Second hematopoietic stem cell transplantation as salvage therapy for relapsed acute myeloid leukemia/myelodysplastic syndromes after a first transplantation

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Received: August 1, 2022.
Accepted: November 28, 2022.
Early view: December 7, 2022.

https://doi.org/10.3324/haematol.2022.281877

Abstract

Second allogeneic hematopoietic stem-cell transplantation (HSCT2) is a therapeutic option for patients with acute myeloid leukemia (AML)/myelodysplastic syndrome (MDS) relapsing after a first transplant (HSCT1). However, patients allocated to HSCT2 may be a selected group with better prognosis and the added efficacy of HSCT2 is not well established. We retrospectively analyzed 407 consecutive patients with relapsed AML/MDS after HSCT1. Sixty-two patients had HSCT2 (15%) and 345 did not. The 2-year cumulative incidence rates of non-relapse mortality and relapse after HSCT2 were 26% (95% confidence interval [95% CI]: 17-39%) and 50% (95% CI: 39-65%), respectively. The 5-year overall survival rates were 25% (95% CI: 14-36%) and 7% (95% CI: 4-10%) in the HSCT2 and no-HSCT2 groups, respectively. Multivariate analysis identified female gender (hazard ratio [HR]=0.31, *P*=0.001), short remission duration after HSCT1 (HR=2.31, *P*=0.05), acute graft-*versus*-host disease after HSCT1 (HR=2.27, *P*=0.035), HSCT2 from a haplo-identical donor (HR=13.4, *P*=0.001) or matched unrelated donor (HR=4.53, *P*=0.007) and relapse after HSCT1 in earlier years (HR=2.46, *P*=0.02) as factors predicting overall survival after HSCT2. Multivariate analysis of all patients including HSCT2 as a time-dependent variable identified relapse within 6 months after HSCT1 (HR=2.32, *P*<0.001), acute graft-*versus*-host disease before relapse (HR=1.47, *P*=0.005), myeloablative conditioning in HSCT1 (HR=0.67, *P*=0.011), female gender (HR=0.71, *P*=0.007), relapse in earlier years (HR=1.33, *P*=0.031) and not having HSCT2 (HR=1.66, *P*=0.010) as predictive of overall survival after relapse. In conclusion, HSCT2 is associated with longer survival compared to non-transplant treatments and may be the preferred approach in a subset of patients with relapsed AML/MDS after HSCT1.

Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is an effective treatment with a curative potential for patients with acute myeloid leukemia (AML) or myelodysplastic syndromes (MDS). The outcome of HSCT has improved markedly in the last decades due to a significant reduction in the rate of non-relapse mortality after stem cell transplants.[1] However, relapsed disease remains the major cause of treatment failure.[2] Marked changes have been introduced in modern HSCT over the past decades, including transplants in older patients, more common use of unrelated donors as well as haplo-identical donors, and the use of peripheral blood stem cells as the source of stem cells. Novel conditioning regimens and regimens for the prevention of graft-*versus*-host disease (GvHD) have also been introduced. However, these changes did not change the rate of disease relapse substantially, and the prognosis for relapsed patients following HSCT remains dismal with a long-term survival rate of about 10-15%.[3-5] There is no established standard-of-care therapy for patients relapsing after HSCT. The spectrum of management includes palliative care, withdrawal of immune-suppression therapy, low-dose or intensive chemotherapy,

targeted treatments, donor lymphocyte infusion, a second allogeneic transplantation (HSCT2), or a combination of these therapies.[2-5] Prolonged survival can be achieved only by patients who have a second complete remission and are supported by a form of cellular therapy such as donor lymphocyte infusion or HSCT2.[6,7] The role of HSCT2 in the treatment of relapsed AML or MDS patients still needs to be determined, including the indications and predictive factors for, and outcomes after, a second transplant in comparison with those for non-transplant treatments. Several studies have shown that the major predictive factors of outcome of HSCT2 are the duration of remission after the first HSCT and the status of disease at HSCT2.[7-17] Age, gender, choice of stem cell donor and acute and/or chronic GvHD prior to and following HSCT2 have also been described as important predictive factors.[2-17] The long-term survival after HSCT2 is estimated as 25-30%.[2-17] However, patients addressed to HSCT2 may be a selected group with a better prognosis than those not given a second transplant. In addition, survival time between relapse and HSCT2 may bias in favor of HSCT2, as early deaths are not included in the analysis of HSCT2. In the current study we describe the outcomes of patients relapsing after a first allogeneic HSCT whether they did or did not have HSCT2. We consider HSCT2 as a time-dependent variable to reduce these biases.

