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Sorafenib Therapy Is Associated with Improved Outcomes for FMS-like Tyrosine Kinase 3 Internal Tandem Duplication Acute Myeloid Leukemia Relapsing after Allogeneic Hematopoietic Stem Cell Transplantation

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The optimal therapy for patients with acute myeloid leukemia (AML) with FMS-like tyrosine kinase 3 internal tandem duplication (FLT3-ITD) who relapse after allogeneic hematopoietic stem cell transplantation (allo-HSCT) remains unclear. In this study we retrospectively evaluated the efficacy of sorafenib combined with other therapeutic strategies as salvage therapy for these patients. Eighty-three AML patients with FLT3-ITD relapsing after allo-HSCT were enrolled in this study. Fifty-three patients received salvage therapy containing sorafenib and 30 patients did not. Salvage therapy containing sorafenib was superior to that without sorafenib with respect to complete remission rates, overall survival (OS), and progression-free survival (PFS) (66.0% versus 30.0%, 46.8% versus 20.0%, and 44.9% versus 16.7%, respectively; $P = .002$, $P = .003$, and $P = .001$). Further subgroup analysis revealed that the OS and PFS of patients who received sorafenib combined with chemotherapy followed by donor lymphocyte infusion (DLI) were superior to those receiving other therapeutic regimens, including sorafenib combined with chemotherapy, chemotherapy followed by DLI, and monochemotherapy ($P = .003$, $P < .001$). Multivariate analysis revealed that salvage therapy including sorafenib was the only protective factor for longer OS ($P = .035$; hazard ratio [HR], .526); salvage therapy including sorafenib and DLI were the protective factors for longer PFS ($P = .011$, HR, .423; $P = .019$, HR, .508). Our data suggest that sorafenib therapy is associated with improved outcomes for FLT3-ITD AML relapsing after allo-HSCT, and whether sorafenib combined with chemotherapy followed by DLI reveals an optimal efficacy merits further study.

INTRODUCTION

Internal tandem duplication of FMS-like tyrosine kinase 3 (FLT3-ITD) mutations have been reported in approximately 25% of patients with acute myeloid leukemia (AML) [1]. In contrast to patients with FLT3-ITD wild-type, AML with FLT3-ITD mutations have inferior survival, primarily because of a shorter remission duration and higher relapse rate [2]. Although allogeneic hematopoietic stem cell transplantation (allo-HSCT) improves the outcomes for AML patients with FLT3-ITD, they are still associated with a higher relapse rate post-transplant compared with patients who are FLT3-ITD wild-type [3-5].

Patients experiencing leukemia relapse post-transplant have a dismal prognosis. Treatment options, including chemotherapy, donor lymphocyte infusion (DLI), second allo-HSCT, and targeted drugs, are limited [6-11]. Sorafenib (Nexavar; Bayer Pharma AG, Berlin, Germany) is a multikinase inhibitor characterized by its

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activity against FLT3-ITD, RAS/RAF, c-KIT, and the vascular endothelial growth factor and platelet-derived growth factor receptors [12]. Sorafenib has been used in various settings for AML with FLT3-ITD, including induction, maintenance pre- and post-transplant, and salvage therapy for relapsing patients [13-21]. Recent studies have demonstrated that sorafenib monotherapy or in combination with other therapeutic strategies could induce a sustained response for AML with FLT3-ITD relapsing post-transplant [17,18,22,23]. In this report we retrospectively evaluated the efficacy of sorafenib combined with other therapeutic strategies for AML patients with FLT3-ITD who relapsed after allo-HSCT.

METHODS

Study Design and Data Collection

This retrospective study examined all consecutive AML patients with FLT3-ITD who experienced relapse after allo-HSCT at 7 hospitals (Nanfang Hospital, Peking University People's Hospital, First Affiliated Hospital of Soochow University, Institute of Hematology and Blood Diseases Hospital, Xinqiao Hospital, Zhujiang Hospital, and First People's Hospital of Chenzhou) from January 2012 to October 2017. Patients were eligible for this study if they still had FLT3-ITD mutation at relapse after allo-HSCT. In addition, AML patients who did not have FLT3-ITD mutation before transplant but had FLT3-ITD mutation at relapse after allo-HSCT were also included in this study. In view of known resistance to sorafenib, AML patients with FLT3-tyrosine kinase domain (TKD) at relapse were excluded. Patients were also excluded from the study if they gave up further treatment after relapse post-transplant.

Data were obtained from patients' medical records. Variables collected for all patients included demographic information, pretransplant-related parameters including sorafenib therapy, transplant-related parameters, post-transplant–related parameters including sorafenib maintenance therapy and graft-versus-host disease (GVHD), relapse-related parameters post-transplant, treatment-related parameters, and survival. This study was performed in accordance with modified Helsinki Declaration, and the protocol was approved by respective ethical review boards before study initiation.

