Prognosis and outcome of patients with acute myeloid leukemia based on *FLT3-ITD* mutation with or without additional abnormal cytogenetics

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Abstract. The FMS-like tyrosine kinase 3-internal tandem duplication (*FLT3-ITD)* gene mutation is present in ~20% of patients with *de novo* acute myeloid leukemia (AML). Patients with an *FLT3-ITD* mutation have a poor prognosis. However, the prognostic function of *FLT3-ITD* combined with other cytogenetic abnormalities are not clear. In the present study, a retrospective analysis of 103 newly diagnosed patients with AML was performed. The results revealed that the overall survival (OS) and recurrence-free survival (RFS) times were significantly longer in patients with an *FLT3-ITD* mutation combined with other favorable risk genes, compared with in those patients with a single *FLT3-ITD* mutation (P=0.0361 and P=0.0426). Sorafenib combined with chemotherapy significantly improved the overall response rate (ORR) when compared with mono-chemotherapy (P=0.039), but no significant differences were observed in the OS and RFS. In conclusion, favorable-risk cytogenetics may improve the clinical outcomes of patients with *FLT3-ITD*-mutated AML, but adverse-risk cytogenetics may not further worsen the prognosis. Sorafenib combined with chemotherapy may increase the ORR but would not result in a longer OS and RFS.

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Abbreviations: AML, acute myeloid leukemia; CR, complete remission; OS, overall survival; RFS, recurrence free survival; *NPM1*, nucleophosmin 1; *FLT3-ITD*, FMS-like tyrosine kinase 3-internal tandem duplication; *FLT3-TKD*, FMS-like tyrosine kinase 3-tyrosine kinase domain

Introduction

Acute myeloid leukemia (AML), a highly heterogeneous disease, is characterized by uncontrolled proliferative blasts (1). At present, the treatment of AML is challenging due to patient characteristics and intrinsic biological factors (2). FMS-like tyrosine kinase 3 (*FLT3*) gene mutations are present in ~30% of patients with *de novo* AML; internal tandem duplication (*ITD*) mutations in the juxtamembrane domain of *FLT3* account for two-thirds of *FLT3* mutations (3). Patients with an *FLT3-ITD* mutation have a poor prognosis, with a shorter remission duration and higher relapse rates compared with patients with *FLT3*-unmutated AML (3). The *FLT3-ITD* mutation usually coexists with other gene mutations or fusion genes; it has been reported that the prognosis of patients with *FLT3-ITD* and nucleophosmin (*NPM1*) double mutations was better compared with that in those with a *FLT-ITD* mono-mutation (4). Previously, research has indicated that the occurrence of *FLT3*-tyrosine kinase domain (*TKD*) and *NPM1* double mutations were a highly favorable prognostic factor (5). However, the prognostic function of *FLT3-ITD* combined with other gene mutations or fusion genes is not clear.

Small molecule inhibitors that target *FLT3*, including sorafenib and midostaurin, have been revealed to be effective in the treatment of patients with *FLT3-ITD*-mutated AML; the multitargeted kinase inhibitor midostaurin combined with standard chemotherapy prolonged the overall survival (OS) and event-free survival times in patients with *FLT3*-mutated AML who were in remission after consolidation therapy and entered a maintenance phase compared with patients who received placebo plus chemotherapy (2). In addition, a novel irreversible *FLT3* inhibitor, FF-10101, also demonstrated excellent efficacy against *FLT3*-mutated AML cells (6). However, the long-term efficacy of these novel *FLT3* inhibitors, and whether they may be used as substitutes for hematopoietic stem cell transplantation (HSCT) for patients with *FLT3-ITD* mutations is currently unclear.

In the present study, a retrospective analysis was performed to examine the complete remission (CR), relapse and survival of newly diagnosed patients with *FLT3-ITD*-mutated AML with or without other gene mutations or fusion genes, and the effect of sorafenib was evaluated in patients with *FLT3-ITD* mutated AML.

Patients and methods

Patient population. Adult patients with AML (n=103; age range 18-87 years; mean age, 50 years; 62 men and 41 women) diagnosed between January 2013 and June 2018 at Huai'an No. 1 People's Hospital, Nanjing Medical University (Nanjing, China), including 23 patients harboring an *FLT3-ITD* mutation, who were treated with different treatment regimens, were retrospectively included in the present study. A total of 45 patients were revealed to be alive at the time of data collection. Patients with acute promyelocytic leukemia were excluded. The study was ethically approved by the Institutional Review Committee of Huai'an No. 1 People's Hospital, and written informed consent was obtained from all patients. The diagnosis of AML was established according to the criteria of the WHO classification (7), including clinical presentations and morphological, immunophenotype and recurrent cytogenetic abnormalities. All patients were analyzed based on their response to induction therapy, CR, relapse, OS and recurrence-free survival (RFS) rates.