Methods

Study design and data collection

This is a retrospective, single-center analysis. The study included adult patients with AML or MDS who relapsed after a first allogeneic HSCT from an HLA-matched sibling or unrelated donor between the years 2000-2018. Patients given a first HSCT from haplo-identical donors were not included, because of their small number during that period and to the different biology of relapse after haplo-identical transplants. Patients were divided into two subgroups according to whether they did or did not have HSCT2. Only patients who underwent HSCT2 from a different donor were considered in the HSCT2 subgroup.

All patients provided written informed consent authorizing the use of their personal information for research purposes and the study was approved by the institutional review board.

Conditioning regimens

The conditioning regimen was selected at the attending physicians' discretion. Dose intensity was defined as myeloablative, reduced toxicity (intermediate intensity) or reduced intensity according to standard criteria.[18,19] GvHD prophylaxis consisted of cyclosporine with short-term methotrexate or mycophenolate mofetil in most cases. Antithymocyte globulin was allowed at the attending physicians' discretion. No *ex-vivo* manipulation of cells was used.

Evaluation of outcomes

Active disease was defined as no complete remission (CR) or complete remission with incomplete count recovery (CRi) in AML and >5% marrow blasts in MDS. Disease relapse and transplant engraftment were defined according to standard hematologic criteria. In the analysis of outcomes after HSCT2, non-relapse mortality was defined as death of any cause in the absence of disease recurrence. Leukemia-free survival was defined as survival without relapse. Overall survival was calculated from the day of HSCT2 until death of any cause or the date of last follow-up. In the analysis of outcomes in the entire group of patients all outcomes were calculated from the day of relapse. Acute GvHD was graded and staged by the consensus criteria.[20] Chronic GvHD was graded and staged according to National Institute of Health consensus criteria.[21]

Statistical analysis

This study had two parts. In the first part we analyzed the group of patients who underwent HSCT2. Overall survival and leukemia-free survival were analyzed by the Kaplan-Meier method.[22] Non-relapse mortality, relapse, and acute and chronic GvHD were evaluated by cumulative incidence analysis considering competing risks.[23] Univariate analysis of predictive factors was done by log-rank tests for overall survival and leukemia-free survival and by the Gray test for the other outcomes. Multivariate analysis was done using the Cox proportional-hazard method. In the second part of the study, we evaluated the role of HSCT2 in the entire group of patients who relapsed after their first HSCT. The primary endpoint of this part was overall survival after relapse. The two treatment groups (HSCT2 and no-HSCT2) were compared by the χ^2 method for qualitative variables, and the Mann-Whitney test for continuous parameters. Outcomes were analyzed by the same methodologies. Multivariate analyses were performed using Cox proportional hazards with stepwise backward selection including performing HSCT2 as a time-dependent variable. We also used a landmark analysis at 60 days after relapse and included only patients alive at the landmark. Statistical analyses were performed with SPSS 24.0 (SPSS Inc., Chicago, IL, USA) and R 3.4.1 software packages (Vienna, Austria; URL *https://www.R-project.org/*).

Results

Patients' characteristics

The study included 407 patients with AML (n=338) and

MDS (n=69) who relapsed after a first allogeneic HSCT that was carried out during the years 2000-2018. The median year of relapse was 2012 (range, 2001-2021). The patients' characteristics are outlined in Table 1. A total of 62 patients were given HSCT2. Among the other 345 patients, 98 patients had cellular therapy that was not considered HSCT2, including donor lymphocyte infusion (n=40), granulocyte colony-stimulating factor-mobilized donor lymphocyte infusion given after salvage chemotherapy (n=50) or a second transplant from the same donor (n=7). The patients' median age was 56 years (range, 18-78); 49 years (range, 18-76) in the HSCT2 group and 58 years (range, 18-78) in the no-HSCT2 group (P<0.001). Forty-two percent of patients were males in the HSCT2 group and 61% in the no-HSCT2 group (P=0.006). The conditioning regimens for the first HSCT were myeloablative (n=153), reduced intensity (n=118) and reduced toxicity (n=136) with a higher rate of myeloablative conditioning and a lower rate of reduced intensity conditioning in those receiving HSCT2. The GvHD prophylaxis regimen at first HSCT was cyclosporine A/methotrexate in most patients. In the HSCT2 and no-HSCT2 groups, 44% and 37% of the patients, respectively, were in first complete remission/MDS with no blasts and 11% and 12% were in second complete remission. Data on measurable residual disease at the time of HSCT were not available as it was not routine practice to determine residual disease in AML at the beginning of the study. The median time from the first HSCT to first relapse was 4.5 months (range, 0.4-143.1 months). It was longer in the HSCT2 group (10.5 months

Table 1. Patients' characteristics.