Mutation Analysis of FLT3-ITD Gene

Genomic DNA was extracted from bone marrow specimens at diagnosis and at relapse after allo-HSCT using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Mutation analysis of *FLT3-ITD* (exons 14 and 15) gene was performed by PCR and direct sequencing. The sequences of *FLT3-ITD* primers and cycling conditions were designed according to our previous literature [16]. An aliquot of each PCR product (*FLT3-ITD* $\geq$ 329 bp) was confirmed by gel electrophoresis. The rest was subjected to bidirectional sequencing using an ABI 3130xl genetic analyzer (Applied Biosystems, Foster City, CA). Sequence spanning was analyzed for mutation with software-assisted review (Mutation Surveyor 4.0.9; SoftGenetics, State College, PA) in comparison with GenBank reference sequence (https://www.ncbi.nlm.nih.gov/nuccore; accession number NM_004119). Seven hospitals adopted a uniform approach for the diagnosis of FLT3-ITD, and the test was performed in the respective laboratory.

Evaluation Points and Definitions

Our study data were analyzed on October 31, 2018. All living patients had a minimum follow-up of 12 months at the time of analysis. This study mainly focused on the efficacy of salvage therapy for leukemia relapse post-transplant and survival. Leukemia relapse was defined as bone marrow, extramedullary, or both according to common morphologic criteria. Flow cytometry was used for minimal residual disease detection, and minimal residual disease positive was defined as >.001% of cells with leukemia-associated aberrant immune phenotypes in bone marrow. A total of 1,000,000 events were collected for analysis routinely. When cell numbers were limited, a minimal 750,000 events were collected.

Treatment response was defined according to Cheson criteria [24]: complete remission (CR), <5% blasts with no evidence of dysplasia in the bone marrow and no manifestations of leukemia outside the hematopoietic system; partial remission (PR), <20% blasts with or without extramedullary leukemia; no response, a failure to meet the criteria for CR or PR; and complete molecular remission, flow cytometry-minimal residual disease negativity accompanied by molecular negativity for FLT3-ITD by PCR. The overall response (OR) included CR and PR.

Acute GVHD (aGVHD) and chronic GVHD (cGVHD) were graded as described previously [25,26]. The time of GVHD before relapse was defined as the time interval from HSCT to the onset of GVHD. The time of GVHD after relapse was defined as the time interval from the initiation of salvage therapy to the onset of GVHD. Overall survival (OS) was defined as the time interval from the initiation of salvage therapy to death or last follow-up. Progression-free survival (PFS) was defined as the time of salvage therapy initiation until disease progression or death. GVHD-free relapse-free survival (GRFS) was defined as the time from salvage therapy initiation to disease progression or death or severe aGVHD or extensive cGVHD.

Statistics

Statistical analysis was performed using SPSS 20.0 (SPSS Inc., Chicago, IL) and R version 3.3.0 (R Development Core Team, Vienna, Austria). Comparisons of categorical variables were made by means of chi-square tests, and Bonferroni correction was used for pairwise comparisons involving multiple comparisons. Differences between numerical variables were calculated by means of Mann-Whitney tests. OS, PFS, and GRFS were estimated using the Kaplan-Meier method and compared using the log-rank test. Cumulative incidences of nonrelapse mortality (NRM) and GVHD were calculated by accounting for competing risks. Death due to other causes was considered a competing event for GVHD, and death due to relapse was considered a competing event for NRM. Multivariate analyses were performed using a Cox proportional hazards model. $P < .05$ was considered statistically significant ($P < .008$ was considered statistically significant with Bonferroni correction).

RESULTS

Patient Clinical and Transplant Characteristics

With the exception of 18 patients who abandoned further treatment after relapse, 83 AML patients with FLT3-ITD relapsing after allo-HSCT were enrolled in this study, 61 patients who had FLT3-ITD mutation both before transplant and at relapse post-transplant and 22 who developed FLT3-ITD mutation at relapse post-transplant. The median age at the time of transplant was 36 years (range, 14 to 59), and there were 43 male and 40 female patients. Fifty-eight patients were in CR, 7 in PR, and 18 had no response at the time of transplant. Thirty-one patients received HLA-matched sibling donor, 10 HLA-matched unrelated donor, and 42 HLA-haploidentical related donor transplant. The 83 patients all achieved CR and complete chimerism by day +30 post-transplant. Of the 61 patients diagnosed as AML with FLT3-ITD pretransplant, 33 patients had used sorafenib before relapse post-transplant, including 17 with sorafenib pretransplant, 10 with sorafenib maintenance post-transplant, and 6 with sorafenib both pretransplant and maintenance post-transplant. Of the 23 patients receiving sorafenib pretransplant, 11 patients received chemotherapy combined with sorafenib as initial induction and maintenance postremission, 5 as reinduction after relapse and maintenance postremission, and 7 as maintenance postremission alone. The median time of sorafenib therapy pretransplant was 72 days (range, 29 to 169). Ten patients (62.5%) achieved CR among the 16 untreated or refractory relapsed patients receiving sorafenib as induction/reinduction therapy. Of the 16 patients who received sorafenib as maintenance post-transplant, sorafenib was initiated at a median of 30 days (range, 30 to 102) post-transplant, and the median time of sorafenib maintenance post-transplant was 141 days (range, 65 to 215).

