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Allogeneic hematopoietic stem cell transplantation at the first remission for younger adults with *FLT3*-internal tandem duplication AML: The JALSG AML209-FLT3-SCT study

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Abstract

In this phase II multicenter study (JALSG AML209-FLT3-SCT), we aimed to prospectively elucidate the role of allogeneic hematopoietic stem cell transplantation (allo-HSCT) at first complete remission (CR1) for *FLT3*-internal tandem duplication (ITD)-positive AML. Newly diagnosed de novo AML patients with *FLT3*-ITD were enrolled at the achievement of CR1 and received allo-HSCT as soon as possible after the first consolidation therapy. Mutations of 57 genes in AML cells at diagnosis were

also analyzed. Among 48 eligible patients with a median age of 38.5 (17-49) years, 36 (75%) received allo-HSCT at a median of 108 days after CR1. The median follow-up was 1726 days. The primary end-point, 3-year disease-free survival (DFS) based on an intent to treat analysis, was 43.8% (95% confidence interval [CI], 30%-57%), suggesting the efficacy of this treatment because the lower limit of the 95% CI exceeded the threshold response rate of 20%. The 3-year overall survival, post-transplant DFS, and non-relapse mortality rates were 54.2% (95% CI, 39%-67%), 58.3% (95% CI, 41%-72%), and 25.0% (95% CI, 12%-40%), respectively. The median ITD allelic ratio (AR) was 0.344 (0.006-4.099). Neither *FLT3*-ITD AR nor cooccurring genetic alterations was associated with a poor DFS. This prospective study indicated the efficacy and safety of allo-HSCT for *FLT3*-ITD AML patients in CR1. This study was registered at: www.umin.ac.jp/ctr/ as #UMIN000003433.

KEYWORDS

acute myeloid leukemia, allogeneic hematopoietic stem cell transplantation, FLT3-ITD

1 | INTRODUCTION

Internal tandem duplication mutations of FMS-like kinase 3 (*FLT3*-ITD) are frequently identified in 20%-30% of adult AML patients.[1-3] Mutations of the *FLT3* gene result in constitutive activation of FLT3 kinase and its downstream signaling pathway, causing ligand-independent cell proliferation and resistance to apoptosis, which leads to the development and progression of AML.[4-7] *FLT3*-ITD is widely accepted as an independent adverse prognostic factor associated with high relapse and poor survival rates.[8]

A number of studies reported that cytotoxic chemotherapy alone achieves stable remission in only 20% of patients with *FLT3*-ITD-positive AML, and that allogeneic hematopoietic stem cell transplantation (allo-HSCT) could improve the outcomes.[9-11] Therefore, allo-HSCT is generally accepted as the standard of care when feasible for AML patients with *FLT3*-ITD. However, *FLT3*-ITD mutations are heterogeneous with respect to the allelic ratio (AR) and/or length of the insertion, and the poor prognosis is largely dependent on ITD AR.[12] Guidelines of the National Comprehensive Cancer Network (NCCN) and European LeukemiaNet (ELN) have recommended risk stratification systems according to the AR of *FLT3*-ITD and mutational status of the *NPM1* gene.[13,14] However, more recently, controversy has arisen regarding the indication of allo-HSCT according to ITD AR or cooccurring genetic abnormalities. Previous studies suggested that patients with *NPM1* mutation and *FLT3*-ITD with a low (less than 0.5) AR (*FLT3*-ITD^{low}) have as favorable outcomes as those with *NPM1* mutation, but not *FLT3*-ITD.[15-19] However, an unfavorable prognosis was reported to be associated with *NPM1*-mutated AML with *FLT3*-ITD^{low} when allo-HSCT was not carried out in the first complete remission (CR1).[20] It remains to be prospectively elucidated which patients with AML with *FLT3*-ITD benefit from allo-HSCT. In addition, as FLT3 kinase inhibitors have been recently approved, further insight into the genetic background of AML with *FLT3*-ITD and prognostic factors after allo-HSCT for constructing risk-stratified treatment strategies in the era of FLT3 inhibitors will be valuable.

As there are few data on the usefulness of allo-HSCT in prospective settings, we undertook a multicenter prospective study to clarify the role of allo-HSCT at CR1 in younger adult AML patients with *FLT3*-ITD.

2 | MATERIALS AND METHODS

2.1 | Patients

Patients aged 16-49 years with newly diagnosed de novo AML according to the WHO 2008 classification and an ECOG performance status of 2 or lower were eligible for enrollment if they had a *FLT3*-ITD mutation, achieved CR within 2 courses of standard induction therapies, and had not received further postremission therapies. Although the regimen for induction therapy was not predetermined, combination therapy consisting of cytarabine (Ara-C) and either daunorubicin (DNR) or idarubicin (IDR) was recommended (Table S1). FLT3 inhibitors were not given during either induction or postremission therapies because they were not available at the time of enrollment of this study. Regimens including gemtuzumab ozogamicin were excluded. Other inclusion criteria included sufficient organ functions: serum alanine aminotransferase or serum aspartate aminotransferase up to 2.5-times the institutional upper limit of normal (ULN); serum bilirubin up to 2.0 mg/dL; serum creatinine up to 1.5-times the institutional ULN; left ventricular ejection fraction greater than 50% on echocardiography; and PaO2 greater than 60 Torr or SpO2 > 90% in room air. We excluded patients with a *RUNX1-RUNX1T1*, *CBFB-MYH11*, or *PML-RARA* chimeric transcript, secondary AML, a history of hematological abnormalities, other

types of malignant tumors, cardiac dysfunction, digestive tract ulcer of higher than A2 stage, ileus, uncontrolled diabetes mellitus, active uncontrolled infections, hepatitis B reactivation, uncontrolled psychiatric disorders, or pregnancy. Written informed consent was received from all patients. The protocol was approved by the ethics committees of all participating institutions. This study was registered in the UMIN Clinical Trials Registry (UMIN000003433, http://www.umin.ac.jp/ctr/).