	Second HSCT	No second HSCT	*P* value
Number	62	345	
Age, years, median (range) Age >55 years, N (%)	49 (18-76) 21 (34)	58 (18-78) 199 (58)	<0.001
Male gender, N (%)	26 (42)	209 (61)	0.006
Diagnosis, N (%) AML MDS	 47 (76) 15 (24)	 291 (84) 54 (16)	0.09
ELN risk (AML only), N (%) Good Intermediate Poor Missing	 7 (15) 17 (36) 16 (34) 7 (15)	 28 (10) 119 (62) 107 (56) 37 (19)	0.29
Status at 1st HSCT, N (%) CR1/MDS no blasts CR2 Active disease	 27 (44) 5 (11) 30 (48)	 127 (37) 41 (12) 177 (51)	0.51
Conditioning for 1st HSCT, N (%) MAC RIC RTC	 30 (48) 9 (15) 23 (37)	 123 (36) 109 (32) 113 (33)	0.02
GvHD prophylaxis, N (%) CSA/MTX CSA/Cellcept	 55 (89) 7 (11)	 266 (77) 79 (23)	0.04
Source of stem cells, N (%) Peripheral blood stem cells Bone marrow	 59 (95) 3 (5)	 340 (99) 5 (1)	0.08
Donor for 1st HSCT, N (%) Sibling Matched unrelated	 31 (50) 31 (50)	 169 (49) 176 (51)	0.49
F to M, N (%)	10 (17)	69 (20)	0.51
Prior acute GvHD, N (%)	16 (26)	110 (32)	0.3
Prior chronic GvHD, N (%)	17 (27)	65 (19)	0.12
Time to 1st relapse, months, median (range) 1st relapse in <6 months, N (%)	10.5 (1.3-143) 19 (31)	3.8 (0.4-112) 224 (65)	<0.001
Year of relapse, median (range)	2013 (2002-2021)	2012 (2001-2020)	0.02

HSCT: hematopoietic stem cell transplantation; AML: acute myeloid leukemia; MDS: myelodysplastic syndrome; ELN: European Leukemia Network; CR1: first complete remission; CR2: second complete remission; MAC: myeloablative conditioning; RIC: reduced-intensity conditioning; RTC: reduced toxicity myeloablative conditioning; GvHD: graft-*versus*-host disease; CSA: cyclosporine A; MTX: methotrexate; F to M: female donor to male recipient.

[range, 1.3-143]) than in the no-HSCT2 group (3.8 months [range, 0.4-112]) (P<0.001). Of all relapses, 31% and 65% occurred within the first 6 months after the first HSCT in the HSCT2 and no-HSCT2 groups, respectively. A similar percent of patients had acute GvHD and/or chronic GvHD prior to relapse.

Characteristics of the second hematopoietic stem cell transplantation

The median time from relapse to HSCT2 was 4.5 months (range, 0.3-91). Eighteen patients (29%) underwent HSCT2 within 3 months of relapse. The median time from first to second HSCT was 23 months (range, 3.3-146). Seventeen patients (27%) underwent HSCT2 within less than 1 year of the first HSCT. At the time of HSCT2, 29 patients (47%) were in complete remission, and 33 patients (53%) had active disease. The donor for HSCT2 was a different donor in all second transplants; either a second HLA-matched sibling (n=13, 21%), a matched unrelated donor (n=39, 63%), or a haplo-identical donor (n=10, 16%). The conditioning regimen in the matched transplants included a sequential salvage and reduced intensity conditioning (FLAMSA-like) in six patients (treosulfan-based in 2 of these patients), fludarabine with 2 days of busulfan in seven patients, fludarabine with 4 days of busulfan in five patients, and fluradabine-treosulfan in 34 patients. Among the ten recipients of haplo-identical transplants, five were given thiotepa/busulfan/fludarabine with post-transplant cyclophosphamide and five were given T-cell-depleted transplants. In all, conditioning intensity was determined as low intensity in 29 patients and intermediate intensity in 33 patients according to the redefined European Society for Blood and Marrow Transplantation (EBMT) criteria.