With a median time of 153 days (range, 54 to 602) after allo-HSCT, 76 patients experienced hematologic relapse, 5 extramedullary relapse (3 central nervous system leukemia and 2 skin infiltration), and 2 hematologic accompanied by extramedullary relapse. Based on sorafenib inclusion in salvage therapy, patients were divided into 2 groups: sorafenib (n = 53) and nonsorafenib (n = 30). If patients took sorafenib after relapse for more than 2 weeks, they were included into the sorafenib group. One patient took sorafenib only for 5 days and discontinued sorafenib because of a severe skin rash and was thus moved into the nonsorafenib group. The other 29 patients in the nonsorafenib group did not take sorafenib after relapse post-transplant. In addition, patients were divided into DLI (n = 58) and non-DLI (n = 25) groups on the basis of DLI inclusion in salvage therapy. The patients' clinical and transplant characteristics are shown in Table 1.

Table 1
Patients' Clinical and Transplant Characteristics

	Entire sample (N = 83)	Sorafenib Group (n = 53)	Nonsorafenib Group (n = 30)	*P*	DLI Group (n = 58)	Non-DLI Group (n = 25)	*P*
Median age at transplant, yr (range)	36 (14-59)	37 (15-59)	35 (14-57)	.758	37 (14-57)	35 (17-59)	.506
Gender				.834			.982
Male	43 (51.8)	27 (50.9)	16 (53.3)		30 (51.7)	13 (52.0)	
Female	40 (48.2)	26 (49.1)	14 (46.7)		28 (48.3)	12 (48.0)	
Karyotype				.140			.189
Favorable	2 (2.4)	2 (3.8)	0 (.0)		2 (3.4)	0 (.0)	
Intermediate	69 (83.1)	46 (86.8)	23 (76.7)		50 (86.2)	19 (76.0)	
Unfavorable	12 (14.5)	5 (9.4)	7 (23.3)		6 (10.3)	6 (24.0)	
Disease status at transplant				.677			.091
CR	58 (69.9)	38 (71.7)	20 (66.7)		41 (70.7)	17 (68.0)	
PR	7 (8.4)	5 (9.4)	2 (6.7)		7 (12.1)	0 (.0)	
NR	18 (21.7)	10 (18.9)	8 (26.7)		10 (17.2)	8 (32.0)	
Donor type				.685			.481
HLA-matched sibling	31 (37.3)	18 (34.0)	13 (43.3)		24 (41.4)	7 (28.0)	
HLA-matched unrelated	10 (12.0)	7 (13.2)	3 (10.0)		7 (12.1)	3 (12.0)	
HLA-haploidentical related	42 (50.6)	28 (52.8)	14 (46.7)		27 (46.5)	15 (60.0)	
Median relapse time post-transplant, days (range)				.269			.332
	153 (54-602)	155 (54-602)	146 (60-414)		141 (54-602)	195 (57-589)	
Sorafenib use before relapse*				.627			.814
Use	33 (54.1)	18 (51.4)	15 (57.7)		21 (55.3)	12 (52.2)	
No use	28 (45.9)	17 (48.6)	11 (42.3)		17 (44.7)	11 (47.8)	
aGVHD before relapse				.588			.001†
No aGVHD	55 (66.3)	34 (64.2)	21 (70.0)		45 (77.6)	10 (40.0)	
aGVHD	28 (33.7)	19 (35.8)	9 (30.0)		13 (22.4)	15 (60.0)	
cGVHD before relapse‡				.109			.311
No cGVHD	48 (78.7)	28 (71.8)	20 (90.9)		35 (83.3)	13 (68.4)	
cGVHD	13 (21.3)	11 (28.2)	2 (9.1)		7 (16.7)	6 (31.6)	
Relapse type post-transplant				.688			.272
Hematologic	76 (91.6)	48 (90.6)	28 (93.3)		52 (89.7)	24 (96.0)	
Extramedullary	5 (6.0)	4 (7.5)	1 (3.3)		5 (8.6)	0 (.0)	
Hematologic + extramedullary	2 (2.4)	1 (1.9)	1 (3.3)		1 (1.7)	1 (4.0)	

Values are n (%) unless otherwise defined. NR indicates nonremission.
* Indicated by 61 patients diagnosed as AML with FLT3-ITD pre-transplant.
† $P < .05$.
‡ Excluding the 22 patients who relapsed before 100 days of transplant.