2.2 | Postremission chemotherapy

Patients received allo-HSCT as soon as possible after the first consolidation therapy. Multiagent consolidation therapy was continued for up to 4 courses until allo-HSCT, as previously described.[21,22] They consisted of mitoxantrone (7 mg/m^2 for 3 days) and Ara-C (200 mg/m^2 by 24-hour continuous infusion for 5 days) in the first course, DNR (50 mg/m^2 for 3 days) and Ara-C (200 mg/m^2 by 24-hour continuous infusion for 5 days) in the second course, aclarubicin (ACR, 20 mg/m^2 for 5 days) and Ara-C (200 mg/m^2 by 24-hour continuous infusion for 5 days) in the third course, and Ara-C (200 mg/m^2 by 24-hour continuous infusion for 5 days), etoposide (VP, 100 mg/m^2 for 5 days), vincristine (0.8 mg/m^2 on day 8), and vindesine (2 mg/m^2 on day 10) in the fourth course. Each consolidation was started as soon as possible after neutrophils, white blood cells, and platelets recovered to greater than 1.5 × 10^9/L, 3.0 × 10^9/L, and 100.0 × 10^9/L, respectively. Intrathecal injection of Ara-C (40 mg/body), methotrexate (MTX, 15 mg/body), and prednisolone (10 mg/body) was carried out after the first course of consolidation therapy and platelets recovered to greater than 100 × 10^9/L. When a nonhematological toxicity more severe than grade 3 was observed according to NCI-Common Terminology Criteria for Adverse Events version 3.0 and it took longer than 60 days to recover, or it took longer than 60 days after the start of consolidation for neutrophils, white blood cells, and platelets to recover to more than 1.5 × 10^9/L, 3.0 × 10^9/L, and 100.0 × 10^9/L, respectively, the dosages of DNR, ACR, and VP were reduced to 40 mg/m^2, 16 mg/m^2, and 80 mg/m^2, respectively. Patients received no further chemotherapy after completing 4 courses of consolidation therapy.

2.3 | Hematopoietic stem cell transplantation

Allogeneic HSCT was undertaken for patients younger than 50 years maintaining CR1 at the time of transplantation. In addition to the inclusion criteria of this study, sufficient pulmonary function with higher than 66% predicted Forced Expiratory Volume 1.0 was required before allo-HSCT. Allogeneic HSCT was carried out as soon as possible within 180 days after registration when suitable donors were available. Conditioning was started 28-60 days after the final administration of consolidation chemotherapy. Recommended donors/stem cell sources included human leukocyte antigen (HLA)-A/B/DR-matched sibling bone marrow (Rel-BM) or peripheral blood stem cells (PB), HLA-A/B/C/DRB1 allele 8/8-matched unrelated BM (UR-BM), HLA-DRB1 allele 1 locus-mismatched UR-BM, HLA-C allele 1 locus-mismatched UR-BM, a single cord blood (CB) unit serologically matched in more than 4/6 HLA-A/B/DR loci with more than 2 × 10^7 total nucleated cell (TNC) count/body weight (kg), HLA-C serologically 1 locus and HLA-DRB1 allele 1 locus-mismatched UR-BM, or HLA serologically 1 locus-mismatched Rel-BM or PB. The conditioning regimen was selected according to each institutional standard from myeloablative regimens such as total body irradiation (TBI)/cyclophosphamide (Cy). Reduced-intensity conditioning was permitted only for patients older than 40 years or with a hematopoietic cell transplantation-specific comorbidity index higher than 1 point. Prophylaxis for graft versus host disease (GvHD) was performed according to each institutional standard based on cyclosporine A or tacrolimus combined with short-term MTX. Treatment after relapse was not determined. All transplantation data for the current study were obtained from the Transplant Registry Unified Management Program (TRUMP) database.[23]

2.4 | Cytogenetic and molecular analyses

Cytogenetic G-banding analysis was undertaken using AML cell samples at diagnosis by standard methods. *FLT3*-ITD mutation and 11 chimeric gene transcripts (major *BCR-ABL1*, minor *BCR-ABL1*, *PML-RARA*, *RUNX1-RUNX1T1*, *CBFB-MYH11*, *DEK-NUP214*, *NUP98-HOXA9*, *MLLT1-KMT2A*, *MLLT2-KMT2A*, *MLLT3-KMT2A*, and *MLLT4-KMT2A*) were centrally examined using BM or PB samples at diagnosis, and the results were immediately reported to each institute. The ITD AR was calculated as the ratio of the height of *FLT3*-ITD divided by that of the WT using DNA fragment analysis as previously described.[1] Residual DNA and RNA samples were preserved at the Japan Adult Leukemia Study Group (JALSG) sample storage center. In addition, target sequencing of 57 genes, which are frequently identified in myeloid malignancies, was undertaken using TruSight Myeloid Sequencing Panel or TruSeq Custom Amplicon Kit version 1.5 (Illumina) as previously reported.[24,25]

2.5 | Definitions and study end-points

Relapse after CR was defined as the presence of at least 1 of the following: reappearance of leukemic blasts in the PB, recurrence of more than 5% blasts in BM not attributable to another cause, such as BM regeneration after chemotherapies, or the development of extramedullary leukemia. The overall survival (OS) was defined as the time from registration to death due to any cause or the last follow-up. The disease-free survival (DFS) was defined as the time from the date of CR1 to relapse or death by any cause or the last follow-up. The primary end-point was the 3-year DFS. The secondary end-points included the 3-year OS, 3-year DFS post-allo-HSCT, outcomes according to karyotypes, genetic alterations and stem cell sources, time from CR1 to allo-HSCT, cumulative incidence of

relapse, transplantation-associated complications, and nonrelapse mortality (NRM).

2.6 | Statistical analysis

Protocol compliance was reviewed in the independent data monitoring committee.

With a sample size of 47 patients, the study had a power of more than 90% at a 5% level of significance to show significance by a binomial distribution calculation with the expected and threshold 3-year DFS of 40% and 20%, respectively. The threshold value was estimated according to previous reports of patients with *FLT3*-ITD-positive AML who were younger than 50 years of age and did not receive allo-HSCT at CR1.[26-28] The treatment was considered effective if the lower limit of the 3-year DFS exceeded the threshold response rate of 20%. The probabilities of DFS and OS were estimated using the Kaplan-Meier method, and the log-rank test was used for univariate comparisons. The survival time post-allo-HSCT was calculated from the date of allo-HSCT to the date of death or last contact. The prognostic significance of the clinical variables was assessed using the Cox proportional hazards model. The cumulative incidence of NRM was estimated, treating relapse as a competing risk. The Fine and Gray proportional hazard models were used for the incidence of NRM. Differences in continuous variables were analyzed by the Mann-Whitney *U* test for distribution between 2 groups. Analysis of frequencies was carried out using Fisher's exact test for 2 × 2 tables or Pearson's χ^2 test for larger tables. Two-sided *P* values less than .05 were considered significant. All statistical analyses were carried out using Stata version 12 and EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan).[29]