Outcome after a second hematopoietic stem cell transplantation

Fifty-seven of the 62 patients who were given HSCT2 following relapse engrafted at a median of 12 days (range, 9-32). Five patients died prior to engraftment. The median follow-up was 55 months (range, 8-188 months). The 100-day cumulative incidence of acute GvHD was 31% (95% confidence interval [95% CI]: 21-46%) and the 1-year cumulative incidence of chronic GvHD was 15% (95% CI: 8-27%). In all, at last follow-up, 17 patients were alive, and 45 patients had died. Sixteen patients died of non-relapse causes including GvHD (n=3), infection (n=4), and organ toxicity (n=9). The 2-year cumulative incidence of non-relapse mortality was 26% (95% CI: 17-39%) (Figure 1A). Forty-nine patients relapsed after HSCT2, with a 2-year cumulative incidence of relapse of 50% (95% CI: 39-65%) (Figure 1B). Four of these patients were alive after a second relapse, three of them in the long-term. The 2-year and 5-year overall survival rates were 38%

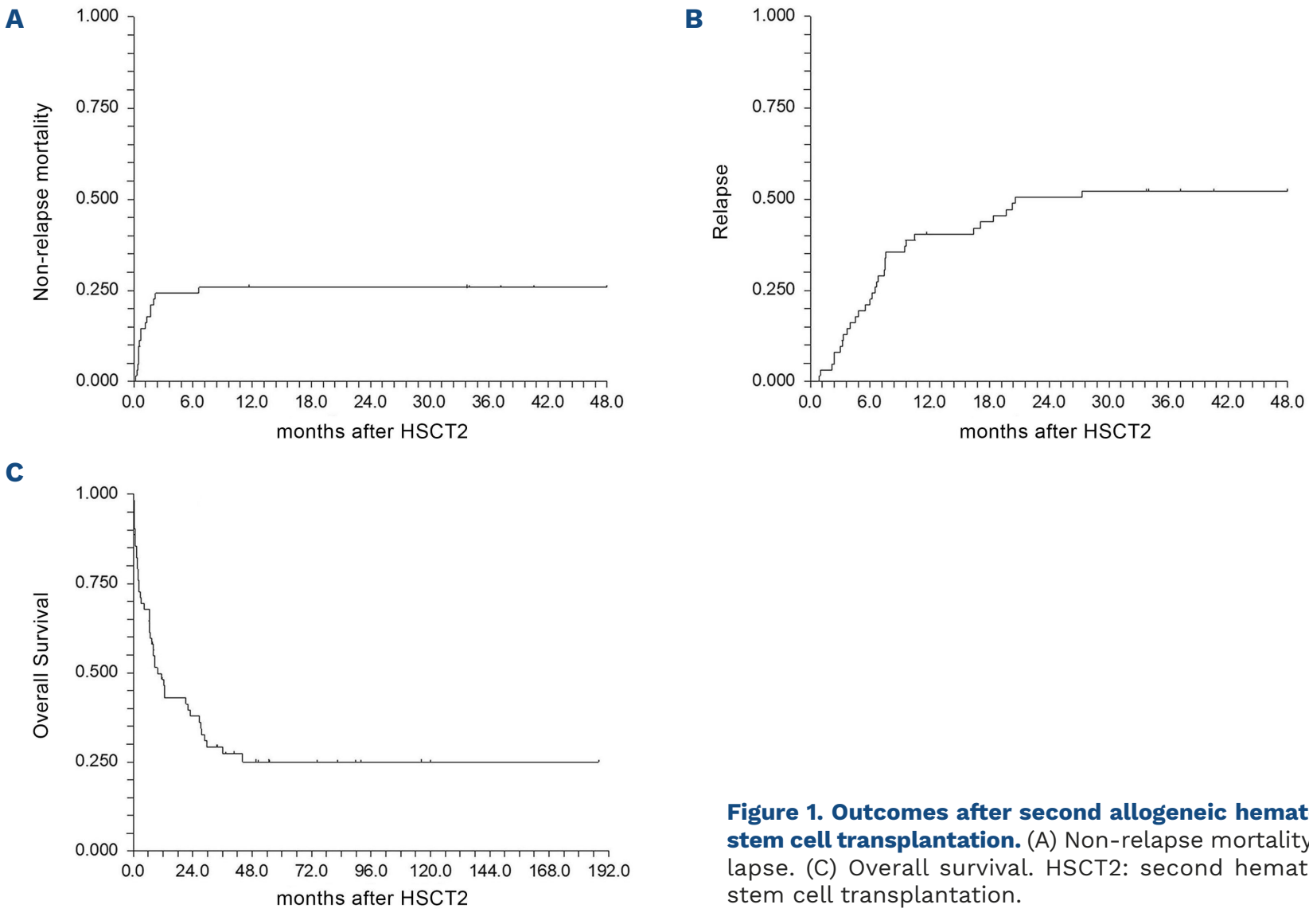

Figure 1. Outcomes after second allogeneic hematopoietic stem cell transplantation. (A) Non-relapse mortality. (B) Relapse. (C) Overall survival. HSCT2: second hematopoietic stem cell transplantation.

Table 2. Univariate analysis of factors predicting 2-year overall survival after second hematopoietic stem cell transplantation.

	N	Alive (N)	2-year OS, percent (95% CI)	*P* value
All patients	62	17	38 (26-50)	
Age				
<55 years	41	12	40 (25-55)	0.34
>55 years	21	5	33 (13-54)	
Gender				
Female	36	12	49 (33-66)	0.001
Male	26	5	22 (5-40)	
Diagnosis				
AML	47	16	44 (29-58)	0.04
MDS	15	1	20 (0-40)	
ELN risk (AML only)				
Good	7	5	71 (38-100)	
Intermediate	17	5	39 (16-69)	0.02
Poor	16	3	20 (0-39)	
Missing	8	3	40 (19-61)	