Detection of FLT3-ITD and other associated genes. Multiple markers for the diagnosis of AML were identified, along with gene mutations, including *AML1-ETO, CBFB/MYH11, MLL/AF6, MLL/AF9, MLL/ENL, dupMLL, DEK/CAN, EVI1, HOX11, BCR/ABL, FLT3-ITD, NPM1, CEBPA, C-kit* and *DNMT3A*. Fusion genes were detected using multiple nested RT-PCR. Total RNA was extracted using Omega whole-blood RNA extraction kit (cat. no. R6616-02; Omega Bio-Tek, Inc., Norcross, GA, USA). Nested RT-PCR detection method and reaction system were used as previously reported (8,9). AML-associated mutated genes were detected using high-throughput sequencing technology. Total DNA was extracted using whole blood DNA extraction kit (cat. no. D3392-02; Omega Bio-Tek, Inc.), and genetic mutations were detected by Kindstar Global Medical Laboratory Center (http://www.kindstar.com.cn/kindstar/cn/platform.html) using Sanger sequencing, as previously described (9,10). Bone marrow samples were used for this, and each patient signed a bone marrow puncture written informed consent form.

Treatment regimens. All 103 patients received a '3+7' chemotherapy regimen (60 mg/m² /day daunorubicin or 10 mg/m² /day idarubicin for 3 days, and 100 mg/m² /day cytarabine for 7 days with a 24-h intravenous pump maintenance). Out of the 23 patients harboring *FLT3-ITD* mutations, 7 were treated with sorafenib combined with a '3+7' chemotherapy regimen for induction therapy. If the interim bone marrow (BM) examination, which was performed between days 14 and 21 of induction therapy, revealed residual leukemic blasts, a second course of induction chemotherapy comprising cytarabine (100 mg/m² /day) plus 2 mg/m² /day homoharringtonine was administered for 5 days. Patients who achieved CR usually received four to six courses of consolidation chemotherapy or allo-HSCT. Consolidation chemotherapy regimens included high-dose cytarabine (3 g/m² twice a day on days 1 to 3) or intermediate-dose cytarabine (1 g/m² for 4 days) plus daunorubicin (45 mg/m² /day for 3 days) or mitoxantrone (4 mg/m² /day for 3 days). The patients who achieved CR whose induction regimen contained sorafenib with chemotherapy received a continuous maintenance of sorafenib during the chemotherapy interval. Allo-HSCT was performed in patients who achieved CR at the discretion of the attending physician, usually following two courses of consolidation chemotherapy; however, decisions were often made on the basis of the patients' willingness, disease status, risk classification, human leukocyte antigen (HLA) matching and financial status.

Evaluation. CR was defined according to the standard criteria of <5% blasts in BM. Hematologic recovery was measured in terms of the absolute neutrophil (>1x10⁹/l) and platelet (>100x10⁹/l) counts in the peripheral blood. Clinical recurrence following CR was defined as the presence of ≥5% blasts in BM or re-appearance of leukemic blasts in the peripheral blood, or the presence of extramedullary disease. OS and RFS were calculated from the date of diagnosis. An event was defined as induction therapy failure, relapse following CR or mortality from any cause. Relapse was evaluated in patients who achieved CR using a cumulative incidence function with respect to competing risks.

Statistical analysis. Statistical analysis was performed using SPSS 24.0 (IBM Corp., Armonk, NY, USA) and data were presented as the means ± standard deviation/standard error of the mean. Pearson χ^2 survival distributions were estimated using the Kaplan-Meier method and the differences were compared using the log-rank (Mantel-Cox) test. Categorical variables were compared using Pearson χ^2 or Fisher Exact test. P<0.05 was considered to indicate a statistically significant difference.

Results

Patient characteristics. A total of 103 patients with AML were investigated during this period, 23 (22.3%) of which had an *FLT3-ITD* mutation. The median age of the entire cohort was 50 years old (age range, 18-87 years); 31 (30.1%) patients were aged ≥60 years. Patient characteristics and *FLT3-ITD* mutation statuses are summarized in Table I. Eleven out of 23 (47.83%) patients had both *NPM1* and *FLT3-ITD* mutation. High leukocyte counts usually occurred in patients with *FLT3-ITD* mutations. In addition to *NPM1*, other gene mutations combined with the *FLT3-ITD* mutation were observed as follows: *CEBPA* (2/23, 8.69%), *AML1-ETO* (1/23, 4.35%), *EVI1* (1/23, 4.35%), *HOX11* (5/23, 21.74%) and *dupMLL* (2/23, 8.96%) mutations.

Treatment response. All 103 patients received a standard '3+7' regimen of induction chemotherapy. There were no significant differences in the CR rate between the *FLT3-ITD*-positive and *FLT3-ITD*-negative groups (65.22 vs. 62.5%; P=0.812; Table II). The overall response rate (ORR) was 78.26% for the *FLT3-ITD*-positive group and 86.25%

Table I. Patient characteristics.