Salvage Therapy after Relapse Post-Transplant

Once the patients relapsed after allo-HSCT, immunosuppressants were withdrawn/stopped immediately, and salvage therapy was taken. Four salvage regimens were administered after relapse post-transplant, including sorafenib combined with chemotherapy followed by DLI (group A, n = 41), sorafenib combined with chemotherapy (group B, n = 12), chemotherapy followed by DLI (group C, n = 17), and monochemotherapy (group D, n = 13). The 83 patients all received salvage chemotherapy (median, 2 cycles; range, 1 to 4). The chemotherapy regimens included the aclacinomycin, cytarabine, and granulocyte colony-stimulating factor regimen (n = 41), the idarubicin and cytarabine regimen (n = 25), and other regimens (n = 17). Fifty-three patients were treated with sorafenib for a median duration of 175 days (range, 31 to 396) after relapse post-transplant. Generally, sorafenib was started at 400 mg twice daily and adjusted based on suspected toxicity (dose range, 200 to 800 mg daily). For patients without grades II to >II aGVHD or extensive cGVHD at the time of relapse, granulocyte colony-stimulating factor–mobilized DLI was administered at the following day of chemotherapy end if donor lymphocytes were available. Seventy-eight DLI doses were administered to 58 patients, with a median of once per patient (range, 1 to 3 times) and a median dosage of 3.2×10^7 $CD3^+$ T cells/kg (range, 1.5 to 7.1).

Response

Forty-four patients achieved CR and 15 achieved PR after salvage therapy, with CR and OR rates of 53.0% and 71.1%, respectively. Of the 44 patients who achieved CR, 35 underwent molecular biologic evaluations, and 28 of these patients achieved complete molecular remission. The CR and OR rates were 66.0% and 83.0%, respectively, for the sorafenib group, compared with 30.0% and 50.0%, respectively, for the nonsorafenib group ($P = .002$ and $P = .001$). The CR and OR rates in the DLI group were higher than those in the non-DLI group ($P = .041$ and $P = .012$). Subgroup analysis demonstrated significant differences in the CR and OR rates among the 4 groups ($P = .007$ and $P = .003$) (Table 2). The CR and OR rates in group A were higher than those in group D ($P = .002$ and $P = .001$) but were similar to those in groups B and C (all $P > .008$). There were also no significant differences in the CR and OR rates among groups B, C, and D (all $P > .008$).

Table 2
Outcomes after Salvage Therapy

	Entire Sample (N = 83)	Sorafenib + Chemotherapy + DLI (Group A) (n = 41)	Sorafenib + Chemotherapy (Group B) (n = 12)	Chemotherapy + DLI (Group C) (n = 17)	Monochemotherapy (Group D) (n = 13)	*P*
CR, n (%)	44 (53.0)	29 (70.7)	6 (50.0)	6 (35.3)	3 (23.1)	.007*
OR, n (%)	59 (71.1)	36 (87.8)	8 (66.7)	10 (58.8)	5 (38.5)	.003*
1-year incidence of aGVHD after salvage therapy, % (range)	32.0 (22.1-42.4)	39.5 (24.4-54.3)	29.4 (9.4-51.9)	34.7 (10.2-62.4)	7.7 (.4-30.4)	.242
1-year incidence of cGVHD after salvage therapy, % (range)	28.1 (17.8-39.3)	32.8 (18.2-48.3)	27.1 (5.6-55.4)	35.8 (8.9-64.6)	.0 (.0-.0)	.276
1-year mortality of GVHD after salvage therapy, % (range)	3.6 (1.0-9.4)	2.4 (.2-11.2)	8.3 (.3-33.7)	5.9 (.3-25.1)	.0 (.0-.0)	.662
1-year OS, % (range)	37.2 (26.8-47.5)	53.2 (36.9-67.1)	25.0 (6.0-50.5)	23.5 (7.3-44.9)	15.4 (2.5-38.8)	.003*
1-year PFS, % (range)	34.7 (24.7-45.0)	50.8 (34.6-64.9)	25.0 (6.0-50.5)	23.5 (7.3-44.9)	7.7 (.5-29.2)	<.001*
1-year GRFS, % (range)	30.2 (20.6-40.4)	41.8 (26.3-56.6)	25.0 (6.0-50.5)	23.5 (7.3-44.9)	7.7 (.5-29.2)	.018*
1-year NRM, % (range)	15.7 (8.8-24.3)	12.4 (4.4-24.7)	25.0 (5.0-52.6)	23.5 (6.7-46.1)	15.4 (2.1-40.5)	.763

* $P < .05$

Among the 44 patients who achieved CR after salvage therapy, 12 patients experienced re-relapse, including 6 in group A, 2 in group B, 2 in group C, and 2 in group D. The median duration of CR was 152 days (range, 51 to 359). In the sorafenib group, with a median duration of 147 days (range, 51 to 309) of sorafenib maintenance after CR, 27 patients remained in CR, 5 re-relapsed during sorafenib maintenance after CR, and 3 re-relapsed at 3, 5, and 6 months after sorafenib was discontinued. The leukemia re-relapse rate did not differ significantly between sorafenib and nonsorafenib groups ($P = .227$). There was also no significant difference in the leukemia re-relapse rate among the 4 groups ($P = .361$).