Status at 1st HSCT				
CR1/MDS no blasts	27	9	47 (28-66)	0.26
CR2	5	2	30 (0-77)	
Active disease	30	6	30 (14-46)	
Conditioning 1st HSCT				
MAC	30	12	52 (34-71)	0.11
RIC (low intensity)	9	3	33 (3-64)	
RTC (intermediate intensity)	23	2	22 (5-39)	
GvHD prophylaxis				
CSA/MTX	55	17	43 (30-56)	0.09
Other	7	0	0 (0-40)	
Stem cell source				
Peripheral blood stem cells	59	16	38 (26-51)	0.76
Bone marrow	3	1	33 (0-87)	
Donor for 1st HSCT				
Sibling	31	10	41 (23-59)	0.67
Matched unrelated	31	7	35 (18-52)	
F to M				
Yes	10	2	15 (0-40)	0.02
No	52	15	43 (29-56)	
Prior acute GvHD				
Yes	16	4	28 (5-51)	0.42
No	46	13	41 (27-55)	
Prior chronic GvHD				
Yes	17	6	43 (18-68)	0.40
No	45	11	36 (22-50)	
Median year of relapse				
≤ 2012	27	5	26 (9-43)	0.15
> 2012	35	12	48 (31-65)	
Time from relapse to 2nd HSCT				
<3 months	18	5	36 (13-59)	0.68
>3 months	44	12	36 (21-50)	
Time from 1st to 2nd HSCT				
<1 year	17	4	23 (3-44)	0.05
>1 year	45	13	43 (28-58)	
Status at 2nd HSCT				
Active disease	33	7	33 (17-49)	0.23
Complete remission	29	10	40 (22-59)	
Donor for 2nd HSCT				
Sibling	13	6	42 (14-70)	0.02
Matched unrelated	39	10	25 (11-38)	
Haplo-identical	10	1	10 (0-20+)	
Conditioning for 2nd HSCT				
Low intensity	29	7	27 (23-58)	0.45
Intermediate intensity	33	10	24 (9-40)	
Treosulfan in 2nd HSCT				
Yes	36	14	36 (20-53)	0.007
No	26	3	11 (0-24)	

HSCT: hematopoietic stem cell transplantation; AML: acute myeloid leukemia; MDS: myelodysplastic syndrome; ELN: European Leukemia Network; CR1: first complete remission; CR2: second complete remission; MAC: myeloablative conditioning; RIC: reduced-intensity conditioning; RTC: reduced toxicity myeloablative conditioning; GvHD: graft-*versus*-host disease; CSA: cyclosporine A; MTX: methotrexate; F to M: female donor to male recipient.