Characteristics	Total (n=103)	*FLT3-ITD*(+) (n=23)	*FLT3-ITD*(-) (n=80)	P-value
Age (years), median (range)	50 (18-87)	51 (18-87)	49.5 (18-76)	0.138
<60 years, no. (%)	82 (79.61)	15 (65.22)	57 (71.25)	
≥60 years, no. (%)	31 (20.39)	8 (34.78)	23 (28.75)	0.578
Male, no. (%)	62 (60.19)	12 (52.17)	50 (62.50)	
Female, no. (%)	41 (39.81)	11 (47.83)	30 (37.50)	0.372
WBC (x10^9/l), median (range)	15.29 (0.63-257.0)	40.44 (1.55-257.0)	9.71 (0.63-211.0)	<0.001[a]
HB (g/l), median (range)	74 (40-134)	65 (46-127)	79.5 (40-134)	0.842
PLT (x10^9/l), median (range)	44.0 (1-461)	48 (4-219)	41.5 (1-461)	0.290
Cytogenetics, no. (%)				
Abnormal karyotype	44 (42.72)	5 (21.74)	39 (48.75)	
Normal karyotype	59 (57.28)	18 (78.26)	41 (51.25)	0.021[a]
Other abnormal genes no. (%)				
NPM1	18 (17.48)	11 (47.83)	7 (13.75)	0.0001[a]
CEBPA	18 (17.48)	2 (8.69)	16 (20.00)	0.208
AML1/ETO	17 (16.50)	1 (4.35)	16 (20.00)	0.074
EVI1	9 (8.74)	1 (4.35)	8 (10.00)	0.397
HOX11	9 (8.74)	5 (21.74)	4 (5.00)	0.012[a]
C-kit/D816V	7 (6.79)	0 (0)	7 (8.75)	0.141
Dup MLL	3 (2.91)	2 (8.96)	1 (1.25)	0.061
CBFB/MYH11	2 (1.94)	0 (0)	2 (2.50)	0.281
BCR/ABL	2 (1.94)	0 (0)	2 (2.50)	0.443
DEK/CAN	2 (1.94)	0 (0)	2 (2.50)	0.443
MLL/AF6	2 (1.94)	0 (0)	1 (1.25)	0.590
MLL/AF9	2 (1.94)	0 (0)	2 (2.50)	0.443
MLL/AF10	1 (0.97)	0 (0)	1 (1.25)	0.590
MLL/ELL	2 (1.94)	0 (0)	2 (2.50)	0.443
NuP98/HOxC11	1 (0.97)	0 (0)	1 (1.25)	0.338

PLT, platelet; WBC, white blood cell count; HB, hemoglobin; *FLT3-ITD*, FMS-like tyrosine kinase 3-internal tandem duplication mutation; *NPM1*, nucleophosmin 1. [a]Statistically significant P-value.

for the *FLT3-ITD*-negative group (P=0.351). The relapse rate was significantly higher in the *FLT3-ITD*-positive group compared with the *FLT3-ITD*-negative group (34.78 vs. 15.0%; P=0.034). One patient harboring an *FLT3-ITD* mutation received allo-HSCT, and still had CR status 6 months later. In the *FLT3-ITD*-negative group, 10 patients received autologous stem cell transplantation, 3 received allo-HSCT and 4 received micro-transplantation (11). Out of the 23 patients harboring *FLT3-ITD* mutations, 7 were treated with sorafenib combined with chemotherapy for induction therapy and 16 with mono-chemotherapy. The ORR was significantly higher in the sorafenib plus chemotherapy group compared with in the mono-chemotherapy group (P=0.039; Table III), but no significant differences in the CR and relapse rates were observed between these two groups.

Sorafenib plus chemotherapy did not improve the OS and RFS of patients with FLT3-ITD-mutations who did not receive allo-HSCT. Patients with *FLT3-ITD*-mutated AML were associated with a poor prognosis due to a high relapse rate (12). In the present study, 103 patients with AML who completed at least one cycle of induction therapy were analyzed, and the OS and RFS rates in 23 patients harboring an *FLT3-ITD* mutation were revealed to be significantly lower compared with those in patients with *FLT3-ITD*-unmutated AML (P=0.0295 and P=0.0238, respectively; Fig. 1A and B). A number of *FLT3* inhibitors have been revealed to specifically inhibit the proliferation of leukemia cells in preclinical models. However, first-generation *FLT3* inhibitors in patients with relapsed/refractory *FLT3*-mutated AML revealed transient reductions in the number of blasts in the blood and bone marrow, but rarely CR (13,14). In the present study, 7 patients with an *FLT3-ITD* mutation received sorafenib plus chemotherapy, and their ORR was revealed to be higher compared with those of patients with *FLT3-ITD*-unmutated AML, but no significant differences in the OS and RFS were observed between the mono-chemotherapy and sorafenib plus chemotherapy groups (P=0.2735 and P=0.7302, respectively; Fig. 1C and D). These results indicate that sorafenib was not able to improve the long-term follow-up results of patients harboring an *FLT3-ITD* mutation, and should receive allo-HSCT as soon as possible.

Table II. Treatment response between the *FLT3-ITD* positive group and *FLT3-ITD* negative group.