Graft-versus-Host Disease

Twenty-eight patients developed aGVHD (grade I, n = 15; grade II, n = 10; grade III, n = 3), and 13 had cGVHD (local, n = 11; extensive, n = 2) before relapse. The cumulative incidences of aGVHD and cGVHD before relapse were 32.5% (95% confidence interval [CI], 22.7% to 42.7%) and 20.4% (95% CI, 10.5% to 32.6%), respectively. The cumulative incidences of aGVHD and cGVHD and mortality from GVHD after salvage therapy were 32.0% (95% CI, 22.1% to 42.4%), 28.1% (95% CI, 17.8% to 39.3%), and 3.6% (95% CI, 1.0% to 9.4%), respectively. The cumulative incidences of aGVHD and cGVHD and the mortality of GVHD were similar between the patients undergoing and not undergoing DLI ($P = .122$, $P = .190$, and $P = .903$, respectively). Subgroup analysis demonstrated that the incidences of aGVHD and cGVHD and GVHD mortality after salvage therapy did not differ significantly among the 4 groups ($P = .242$, $P = .276$, and $P = .662$, respectively) (Table 2).

Survival

With a median follow-up of 251 days (range, 30 to 1992) after relapse, 29 patients were living and 54 deceased. Causes of death included leukemia progression (n = 39), infection (n = 9), GVHD (n = 3), and other causes (n = 3). The 1-year OS, PFS, and GRFS were 37.2% (95% CI, 26.8% to 47.5%), 34.7% (95% CI, 24.7% to 45.0%), and 30.2% (95% CI, 20.6% to 40.4%), respectively. The 1-year OS, PFS, and GRFS of the sorafenib group were superior to those of the nonsorafenib group (46.8% versus 20.0%, $P = .003$; 44.9% versus 16.7%, $P = .001$; and 37.9% versus 16.7%, $P = .015$, respectively; Figure 1A-C). The 1-year OS, PFS, and GRFS of the DLI group were also superior to those of the non-DLI group ($P = .007$, $P = .002$, and $P = .026$, respectively). The 1-year NRM post-transplant was 15.7% (95% CI, 8.8% to 24.3%), and NRM was similar between sorafenib and nonsorafenib groups ($P = .703$; Figure 1D).

Detailed subgroup analysis revealed a significant difference in OS, PFS, and GRFS among the 4 groups ($P = .003$, $P < .001$, and $P = .018$; Figure 2A-C). The OS and PFS of group A were superior to those of groups B, C, and D (all $P < .05$). The GRFS in group A was superior to that of group D ($P = .002$) and was similar to that of groups B and C ($P = .234$ and $P = .139$). There was no significant difference in OS, PFS, and GRFS among groups B, C, and D (all $P > .05$). NRM did not differ significantly among the 4 groups ($P = .763$; Figure 2D).

Moreover, of the 61 relapsed patients diagnosed as AML with FLT3-ITD pretransplant, 16 patients received sorafenib maintenance therapy post-transplant and 45 did not. The 1-year OS, PFS, GRFS, and NRM after salvage therapy did not differ significantly between the relapsed patients with and without sorafenib maintenance therapy post-transplant ($P = .090$, $P = .113$, $P = .449$, and $P = .740$, respectively). Multivariate analysis revealed that salvage therapy including sorafenib was the only protective factor for longer OS ($P = .035$; hazard ratio,