3 | RESULTS

3.1 | Patient and transplant characteristics

Between August 2010 and March 2015, 49 patients from 39 institutes were enrolled in this prospective study. One was excluded because of registration after the start of postremission chemotherapy. In total, 48 patients were included in the primary end-point analysis (Figure 1). Among the 48 patients, 36 (75%) successfully received allo-HSCT in CR1, whereas 11 (22.9%) relapsed during donor search and consolidation chemotherapies, and 1 withdrew consent for allo-HSCT in CR1. Nineteen of 36 patients (53%) fulfilled the criteria for allo-HSCT, and the other 17 patients (47%) had protocol deviations, including the delay in allo-HSCT later than 180 days after registration (n = 11), HLA disparity (n = 5), insufficient organ functions at transplantation (n = 3: liver, 1; pulmonary, 1; cardiac, 1), reduction of the consolidation regimen (n = 1), active infection at transplantation (n = 1), and insufficient TNC count of infused CB unit (n = 1). The data monitoring committee did not consider them as violations but as deviations and suggested that they were not excluded from the final

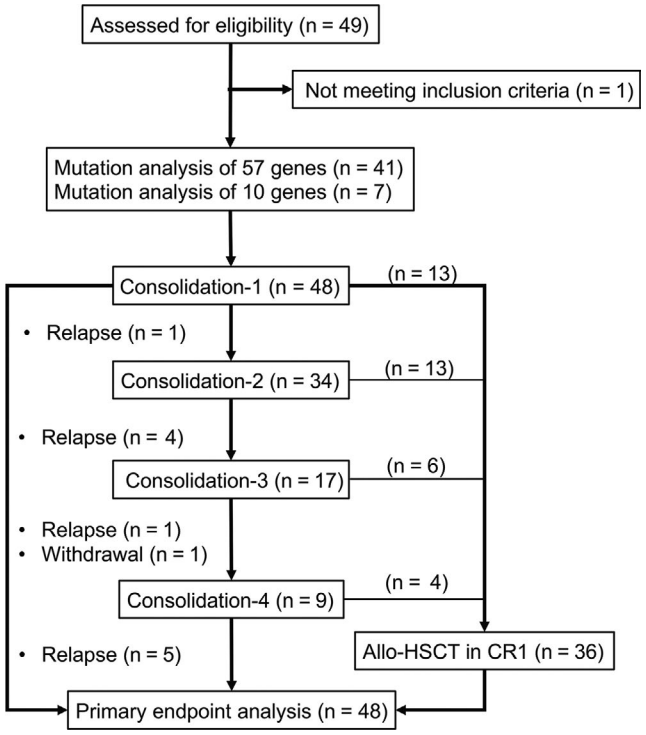


FIGURE 1 CONSORT diagram. The primary end-point, disease-free survival, was evaluated in 48 eligible AML patients with *FLT3*-internal tandem duplication. Allo-HSCT, allogeneic hematopoietic stem cell transplantation; CR1, first complete remission

analyses for the following reason: the aim of this study was to evaluate whether the clinical decision to undergo allo-HSCT as soon as possible led to improved prognosis in AML patients with *FLT3*-ITD. Therefore, this protocol was designed to undertake allo-HSCT immediately when both the donor and the patient were feasible. Each deviation was reviewed and accepted as routinely performed practice by the data monitoring committee. Thus, they were suggested to be included in the analyses.

The patient characteristics are summarized in Table 1. The median age was 38.5 years (range, 17-49 years). The median white blood cell (WBC) count at diagnosis was 61.2 (range, 1.7-316.2) × 10^9/L with 77% (range, 4%-99%) circulating blasts. An extramedullary tumor was observed in 4 patients (8.3%) at diagnosis, including the skin (n = 2), lymph nodes (n = 2), and gingiva (n = 1). According to the refined Medical Research Council (MRC) cytogenetic risk category, 47 patients were classified as intermediate risk; 34 normal karyotype, 3 with *t*(7;11)(p15;p15), and one patient with *t*(6;9)(p23;q34) as adverse risk.[30]

The demographics of 36 patients who received allo-HSCT in CR1 and 19 patients without protocol deviations are presented in Table 2. The median number of days from achieving CR1 to allo-HSCT was 108 (range, 54-228) days for all patients. The median number of days from registration to allo-HSCT was 104 (range, 50-223) days for all patients. Twenty-six of 36 patients (72%) received allo-HSCT within 2 courses of consolidation therapy. When limited to 19 patients without protocol deviations, the median time from CR1 to allo-HSCT was as short as 80 (range, 54-154) days and it was

TABLE 1 Characteristics of 48 patients with *FLT3*-internal tandem duplication AML

Characteristic	
Gender (M/F)	27/21
Age (y), median (range)	38.5 (17-49)
WBC ($\times 10^9$/L), median (range)	61.2 (1.7-316.2)
PB blasts (%), median (range)	77.0 (4-99)
BM blasts (%), median (range)	79.1 (15-98)
FLT3-ITD allelic ratio, median (range)	0.344 (0.006-4.099)
Extramedullary tumor, n (%)	4 (8.3)
FAB classification, n (%)	
M0	2 (4.2)
M1	15 (31.3)
M2	12 (25.0)
M4	12 (25.0)
M5a	2 (4.2)
M5b	5 (10.4)
Chromosomal abnormality	
Intermediate, n (%)	47 (97.9)
Normal	34 (70.8)
t(7;11)(p15;p15)	3 (6.3)
+21	2 (4.2)
+8	1 (2.1)
t(9;11)(p21.3;q23.3)	1 (2.1)
-13/del(13q)	1 (2.1)
Other structural	1 (2.1)
Other numerical	2 (4.2)
Other structural and numerical	2 (4.2)
Adverse, n (%)	1 (2.1)
t(6;9)(p23;q34.1)	1 (2.1)
Induction therapy	
DNR base/IDR base	18/30
1 course/2 courses	39/9

Abbreviations: BM, bone marrow; DNR, daunorubicin; F, female; FAB, French-American-British; IDR, idarubicin; ITD, internal tandem duplication; M, male; PB, peripheral blood; WBC, white blood cells.

within 2 courses of consolidation therapy in 17 of 19 patients (90%). Stem cell sources included those from related donors in 58% and CB in 37%, reflecting their availability for immediate transplantation after achieving CR.