Treatment	Total (n=103)	*FLT3-ITD*(+) (n=23)	*FLT3-ITD*(-) (n=80)	P-value
Therapy regimens				
Mono-chemotherapy, no. (%)	96 (93.2)	16 (69.57)	80 (100.0)	
Sorafenib + chemotherapy, no. (%)	7 (6.8)	7 (30.43)	0 (0)	
Treatment response				
NR, no. (%)	16 (15.53)	5 (21.74)	11 (13.75)	0.351
PR, no. (%)	22 (21.34)	3 (13.04)	19 (23.75)	0.269
CR, no. (%)	65 (63.12)	15 (65.22)	50 (62.5)	0.812
ORR, no. (%)	87 (84.46)	18 (78.26)	69 (86.25)	0.351
Relapse, no. (%)	20 (19.42)	8 (34.78)	12 (15.00)	0.034[a]
Post-remission treatment				
Autologous transplantation (%)	10 (9.71)	0 (0)	10 (12.5)	
Allogeneic transplantation (%)	4 (3.88)	1 (4.35)	3 (3.75)	
Micro transplantation (%)	4 (3.88)	0 (0)	4 (5.00)	

NR, no response; PR, partial remission; CR, complete remission; ORR, overall response rate; *FLT3-ITD,* FMS-like tyrosine kinase 3-internal tandem duplication mutation. [a]Statistically significant P-value.

Table III. Treatment of patients with FMS-like tyrosine kinase 3-internal tandem duplication mutation mutations.

Treatment response (%)	Total (n=23)	Mono-chemotherapy (n=16)	Sorafenib plus chemotherapy (n=7)	P-value
NR, no.	5 (21.74)	5 (31.25)	0 (0)	0.039[a]
PR, no.	3 (13.04)	2 (12.50)	1 (14.28)	0.906
CR, no.	15 (65.22)	9 (56.25)	6 (85.71)	0.172
OR, no.	18 (78.26)	11 (68.75)	7 (100.00)	0.039[a]
Relapse, no.	8 (34.78)	4 (25.00)	4 (57.14)	0.136

NR, no response; PR, partial remission; CR, complete remission; OR, overall response. [a]Statistically significant P-value.

Table IV. *FLT3-ITD* mutation together with other genetic abnormalities in 75 patients with acute myeloid leukemia.

FLT3-ITD status	Total (n=75) (%)	Favourable-risk genes (n=46) (%)	Adverse-risk genes (n=22) (%)	Neither (n=7)	P-value
FLT3-ITD (+)	23 (30.67)	11 (23.91)	5 (22.73)	7 (100.0%)	
FLT3-ITD (-a)	52 (69.33)	35 (76.09)	17 (77.27)	0	0.914

Neither, only *FLT3-ITD* mutation not together with other genes; *FLT3-ITD,* FMS-like tyrosine kinase 3-internal tandem duplication mutation.

Outcome of patients harboring an FLT3-ITD mutation combined with other gene mutations or fusion genes. The prognosis and outcome in patients harboring an *FLT3-ITD* mutation combined with other mutations or fusion genes are unclear. In the present study, two groups of patients harboring an *FLT3-ITD* mutation combined with favorable-risk genes (FRGs; defined as the presence alone of the *AML1-ETO*, *CBFB/MYH11* fusion gene or *NPM1* mutation, and *CEBPA* double mutation) or adverse-risk genes (DRGs; defined as multiple gene mutations, specific genes associated