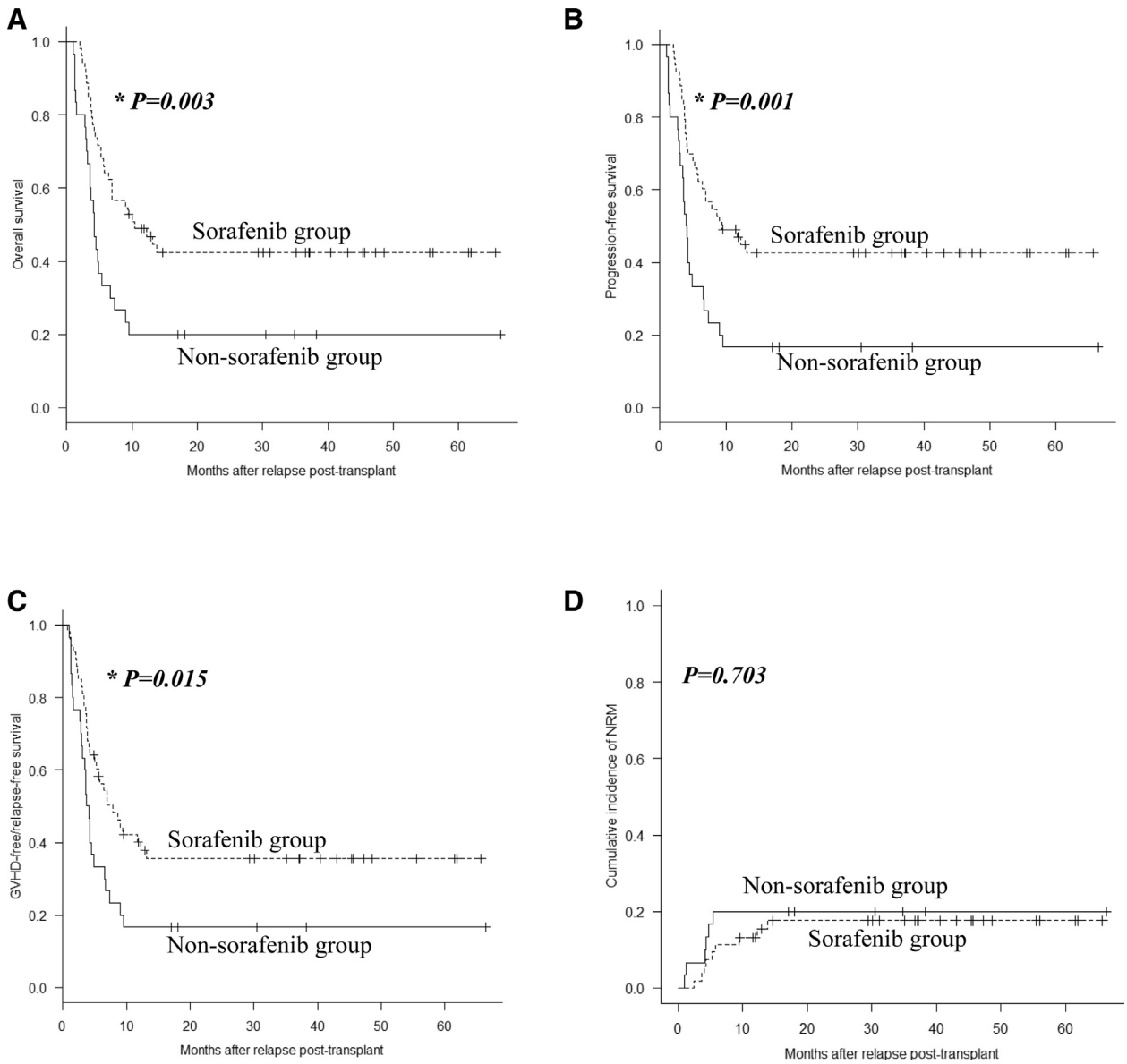

Figure 1. The outcomes of FLT3-ITD AML patients who relapsed post-transplant receiving salvage therapy including or not including sorafenib. OS (A), PFS (B), GRFS (C), and cumulative incidence of NRM (D) between the sorafenib and nonsorafenib groups.

.526); salvage therapy including sorafenib and DLI were the protective factors for longer PFS ($P = .011$, hazard ratio, .423; $P = .019$, hazard ratio, .508) (Table 3).

DISCUSSION

It has been demonstrated that allo-HSCT is an effective strategy for improving prognosis, but relapse is a major cause of treatment failure for AML patients with FLT3-ITD [3-5]. Our group and others have suggested that the use of sorafenib pre- and/or post-transplant could reduce relapse and improve the survival of AML patients with FLT3-ITD [13-21]. Generally, once patients relapse after transplant, the prognosis is dismal. Thus far, treatment options for these relapsed patients are limited, and no standard approach has been established. In this retrospective study we investigated the effects of sorafenib combined with other therapeutic strategies on the outcomes of AML patients with FLT3-ITD who relapsed after allo-HSCT. Our results demonstrated that salvage therapy containing sorafenib was superior with respect to response rates, OS, PFS, and GRFS. Further subgroup analysis revealed that the OS and PFS of patients who received sorafenib together with chemotherapy followed by DLI were superior to those who received other therapeutic regimens, including chemotherapy alone, chemotherapy followed by DLI, and sorafenib combined with chemotherapy.