3.2 | Mutational landscape of *FLT3*-ITD AML

Genetic alterations detected in the 48 patients at diagnosis are shown in Figure 2 and Table S2. Mutation in *NPM1* was the most frequent, found in 31 of the 48 patients (65%). Mutations in genes associated with DNA methylation were frequently identified, including

TABLE 2 Patient and transplantation characteristics of allogeneic hematopoietic stem cell transplantation recipients

	Total		Patients without protocol deviations	
Number	36		19	
Gender (M/F)	20/16		11/8	
Age (y), median (range)	38.5	(18-50)	42	(26-48)
Days from diagnosis to SCT, median (range)	146.5	(76-264)	134	(76-188)
Days from CR1 to SCT, median (range)	108	(54-228)	80	(54-154)
Days from registration to SCT, median (range)	104	(50-223)	85	(50-153)
Postremission therapy, n (%)				
1 course	13	(36.1)	8	(42.1)
2 courses	13	(36.1)	9	(47.4)
3 courses	6	(16.7)	1	(5.3)
4 courses	4	(11.1)	1	(5.3)
Stem cell source, n (%)				
Rel-BM	3	(8.3)	3	(15.8)
Rel-PB	12	(33.3)	8	(42.1)
UR-BM	11	(30.6)	1	(5.3)
CB	10	(27.8)	7	(36.8)
HCT-CI 0/1-2/≥3	20/11/5		10/7/2	
Conditioning, n (%)				
CY + TBI	18	(50)	6	(31.6)
BU + CY	7	(19.4)	6	(31.6)
CA + CY + TBI	7	(19.4)	5	(26.3)
VP + CY + TBI	1	(2.8)	0	(0.0)
Flu + BU	2	(5.6)	1	(5.3)
Flu + TBI	1	(2.8)	1	(5.3)
GvHD prophylaxis, n (%)				
Tac + sMTX	16	(44.4)	4	(21.1)
CsA + sMTX	15	(41.7)	12	(63.2)
CsA + MMF	1	(2.8)	1	(5.3)
CsA	1	(2.8)	0	(0.0)
Tac	1	(2.8)	1	(5.3)
Tac + MMF	1	(2.8)	1	(5.3)
Tac + MTX + PTCY	1	(2.8)	0	(0.0)

Abbreviations: BU, busulfan; CA, cytarabine; CB, cord blood; CR1, first complete remission; CsA, cyclosporine A; CY, cyclophosphamide; F, female; Flu, fludarabine; GvHD, graft versus host disease; HCT-CI, hematopoietic cell transplant comorbidity index; M, male; MMF, mycophenolate mofetil; MTX, methotrexate; PTCY, posttransplant cyclophosphamide Rel-BM, bone marrow from relative donor; Rel-PB, peripheral blood stem cells from relative donor; SCT, stem cell transplantation; Tac, tacrolimus; TBI, total body irradiation; UR-BM, bone marrow from unrelated donor; VP, etoposide.

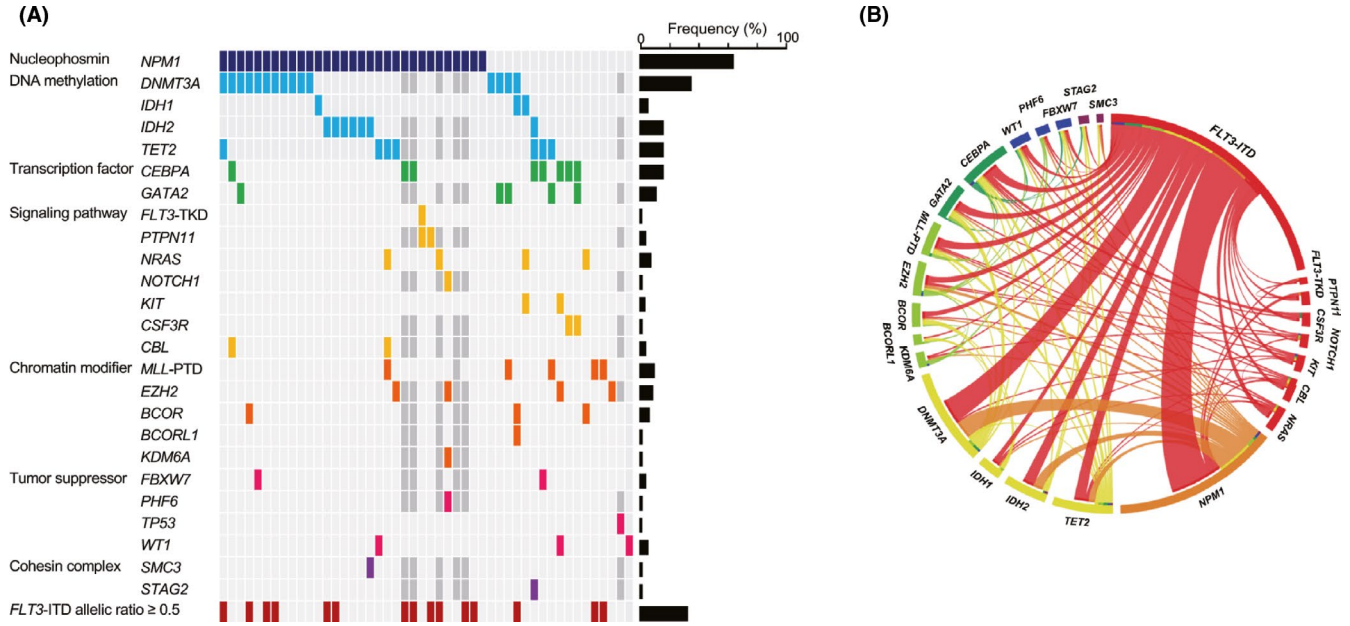
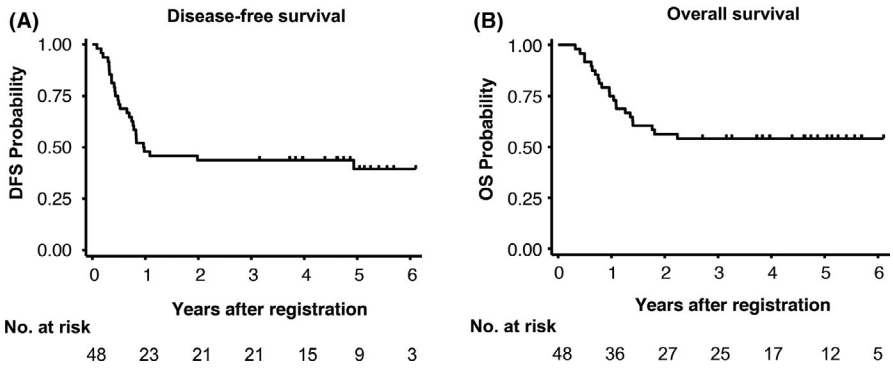


FIGURE 2 Mutational landscape of AML patients with *FLT3*-internal tandem duplication. A, Somatic mutations in analyzed genes grouped into functional categories. The columns in the graph represent patients in the study. Gray boxes indicate the patients whose samples were not analyzed. Frequency of analyzed gene mutations is shown in the bar graph. B, The Circos plot illustrates the association of mutations. The width of the arches indicates the percentage of mutations.[47]

FIGURE 3 Disease-free survival (DFS) and overall survival (OS) of AML patients with *FLT3*-internal tandem duplication (ITD). Kaplan-Meier estimates of DFS (A) and OS (B) in 48 AML patients with *FLT3*-ITD



DNMT3A (36%), *IDH1* (6%), *IDH2* (17%), and *TET2* (17%) (Figure 2A,B). The median ITD AR was 0.344 (range, 0.006-4.099). There was no association of high ITD AR (≥0.5) with specific gene variants (Figure 2A and Table S2).