with a poor prognosis for AML, and complex chromosomal karyotype abnormalities, for example *FLT3-ITD*, *C-kit* and *DNMT3A* mutations) were analyzed. A total of 75 patients with genetic abnormalities received standard regimens of chemotherapy. The survival of all patients with an *FLT3-ITD* mutation combined with FRGs were compared with those of patients with an *FLT3-ITD* mutation combined with DRGs; no significant differences in OS and RFS were observed between these two groups (P=0.3422 and P=0.6599; Fig. 2E and F). Among the 75 patients who
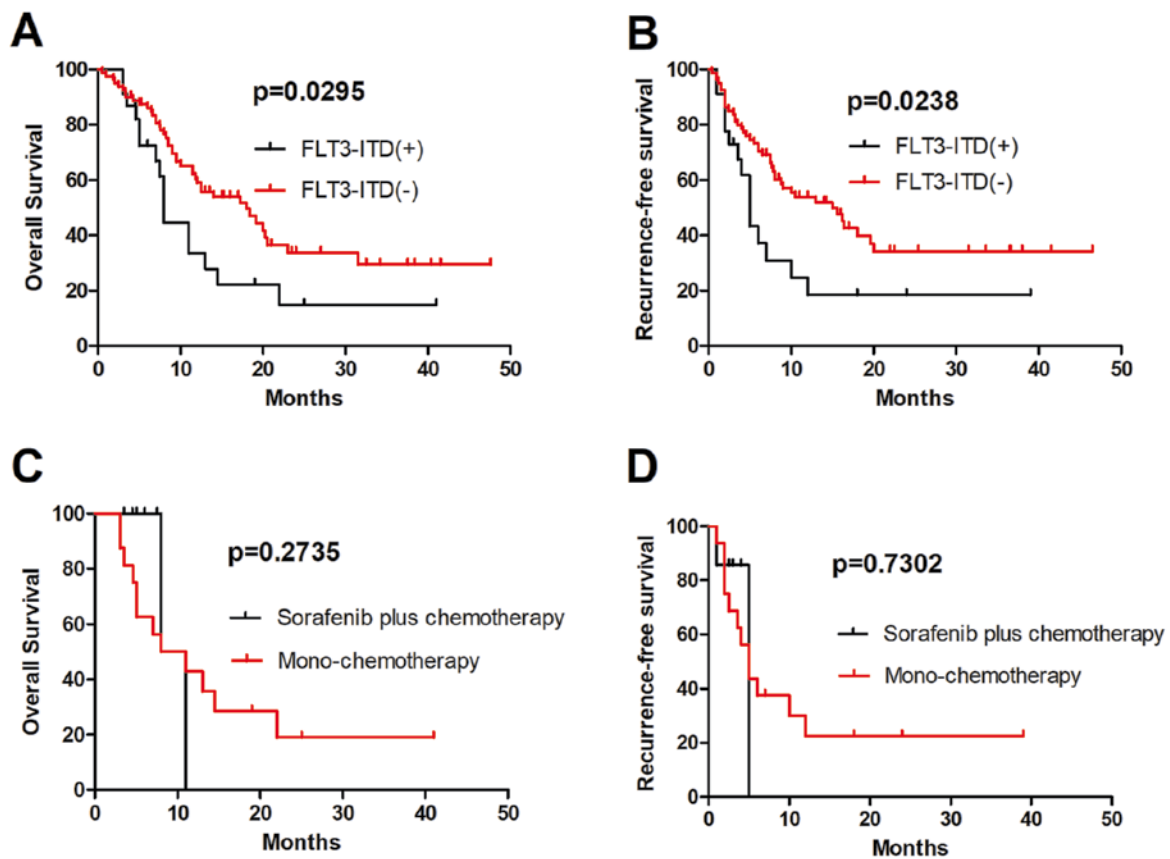
Figure 1. Comparison of OS and RFS in patients with *FLT3-ITD*-positive and -negative AML. (A) OS and (B) RFS in *FLT3-ITD*-positive and -negative groups. OS and RFS rates in 23 patients harboring an *FLT3-ITD* mutation were lower compared with those in *FLT3-ITD* unmutated patients (P=0.0295 and P=0.0238, respectively). (C) OS and (D) RFS in the mono-chemotherapy and sorafenib plus chemotherapy groups. There was no significant difference in OS and RFS between the mono-chemotherapy and sorafenib plus chemotherapy groups (P=0.2735 and P=0.7302, respectively). OS, overall survival; RFS, recurrence free survival; AML, acute myeloid leukemia; *FLT3-ITD*, FMS-like tyrosine kinase 3-internal tandem duplication mutation.

received standard treatment, 35 had a single FRG, 7 had a single *FLT3-ITD* mutation, 11 had an *FLT3-ITD* mutation and FRGs, 17 had a single DRG, and 5 had a combination of *FLT3-ITD* mutation and DRGs (Table IV). Compared with patients with a single *FLT3-ITD* mutation, the OS and RFS were significantly longer in patients with an *FLT3-ITD* mutation together with FRGs (P=0.0361 and P=0.0426, respectively; Fig. 2A and B). No significant differences in OS and RFS were observed between patients with a single *FLT3-ITD* mutation and those with DRGs (P=0.8549 and P=0.7879, respectively; Fig. 2C and D).

FLT3-ITD mutations in normal karyotype (NK) and abnormal karyotype (AK). The *FLT3-ITD* mutation was common in patients with NK. A number of patients with AK also had *FLT3-ITD* mutations. Out of the 23 patients with an *FLT3-ITD* mutation were analyzed, 6 were AK and 17 NK; no significant differences in OS and RFS were observed between these two groups (Fig. 3A and B). Further analysis revealed that the 3-year OS and RFS were longer in patients with *FLT3-ITD* combined with *NPM1* mutations compared with in patients with a single *FLT3-ITD* mutation, but there were no statistically significant differences (Fig. 3C and D). This result may be associated with the relatively small number of cases; further large sample studies are therefore required to confirm it.

Discussion

AML is a heterogeneous disorder of hematopoietic progenitor cells, characterized by blocked differentiation and uncontrolled proliferation (15). Sequencing of AML genomes has revealed that AML is genetically diverse and clonally heterogeneous with multiple mutations, with a majority of patients having >2 gene mutations and being clonally represented (16,17). The most frequently mutated genes in AML are *NPM1* (25-35%), *CEBPA* (6-10%), *RUNX1* (5-15%), *FLT3-ITD* (~20%), *DNMT3A* (18-22%), *ASXL1* (5-17%), *KIT* (<5%) and *TET2* (7-25%) (1). *AML1-ETO*, mutated *NPM1* without *FLT3-ITD*, biallelic mutated *CEBPA* and *CBFB-MYH11* were associated with a favorable prognosis (1), and *EVI1*, *MLL-AF6*, *DEK-CAN* and *FLT3-ITD* with adverse prognosis. *FLT3-ITD* is a distinct clinical entity associated with a poor prognosis (11,18,19). In accordance with the results of the present study, AML with *FLT3-ITD* tends to affect older patients with higher peripheral white blood cell counts, NK (20), shorter remission duration and higher relapse rates compared with patients with *FLT3*-unmutated AML.