The therapeutic efficacy of allo-HSCT relies on the graft-versus-leukemia (GVL) effects to some extent. Some clinical evidence has demonstrated that the use of sorafenib post-transplant might benefit AML patients with FLT3-ITD, and this might be associated with the synergism of sorafenib and allogeneic immune effects on enhancing the GVL reaction [18,27,28]. Recently, Mathew et al. [28] demonstrated that sorafenib might promote GVL effects by inducing IL-15 production in FLT3-ITD–mutant leukemia cells, thereby leading to the metabolic reprogramming of leukemia-reactive T cells. In this study we found that the response rates (CR and OR) and survival (OS, PFS, and GRFS) of patients undergoing therapy including sorafenib were superior to those without sorafenib treatment. The OS and PFS of patients who received sorafenib

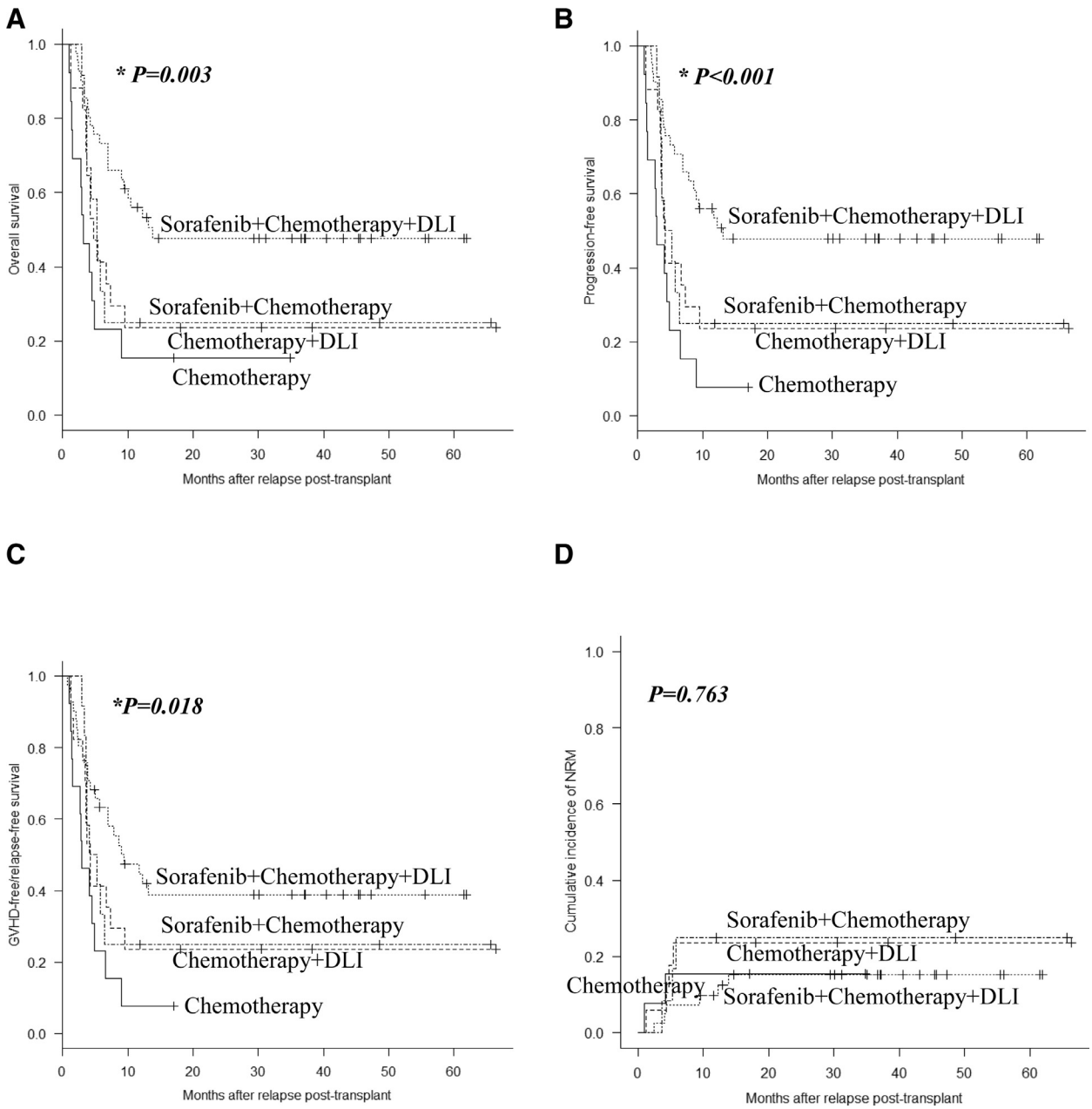

Figure 2. The outcomes of FLT3-ITD AML patients who relapsed post-transplant after 4 different therapeutic regimens. OS (A), PFS (B), GRFS (C), and cumulative incidence of NRM (D) among groups A, B, C, and D.

combined with chemotherapy followed by DLI was superior to those who received sorafenib combined with chemotherapy, although the CR rate was not significant between the 2 groups (70.7% versus 50.0%). We could not rule out the possibility that a difference might be found between the 2 groups if the sample size was larger. The results also demonstrated that sorafenib could synergize with allogeneic immune effects to induce GVL effects.