3.3 | Efficacy of allo-HSCT against *FLT3*-ITD AML

The median follow-up was 1726 (range, 983-2974) days. The primary end-point of this study is shown in Figure 3A. The probability of DFS at 3 years was 43.8% (95% confidence interval [CI], 29.6-57.1). The lower limit of 95% CI exceeded the threshold response rate of 20%, indicating that this treatment was effective. The probability of OS at 3 years was 54.2% (95% CI, 39.2-67.0) (Figure 3B). The cumulative incidence of relapse at 3 years was 47.9% (95% CI, 33.1-61.3). The median follow-up period for allo-HSCT recipients was 1640 (range, 1028-2894) days posttransplantation. The DFS rate posttransplantation was 58.3% (95% CI, 40.7-72.4) at 3 years in a total of 36 recipients (Figure 4A). There was no significant difference in the 3-year DFS rate after allo-HSCT according to stem cell sources: Rel-BM (66.7% [95% CI, 5.4-94.5]), Rel-PB (50.0% [95% CI, 20.9-73.6]), UR-BM (63.6% [95% CI, 29.7-84.5]), or CB (60.0% [95% CI, 25.3-82.7]) (P = .881, Figure 4B). The cumulative incidence of relapse posttransplantation was 30.6% (95% CI, 16.4-46.0) at 3 years.

3.4 | Transplant-related complications and mortality

Neutrophil recovery was achieved in 34 of 36 recipients with an incidence of 94.3% on day 39. One patient received salvage transplantation for graft failure on day 129 and the other died before neutrophil recovery. Cumulative incidences of grade II-IV and III-IV acute GvHD on day 100 were 21.2% (95% CI, 9.2-36.5) and 9.1% (95% CI, 2.3-21.9), respectively. The cumulative incidence of chronic GvHD at 1 year was 25.0% (95% CI, 12.2-40.1). Extensive-type

chronic GvHD developed with an incidence of 19.4% (95% CI, 8.4-33.9) at 1 year. The cumulative incidence of NRM was 5.6% (95% CI, 1.0-16.5) on day 100 and 25.0% (95% CI, 12.2-40.1) at 3 years. Causes of NRM included acute GvHD (n = 2), cardiac failure (n = 2), thrombotic microangiopathy (n = 2), and unknown sudden death (n = 2).

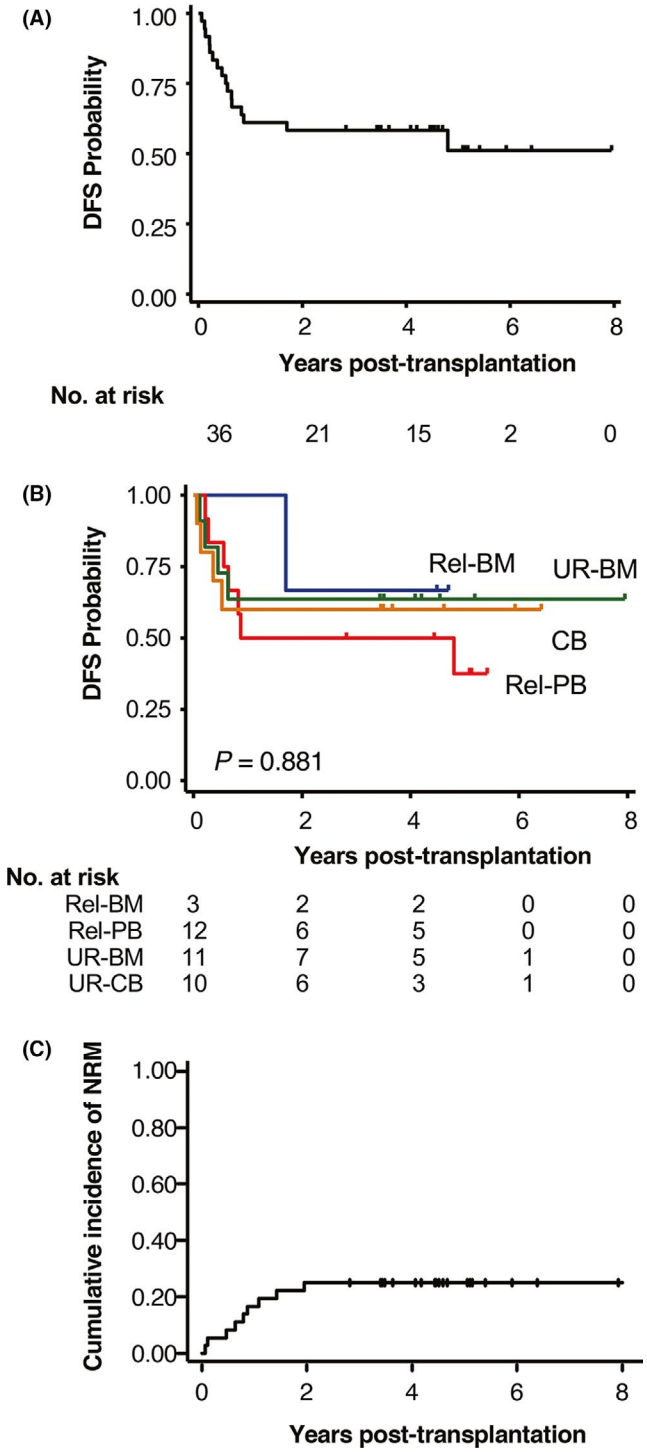


FIGURE 4 Disease-free survival (DFS) and nonrelapse mortality (NRM) post-allogeneic hematopoietic stem cell transplantation (allo-HSCT) of AML patients with *FLT3*-internal tandem duplication. Kaplan-Meier estimates of DFS post-allo-HSCT (A) in total recipients and (B) according to stem cell sources. Cumulative incidence of NRM post-allo-HSCT in all patients