The *FLT3-ITD* mutation has been revealed to usually coexist with other molecular genetics and cytogenetic alterations; however, the prognostic function of *FLT3-ITD* combined with other gene mutations or fusion genes is not clear. In the present retrospective study, 103 patients with
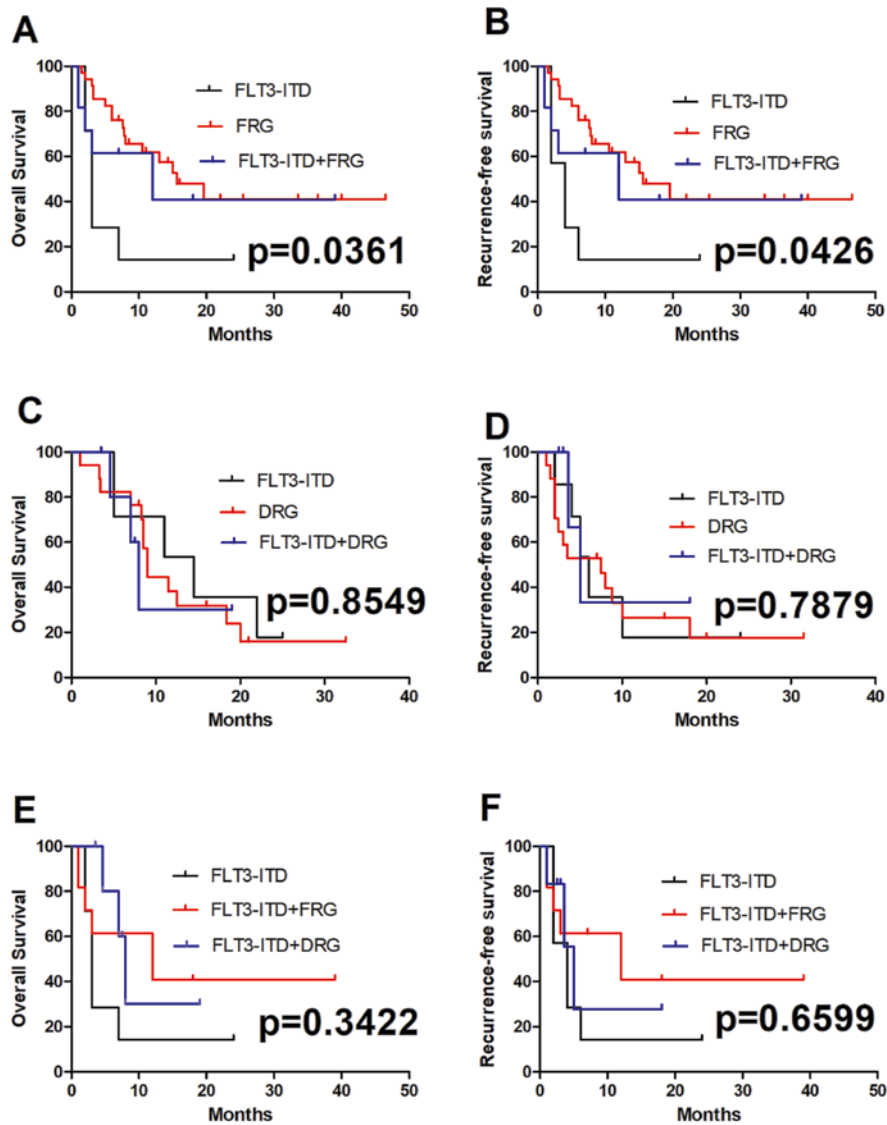
Figure 2. Comparison of OS and RFS between FRGs and DRGs in patients with *FLT3-ITD*-mutated AML. OS in (A) FRGs alone, (C) DRGs alone or (E) compared with each other, with an *FLT3-ITD* mutation. OS was significantly longer in patients with an *FLT3-ITD* mutation combined with FRGs (P=0.0361). There were no significant differences in OS between the patients with a single *FLT3-ITD* mutation and the patients with DRGs (P=0.8549). RFS in (B) FRGs or (D) DRGs combined with an *FLT3-ITD* mutation, or (F) the two compared with each other. RFS were significantly longer in patients with an *FLT3-ITD* mutation combined with FRGs (P=0.0426), but no significant differences in RFS were observed between the patients with a single *FLT3-ITD* mutation and those with DRGs (P=0.7879). OS, overall survival; RFS, recurrence free survival; FRGs, favorable-risk genes; DRGs, adverse-risk genes; AML, acute myeloid leukemia; *FLT3-ITD,* FMS-like tyrosine kinase 3-internal tandem duplication mutation.