DLI has been widely used for the prevention and treatment of leukemia relapse post-transplant, but it is associated with a high incidence and mortality of GVHD [29-32]. The efficacy of DLI alone in relapsed acute leukemia is reported to be very poor, and chemotherapy plus DLI could improve the outcomes compared with chemotherapy or DLI alone [33,34]. Recent studies suggested that immunotherapy treatment such as DLI should preferably be started in leukemia patients with relatively low tumor burden [34,35]. Meanwhile, given that the prognosis of these relapsed patients was dismal, DLI was administered at the following day of chemotherapy end for patients without severe GVHD at relapse in all eligible patients. Our results demonstrated that the response rate and survival of patients undergoing therapy including DLI were superior to those without DLI treatment. The incidences of aGVHD and cGVHD and the mortality of GVHD did not differ significantly in the patients undergoing and not undergoing DLI. The nonincreased incidence of GVHD after DLI might be due to the following 2 reasons: as shown in Table 1 more patients not undergoing DLI had aGVHD at the time of relapse, and some patients not undergoing DLI might have no time to develop GVHD because of a quick death.

Thus far, whether sorafenib maintenance post-transplant increases the risk of GVHD is unknown. Brunner et al. [14] demonstrated no difference in the GVHD incidence of patients

Table 3
Multivariate Analysis of Risk Factors for OS and PFS

Risk Factors	OS		PFS	
	Hazard Risk (95% CI)	*P*	Hazard Risk (95% CI)	*P*
Salvage therapy including sorafenib Sorafenib vs. no sorafenib	.526 (.289-.957)	.035*	.423 (.218-.823)	.011*
Salvage therapy including DLI DLI vs. no DLI	.560 (.281-1.115)	.099	.508 (.289-.893)	.019*
Relapse time post-transplant <6 mo vs. ≥6 mo	.651 (.349-1.212)	.176	.573 (.307-1.068)	.080
aGVHD after salvage therapy No aGVHD Grades I-II aGVHD Grades III-IV aGVHD	1.018 (.825-1.256)	.869	1.108 (.810-1.517)	.520
cGVHD after salvage therapy No cGVHD Limited cGVHD Extensive cGVHD	1.245 (.517-2.994)	.625	1.208 (.551-2.646)	.637

* $P < .05$

with and without sorafenib maintenance post-transplant. In our previous retrospective study, the incidence of aGVHD for patients who underwent sorafenib maintenance post-transplant was higher than that for those without maintenance post-transplant [16]. Whether sorafenib combined with DLI increases the incidence and mortality of GVHD is also a concern of our study. Mathew et al. [28] reported that sorafenib combined with allogeneic T cells did not result in an increased incidence and mortality of GVHD for mouse models of FLT3-ITD$^+$ AML. Our results revealed that sorafenib combined with chemotherapy followed by DLI did not increase the incidence and mortality of GVHD compared with salvage therapy including chemotherapy combined with sorafenib or DLI, which verified the mouse model results [28]. As for the nonincreased GVHD in group A, we also could not exclude the influence of mortality as a competing factor.

There is no consensus regarding the duration of sorafenib maintenance after CR for FLT3-ITD AML patients with relapse after allo-HSCT. Rautenberg et al. [22] observed that 1 patient who relapsed post-transplant achieved complete molecular remission after salvage therapy including sorafenib and azacitidine and remained in complete molecular remission for 168 days after 65 days of sorafenib maintenance. Metzelder et al. [17] reported that 4 patients who relapsed after allo-HSCT were in treatment-free remission for a median of 4.4 years after greater than 20 months of sorafenib monotherapy. Our study demonstrated that with a median duration of 147 days (range, 51 to 309) of sorafenib maintenance after CR, 27 patients remained in CR, 5 relapsed again during sorafenib maintenance, and 3 relapsed within 3, 5, and 6 months of sorafenib discontinuation. Some studies have suggested that long-term sorafenib therapy might induce a secondary FLT3-TKD mutation and give rise to drug resistance, which might be the leading cause of sorafenib treatment failure [36,37]. Our results demonstrated that none of the re-relapse patients who underwent sorafenib salvage therapy acquired FLT3-TKD mutation when leukemia relapse recurred. Whether prolonged administration of sorafenib salvage therapy could further reduce the relapse rate but not increase the risk of mutation is worth further exploration.

There were some limitations of our study. Although it was a large study about salvage therapy in FLT3-ITD AML patients relapsing after allo-HSCT, the number of patients was relatively small, and some patients were followed only for a short time, which might influence the accuracy of our results. Moreover, because it was a retrospective study, the heterogeneity of the population including many centers, FLT3-ITD at diagnosis or acquired at relapse, and inherent selection bias could not be avoided. Well-designed prospective clinical trials are needed to establish the optimal therapy for these relapsed patients.

In conclusions, sorafenib therapy is associated with improved outcomes for FLT3-ITD AML patients relapsing after allo-HSCT. Whether sorafenib combined with chemotherapy followed by DLI reveals an optimal efficacy merits further study.

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