3.5 | Prognostic impact of ITD AR and genetic alterations

We examined prognostic factors for DFS in 48 patients who were eligible for analysis of the primary end-point (Table S3). Univariate analysis revealed no significant prognostic factors among the clinical features including age, gender, WBC count, percentage of BM blasts, an extramedullary tumor, types and courses of induction therapy, karyotype, and cell surface markers. Regarding genetic alterations observed in more than 2 patients, mutations in *BCOR* (hazard ratio [HR] 7.99; 95% CI, 0.72-88.1; P = .09) and *IDH1* (HR 19.5; 95% CI, 1.22-311.7; P = .04) genes were identified as poor prognostic factors for DFS. However, they were not significant on multivariate Cox regression analysis with stepwise selection. The 3-year DFS of patients with *FLT3*-ITD^{high} (43.8%; 95% CI, 19.8-65.6) was the same as those with *FLT3*-ITD^{low} (43.8%; 95% CI, 26.5-59.8) (P = .975, Figure S1). No significant impact on DFS was observed by higher ITD-AR with any cut-off value. According to the ELN2017 risk category, 18, 25, and 5 patients were categorized into favorable, intermediate, and adverse risk groups, respectively. The 3-year OS of the adverse risk group (20.0%; 95% CI, 8.4-58.2) was slightly lower than that of the favorable (55.6%; 95% CI, 30.5-74.8) and intermediate risk groups (60.0%; 95% CI, 38.5-76.1), although not significantly (P = .162; Figure 5).

In terms of prognostic factors for OS, there were no clinical or genetic features identified as independent risk factors (Tables S4).

Prognostic factors for DFS post-allo-HSCT were examined in 36 patients who received allo-HSCT in their CR1 (Table S5). Univariate

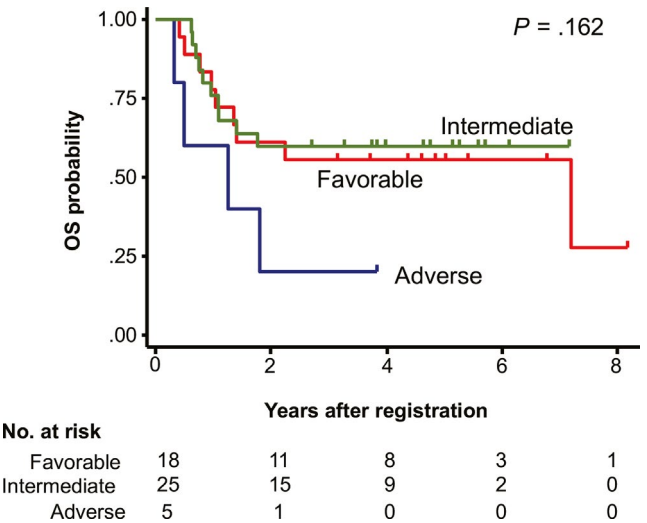


FIGURE 5 Overall survival (OS) of AML patients with *FLT3*-internal tandem duplication (ITD) according to ELN2017 risk stratification. Kaplan-Meier estimates of OS in 48 AML patients with *FLT3*-ITD according to European LeukemiaNet 2017 risk stratification. ITD^{high}, ITD allelic ratio ≥0.5; ITD^{low}, ITD allelic ratio < 0.5

analysis revealed no significant prognostic factors among the clinical features, including the length of time from registration to allo-HSCT.

4 | DISCUSSION

In this study, we prospectively clarified the benefit of allo-HSCT for younger adults with AML with *FLT3*-ITD in CR1 by encouraging patients younger than 50 years old who just achieved their first remission to receive immediate allo-HSCT when feasible during 4 courses of consolidation therapy. The 3-year DFS rate post-allo-HSCT of 58.3% was consistent with a previous retrospective report of 58% at 2 years from the European Group for Blood and Marrow Transplantation (EBMT) registry data that included 120 AML patients with *FLT3*-ITD.[11]

In our cohort, 72.2% of patients received allo-HSCT within 2 courses of consolidation chemotherapy, at a median of 108 days after the first remission. Regarding selection of the donor source, related donors or CB accounted for 69.4%. Unrelated BM was selected for only 5.3% of patients who met the protocol, reflecting the longer time for a donor search in UR-BM in our prospective setting to receive allo-HSCT as soon as possible.[31] Of note, there was no difference in DFS according to stem cell sources ($P = .881$), indicating that CB was not inferior to an HLA-matched sibling. These results suggest that the absence of an HLA-identical donor should not limit the timing for allo-HSCT in CR1 of AML with *FLT3*-ITD, as a recent study found no significant difference in survival outcomes between cord blood transplantation and 8/8 HLA-allele-matched-unrelated HSCT for adult patients with AML.[32] Although a TBI/Cy-based myeloablative regimen was recommended in this protocol, a variety of conditioning regimens and prophylaxis for GvHD were used according to stem cell sources. Therefore, there exist limitations in the understanding of posttransplant outcomes; however, our findings collectively suggest that proceeding faster to allo-HSCT with the first available donor should be considered for younger adult *FLT3*-ITD AML patients.

In the present study, we gained further insight into the clinical and genetic prognostic factors in AML with *FLT3*-ITD. A previous report revealed that risk factors for DFS in AML with *FLT3*-ITD included an older age and higher number of chemotherapy courses before achieving CR.[11] The prognostic impact of ITD-AR was not suggested when this study was designed until the ELN2017 guideline included *FLT3*-ITD^{high} into the unfavorable risk group. Furthermore, recent reports indicated controversial results about the impact of ITD-AR on prognosis, particularly in the setting of allo-HSCT.[17,19,20] Therefore, we additionally assessed the significance of ITD-AR in the final analyses of this study. As previous studies have reported, AML with WT *NPM1* and *FLT3*-ITD^{high} is associated with a poor prognosis and is placed in the adverse-risk group.[13,15] Consistent with previous comprehensive genetic studies, mutations in *NPM1* and *DNMT3A* genes were frequently identified in our analysis.[2,3,33-35] However, neither a high *FLT3*-ITD AR nor *NPM1* mutation status alone impacted outcomes in our cohort comprising younger adults receiving prospective protocol treatment, consistent with recent retrospective studies.[20,36] These results could be affected by the cooccurring risk factors including overlapped mutations or adverse karyotype. Among 16 *FLT3*-ITD^{high} patients in our cohort, as many as 13 harbored *NPM1* mutations, whereas 12 of 32 *FLT3*-ITD^{low} patients carried WT *NPM1*. One harbored *TP53* mutation and the other had the adverse karyotype even though their *FLT3*-ITD AR was low.