AML were analyzed, and it was revealed that the OS and RFS rates in 23 patients harboring *FLT3-ITD* mutations were lower compared with those in patients without *FLT3-ITD* mutations, but no significant differences in the CR and OR rates were observed between these two groups. *NPM1* and *FLT3-ITD* are two of the most common genetic abnormalities in AML. Patients with *NPM1* mutations have a favorable prognosis, older patients with *NPM1*-positive and *FLT3-ITD*-negative genotype have a relatively favorable prognosis, and patients with an *FLT3-ITD* mutation have a poor prognosis (21). The prognostic effect of the *NPM1* mutation in *de novo* AML may also be influenced by the relative abundance of the mutated allele (22). *FLT3-ITD* mutation rates were twice as frequent in *NPM1* mutant AML, when compared with AML with wild-type *NPM1* (22). The *FLT3-ITD* co-mutation has been revealed to diminish the favorable effect of the *NPM1*

mutation (23). Consistent with previous studies, the prognosis of patients with the *FLT3-ITD* and *NPM1* co-mutation in the present study was improved following high intensity chemotherapy, when compared with the patients with a single *FLT3-ITD* mutation. However, no statistically significant differences were observed between these two groups, which may be due to the small patient number. *FLT3-ITD* also co-existed with other genetic abnormalities, including FRGs including *CEBPA* and *AML1-ETO,* and DRGs including *N-RAS, NMT3A, EVI1, DupMLL* and *HOX11* (24,25). In the present study, 76 patients with AML with genetic abnormalities were analyzed and the survival of patients with an *FLT3-ITD* mutation combined with good prognosis genes were compared with those with an *FLT3-ITD* mutation combined with poor prognosis genes. The results revealed no significant differences in OS and RFS between these two
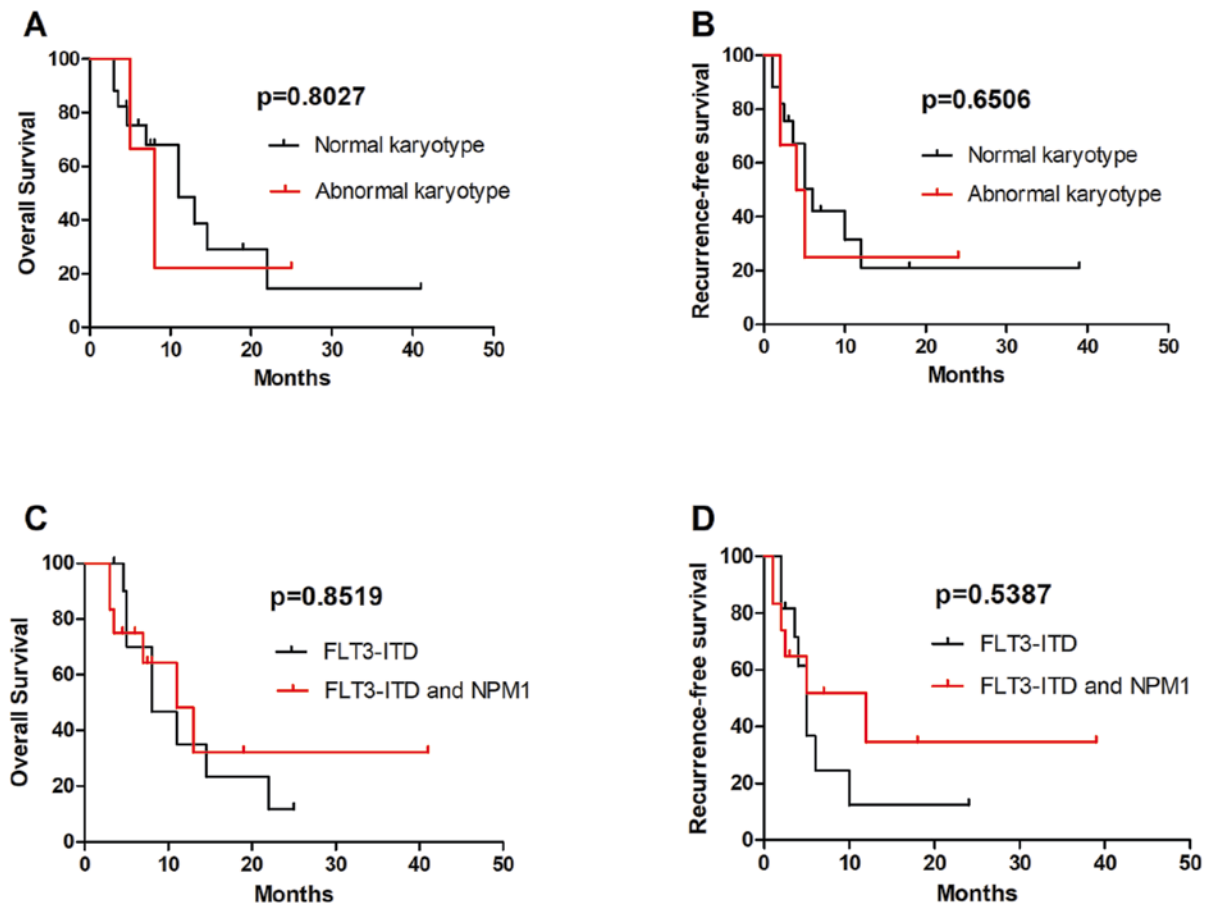
Figure 3. Comparison of OS and RFS in patients with different-karyotype *FLT3-ITD*-mutated AML. Comparison of (A) OS and (B) RFS between patients with *FLT3-ITD*-mutated AML with a normal and abnormal karyotype. There were no significant differences in the OS and RFS between these two groups. Comparison of (C) OS and (D) RFS between patients with a *FLT3-ITD* mono-mutation and *FLT3-ITD* together with *NPM1*-mutations. No significant differences in OS and RFS were observed between these two groups. OS, overall survival; RFS, recurrence free survival; AML, acute myeloid leukemia; *FLT3-ITD*, FMS-like tyrosine kinase 3-internal tandem duplication mutation; *NPM1*, nucleophosmin 1.

