-ITD in the unfavorable risk group by the ELN 2017 guideline. Moreover, there have been conflicting reports regarding the effect of ITD-AR on the prognosis [16-18]. In our study, no statistically significant difference was found between the frequency of ITD mutation and the patient's OS or RFS. Our analyses revealed frequent mutations in the NPM1 and DNMT3A genes, which is consistent with previous comprehensive genetics studies [19,20]. However, the outcomes of our cohort receiving treatment were not affected by either a high FLT3-ITD AR or NPM1 mutation status, as demonstrated by recent retrospective studies [18,21]. Overlapped mutations or adverse karyotypes may impact these results due to the coexisting risk factors. In our study of 55 FLT3-ITD positive patients, 18 (32.7%) had NPM1 mutations, three had TP53 mutations, and two had complex cytotypes, despite having low levels of FLT3-ITD mutation.

Many clinical trials have shown that FLT3 inhibitors may improve the prognosis of patients with FLT3-ITD AML [22,23]. Allo-HSCT combined with FLT3 inhibitors has become the standard of treatment [24]. Clinical trials are being conducted to explore second-generation FLT3 inhibitions (including gilteritinib, crenolanib, and quizartinib), which are currently being investigated to improve the prognosis of patients with FLT3-mutated AML [25-27]. However, it has been shown that using first-generation FLT3 inhibitors resulted in a short-term reduction of blast cells but rarely improved CR in patients with FLT3 mutations [28]. In the current study, 11 patients who were given both sorafenib and chemotherapy had a slightly higher overall response rate (ORR) than those who received only chemotherapy (100% vs. 88.6%). However, no statistically significant difference was observed in OS and RFS between the groups receiving mono-chemotherapy and those treated with sorafenib in combination with chemotherapy. Those results may have been influenced by the small number of patients treated with sorafenib, so future studies should expand the number of cases to confirm the conclusions.

There are still limitations in the present study. The findings of this observational study are based on a small number of patients from a single center. Additionally, the limited number of patients in the subgroups makes it challenging to conduct statistical analysis. Considering the low occurrence of AML with FLT3 mutation, our retrospective study of 55 FLT3-ITD-positive AML cases is significant. It is especially valuable as it covers the use of the FLT3 inhibitor sorafenib as well as HSCT treatment.

In conclusion, our retrospective study suggests that scheduling allo-HSCT immediately following complete remission could improve outcomes for young adults diagnosed with FLT3-ITD-positive AML. It may be advisable to consider maintenance therapy using FLT3 inhibitors following an allo-HSCT. However, we found no statistical difference in ORR and clinical outcome between sorafenib combined with chemotherapy and chemotherapy alone. So, future prospective studies with a large sample size are needed to investigate the efficacy of sorafenib further.

Declaration of Interest:
The authors assert that they have no conflicts of interest.

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