Our results support the report from a large retrospective study assessing the ELN 2017 classification system in patients younger than 60 years who received IDR-based induction chemotherapy for newly diagnosed AML, which found that the ELN 2017 system accurately distinguished their prognosis; however, no significant differences in survival were noted according to *FLT3*-ITD AR.[37] Furthermore, in the era of FLT3-directed therapy, a recent study revealed that the addition of FLT3 inhibitors to chemotherapy nullified the negative prognostic impact of high ITD allele burden.[38]

Among the 48 patients enrolled, 11 relapsed before reaching allo-HSCT, reflecting the aggressive proliferation of AML cells with *FLT3* mutations. As the median WBC count at diagnosis was as high as 61.2×10^9/L in the present study, *FLT3*-ITD mutation results in constitutive activation of FLT3, causing aggressive cell proliferation. Therefore, many FLT3 inhibitors have been developed and are undergoing clinical trials, which could improve outcomes for AML patients with *FLT3*-ITD.[39-42]

Now that allo-HSCT combined with FLT3 inhibitors has become the standard of care, it has become difficult to design a study with arms without FLT3 inhibitors from an ethical point of view.[43] This study was the first prospective evaluation undertaken before the era of FLT3 inhibitors, proposing basic data on constructing treatment strategies for *FLT3*-ITD-positive AML. Our study has prospectively revealed that immediate scheduling of allo-HSCT in CR1 improves the DFS post-allo-HSCT in younger adults with *FLT3*-ITD-positive AML, whereas 23% of the patients relapsed during donor search and failed to receive allo-HSCT in CR1.

Midostaurin given in both induction and maintenance therapy for 12 months followed by allo-HSCT reportedly reduced post-allo-HSCT relapses compared with historical controls.[44] Accordingly, midostaurin is now commonly used combined with chemotherapy and leads to deeper remission for subsequent allo-HSCT.[39] Therefore, the application of FLT3 inhibitors could increase the chance of allo-HSCT by maintaining the CR1 duration. Furthermore, the efficacy of FLT3 inhibitors as maintenance therapy post-allo-HSCT is being evaluated in numerous ongoing clinical trials, most of which prescribe their administration for 24 months or longer.[45,46] However, the timing and duration of the use of FLT3 inhibitors remain to be clarified in postremission/posttransplant therapies. In addition, it was not fully elucidated whether FLT3 inhibitors prior to and/or following allo-HSCT further improve the long-term prognosis of patients with *FLT3*-ITD. Our prospective study provides evidence for the evaluation of treatment strategies before/after allo-HSCT. In the present study, among 16 patients who relapsed after allo-HSCT, 14 (87.5%) relapsed within 1 year post-allo-HSCT (Figure 4A), suggesting that the use of FLT3 inhibitors until 1 year post-allo-HSCT can improve the disease-free interval; however, the necessity of administration for longer than 2 years requires further investigation.

In conclusion, we prospectively clarified that immediate allo-HSCT at CR1 in younger adult AML patients with *FLT3*-ITD is feasible and highly effective. Further investigation of risk factors is required to refine posttransplant management, including the use of FLT3 inhibitors.

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REFERENCES

1. Kihara R, Nagata Y, Kiyoi H, et al. Comprehensive analysis of genetic alterations and their prognostic impacts in adult acute myeloid leukemia patients. *Leukemia*. 2014;28:1586-1595.
2. Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic classification and prognosis in acute myeloid leukemia. *N Engl J Med*. 2016;374:2209-2221.
3. Ley TJ, Miller C, Ding L, et al. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *N Engl J Med*. 2013;368:2059-2074.
4. Kiyoi H, Towatari M, Yokota S, et al. Internal tandem duplication of the FLT3 gene is a novel modality of elongation mutation which causes constitutive activation of the product. *Leukemia*. 1998;12:1333-1337.
5. Kiyoi H, Ohno R, Ueda R, Saito H, Naoe T. Mechanism of constitutive activation of FLT3 with internal tandem duplication in the juxtamembrane domain. *Oncogene*. 2002;21:2555-2563.
6. Kiyoi H, Naoe T. Biology, clinical relevance, and molecularly targeted therapy in acute leukemia with FLT3 mutation. *Int J Hematol*. 2006;83:301-308.
7. Yamamoto Y, Kiyoi H, Nakano Y, et al. Activating mutation of D835 within the activation loop of FLT3 in human hematologic malignancies. *Blood*. 2001;97:2434-2439.
8. Kiyoi H, Naoe T, Nakano Y, et al. Prognostic implication of FLT3 and N-RAS gene mutations in acute myeloid leukemia. *Blood*. 1999;93:3074-3080.
9. Lin P-H, Lin C-C, Yang H-I, et al. Prognostic impact of allogeneic hematopoietic stem cell transplantation for acute myeloid leukemia patients with internal tandem duplication of FLT3. *Leuk Res*. 2013;37:287-292.
10. DeZern AE, Sung A, Kim S, et al. Role of allogeneic transplantation for FLT3/ITD acute myeloid leukemia: outcomes from 133 consecutive newly diagnosed patients from a single institution. *Biol Blood Marrow Transplant*. 2011;17:1404-1409.
11. Brunet S, Labopin M, Esteve J, et al. Impact of FLT3 internal tandem duplication on the outcome of related and unrelated hematopoietic transplantation for adult acute myeloid leukemia in first remission: a retrospective analysis. *J Clin Oncol*. 2012;30:735-741.
12. Harada Y, Nagata Y, Kihara R, et al. Prognostic analysis according to the 2017 ELN risk stratification by genetics in adult acute myeloid leukemia patients treated in the Japan Adult Leukemia Study Group (JALSG) AML201 study. *Leuk Res*. 2018;66:20-27.
13. Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood*. 2017;129:424-447.
14. O'Donnell MR, Tallman MS, Abboud CN, et al. Acute myeloid leukemia, version 3.2017, NCCN clinical practice guidelines in oncology. *J Natl Compr Canc Netw*. 2017;15:926-957.
15. Gale RE, Green C, Allen C, 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;111:2776-2784.
16. Pratcorona M, Brunet S, Nomdedéu J, et al. Favorable outcome of patients with acute myeloid leukemia harboring a low-allelic burden FLT3-ITD mutation and concomitant NPM1 mutation: relevance to post-remission therapy. *Blood*. 2013;121:2734-2738.
17. Schlenk RF, Kayser S, Bullinger L, et al. Differential impact of allelic ratio and insertion site in FLT3-ITD-positive AML with respect to allogeneic transplantation. *Blood*. 2014;124:3441-3449.
18. Linch DC, Hills RK, Burnett AK, Khwaja A, Gale RE. Impact of FLT3(ITD) mutant allele level on relapse risk in intermediate-risk acute myeloid leukemia. *Blood*. 2014;124:273-276.
19. Ho AD, Schetelig J, Bochtler T, et al. Allogeneic stem cell transplantation improves survival in patients with acute myeloid leukemia characterized by a high allelic ratio of mutant FLT3-ITD. *Biol Blood Marrow Transplant*. 2016;22:462-469.
20. Sakaguchi M, Yamaguchi H, Najima Y, et al. Prognostic impact of low allelic ratio FLT3-ITD and NPM1 mutation in acute myeloid leukemia. *Blood Adv*. 2018;2:2744-2754.
21. Miyawaki S, Ohtake S, Fujisawa S, et al. A randomized comparison of 4 courses of standard-dose multiagent chemotherapy versus 3 courses of high-dose cytarabine alone in postremission therapy for