groups, which may be due to the small sample size. Among the 76 patients who received standard treatment, the OS and RFS of patients with an *FLT3-ITD* mutation combined with FRGs were longer compared with those of patients with a single *FLT3-ITD* mutation. No significant differences in OS and RFS were observed between the patients with a single *FLT3-ITD* mutation and those with DRGs.

NK AML accounts for 50% of all AML and represents the third and largest broad cytogenetic category in AML (26). *FLT3-ITD* has been recognized as a marker of poor prognosis in patients with NK AML, but *FLT3-ITD* mutations are also commonly observed in patients with AK AML (27,28), and the prognostic function of the *FLT3-ITD* mutation in these patients was unclear. A total of 23 *FLT3-ITD* mutated patients receiving treatment were analyzed; 6 patients were AK and 17 NK, and no significant differences in the OS and RFS were observed between these two groups.

At present, tyrosine kinase inhibitors have been demonstrated to be effective in the treatment of *FLT3*-mutant AML. The multitargeted kinase inhibitor midostaurin, in combination with chemotherapy, has emerged as a novel standard treatment of patients with *FLT3-ITD* and *TKD*-positive AML. It was approved by the FDA for the treatment of AML

in 2017 (2,29). In order to further improve the prognosis of patients with *FLT3*-mutated AML, selective and potent second-generation *FLT3* inhibitors (including gilteritinib, crenolanib, FF-10101 and quizartinib) are being investigated in clinical trials. FF-10101 and quizartinib have each exhibited an excellent efficacy against AML cells and in patients with *FLT3* mutations (6,29-32). High-dose daunorubicin or cladribine chemotherapy have also improved clinical outcomes following acquired drug resistance in *FLT3-ITD*-mutated AML (3,27). However, first-generation *FLT3* inhibitors in patients with an *FLT3* mutation have resulted in transient reductions in the number of blasts but rarely CR improvement (13,14). In the present study, 7 patients with an *FLT3-ITD* mutation received sorafenib plus chemotherapy and exhibited a slightly higher ORR, when compared with patients who were *FLT3-ITD*-negative; however, no statistically significant difference in OS and RFS was observed between the mono-chemotherapy and sorafenib plus chemotherapy groups. This may be due to the small number of patients that were treated with sorafenib, and future studies should expand the number of cases in order to confirm the conclusions of the present study.

In summary, the *FLT3-ITD* mutation has been identified as a marker of poor prognosis in AML, and the

clinical outcomes of patients with *FLT3-ITD*-mutated AML combined with favorable cytogenetics was improved, when compared with those of patients with *FLT3-ITD* mono-mutated AML. OS and RFS have been prolonged due to the application of *FLT3* inhibitors and adjustments in chemotherapy regimens. However, *FLT3* inhibitors may lack a durable and deep response, which is mainly due to acquired drug resistance (3). Allo-HSCT should be performed as early as possible following CR in patients with *FLT3-ITD*-mutated AML, and maintenance therapy with *FLT3* inhibitors may be recommended following allo-HSCT.

In conclusion, the present data demonstrated that favorable-risk cytogenetics may improve the clinical outcomes of patients with *FLT3-ITD*-mutated AML, but not those of patients with adverse-risk cytogenetic abnormalities. Sorafenib combined with chemotherapy may increase OR rates but could not improve OS and RFS.

Availability of data and materials

Not applicable.

Authors' contributions

ST, CW and LY conceptualized the original idea, designed the experiments and analyzed the data. ST wrote the paper. YC, YD, LS, YS, LL, BD and ZH collected the patient information, treated patients with the regimens and analyzed the data with statistical software. CW and LY revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The protocol was reviewed and ethically approved by the Institutional Review Committee of Huai'an No. 1 People's Hospital (Huai'an, China).

Patients consent for publication

A written informed consent form was obtained from each patient.

Competing interests

The authors declare that they have no competing interests.

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