acute myeloid leukemia in adults: the JALSG AML201 Study. *Blood*. 2011;117:2366-2372.
22. Ohtake S, Miyawaki S, Fujita H, et al. Randomized study of induction therapy comparing standard-dose idarubicin with high-dose daunorubicin in adult patients with previously untreated acute myeloid leukemia: the JALSG AML201 Study. *Blood*. 2011;117:2358-2365.
23. Atsuta Y, Suzuki R, Yoshimi A, et al. Unification of hematopoietic stem cell transplantation registries in Japan and establishment of the TRUMP System. *Int J Hematol*. 2007;86:269-274.
24. Kawashima N, Akashi A, Nagata Y, et al. Clinical significance of ASXL2 and ZBTB7A mutations and C-terminally truncated RUNX1-RUNX1T1 expression in AML patients with t(8;21) enrolled in the JALSG AML201 study. *Ann Hematol*. 2018;98:83-91.
25. Nishiyama T, Ishikawa Y, Kawashima N, et al. Mutation analysis of therapy-related myeloid neoplasms. *Cancer Genet*. 2018;222–223:38-45.
26. Gale RE, Hills R, Kottaridis PD, et al. No evidence that FLT3 status should be considered as an indicator for transplantation in acute myeloid leukemia (AML): an analysis of 1135 patients, excluding acute promyelocytic leukemia, from the UK MRC AML10 and 12 trials. *Blood*. 2005;106:3658-3665.
27. Bornhauser M, Illmer T, Schaich M, Soucek S, Ehninger G, Thiede C. Improved outcome after stem-cell transplantation in FLT3/ITD-positive AML. *Blood*. 2007;109:2264-2265. author reply 2265.
28. Meshinchi S, Arceci RJ, Sanders JE, et al. Role of allogeneic stem cell transplantation in FLT3/ITD-positive AML. *Blood*. 2006;108(1):400-401.
29. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant*. 2013;48:452-458.
30. Grimwade D, Hills RK, Moorman AV, et al. Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. *Blood*. 2010;116:354-365.
31. Kawashima N, Nishiwaki S, Shimizu N, et al. Outcomes of strategic alternative donor selection or suspending donor search based on Japan Marrow Donor Program coordination status. *Int J Hematol*. 2018.
32. Terakura S, Atsuta Y, Tsukada N, et al. Comparison of Outcomes of 8/8 and 7/8 Allele-Matched Unrelated Bone Marrow Transplantation and Single-Unit Cord Blood Transplantation in Adults with Acute Leukemia. *Biol Blood Marrow Transplant*. 2016;22:330-338.
33. Schlenk RF, Döhner K, Krauter J, et al. Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia. *N Engl J Med*. 2008;358:1909-1918.
34. Suzuki T, Kiyoi H, Ozeki K, et al. Clinical characteristics and prognostic implications of NPM1 mutations in acute myeloid leukemia. *Blood*. 2005;106:2854-2861.
35. Ishikawa Y, Kiyoi H, Tsujimura A, et al. Comprehensive analysis of cooperative gene mutations between class I and class II in de novo acute myeloid leukemia. *Eur J Haematol*. 2009;83:90-98.
36. Straube J, Ling VY, Hill GR, Lane SW. The impact of age, NPM1(mut), and FLT3(ITD) allelic ratio in patients with acute myeloid leukemia. *Blood*. 2018;131:1148-1153.
37. Boddu PC, Kadia TM, Garcia-Manero G, et al. Validation of the 2017 European LeukemiaNet classification for acute myeloid leukemia with NPM1 and FLT3-internal tandem duplication genotypes. *Cancer*. 2019;125:1091-1100.
38. Yalniz F, Abou Dalle I, Kantarjian H, et al. Prognostic significance of baseline FLT3-ITD mutant allele level in acute myeloid leukemia treated with intensive chemotherapy with/without sorafenib. *Am J Hematol*. 2019; 94(9):984-991.
39. Stone RM, Mandrekar SJ, Sanford BL, et al. Midostaurin plus chemotherapy for acute myeloid leukemia with a FLT3 mutation. *N Engl J Med*. 2017;377:454-464.
40. Perl AE, Altman JK, Cortes J, et al. Selective inhibition of FLT3 by gilteritinib in relapsed or refractory acute myeloid leukaemia: a multicentre, first-in-human, open-label, phase 1–2 study. *Lancet Oncol*. 2017;18:1061-1075.
41. Cortes JE, Khaled S, Martinelli G, et al. Quizartinib versus salvage chemotherapy in relapsed or refractory FLT3-ITD acute myeloid leukaemia (QuANTUM-R): a multicentre, randomised, controlled, open-label, phase 3 trial. *Lancet Oncol*. 2019;20:984-997.
42. Perl AE. Availability of FLT3 inhibitors–how do we use them? *Blood*. 2019;134(9):741-745.
43. Levis MJ, Chen YB, Hamadani M, Horowitz MM, Jones RJ. FLT3 inhibitor maintenance after allogeneic transplantation: is a placebo-controlled, randomized trial ethical? *J Clin Oncol*. 2019;37:1604-1607.
44. Schlenk RF, Weber D, Fiedler W, et al. Midostaurin added to chemotherapy and continued single-agent maintenance therapy in acute myeloid leukemia with FLT3-ITD. *Blood*. 2019;133:840-851.
45. Burchert A, Bug G, Finke J, et al. Sorafenib as maintenance therapy post allogeneic stem cell transplantation for FLT3-ITD positive AML: results from the randomized, double-blind, placebo-controlled multicentre sormain trial. *Blood*. 2018;132:661-661.
46. Maziarz RTT, Patnaik MM, Scott BL, et al. Radius: A phase 2 randomized trial investigating standard of care ± midostaurin after allogeneic stem cell transplant in FLT3-ITD-mutated AML. *Blood*. 2018;132:662-662.
47. Krzywinski M, Schein J, Birol I, et al. Circos: an information aesthetic for comparative genomics. *Genome Res*. 2009;19:1639-1645.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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