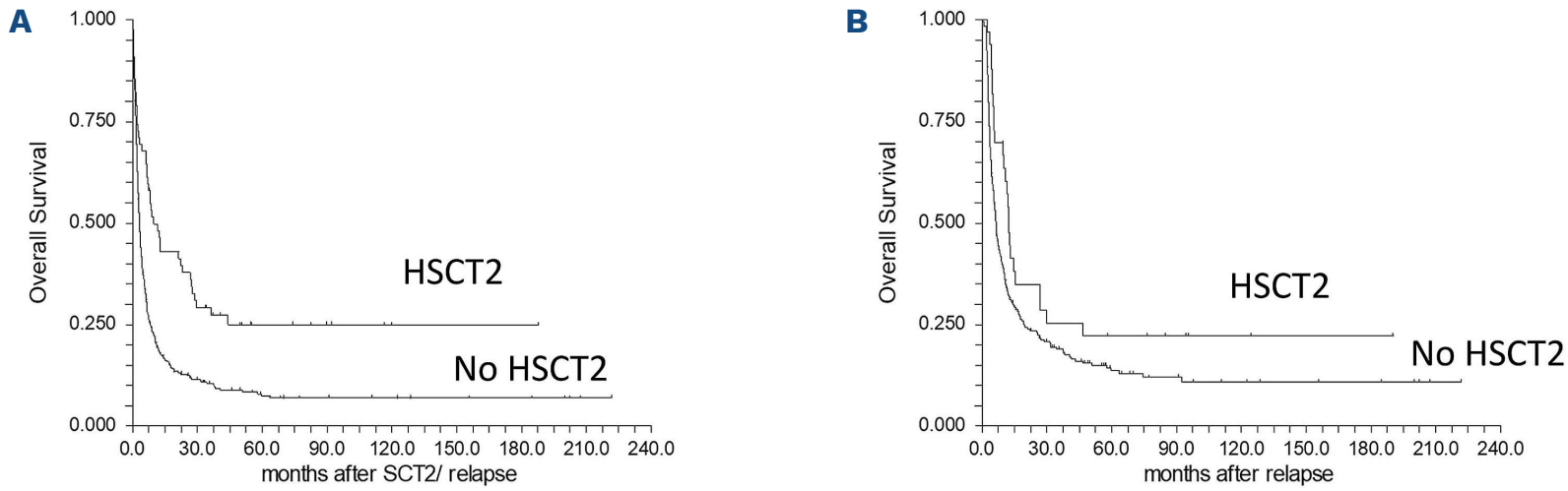

Figure 2. Overall survival after relapse. (A) Overall survival in 345 patients not given a second hematopoietic stem cell transplant (HSCT2) compared with overall survival after HSCT2 in 62 recipients (calculated from the day of the second transplant). (B) Landmark analysis at 60 days after relapse according to whether patients did or did not have a HSCT2.

(95% CI: 26-50%) and 25% (95% CI: 14-36%), respectively (Figure 1C). The 2-year and 5-year leukemia-free survival rates were 24% (95% CI: 13-34%) and 18% (95% CI: 8-29%), respectively. Seven patients were given maintenance treatment after HSCT2, including azacytidine (n=5) and sorafenib (n=2). Two of these patients are long-term survivors.

Factors predicting 2-year overall survival after second hematopoietic stem cell transplantation

Table 2 outlines the univariate analysis of factors predicting 2-year overall survival after HSCT2. A 2-year overall survival advantage was statistically significant for females compared to males (*P*=0.001), AML compared to MDS (*P*=0.04), favorable European LeukemiaNetwork (ELN[24]) risk (*P*=0.02), a prior remission duration from HSCT to first relapse that was longer than 6 months (*P*=0.05), and a matched sibling as a donor at HSCT2 (*P*=0.03) compared to an unrelated or haplo-identical donor. Interestingly, the survival of patients with active disease at HSCT2 was not statistically significantly different from that of patients in complete remission. However, six of the 33 patients with active disease at HSCT2 had not had therapy prior to HSCT. When these patients were excluded the 2-year overall survival was 14% (95% CI: 6-34) compared with 40% (95% CI: 22-59) in patients in complete remission (*P*=0.10). The use of treosulfan in the conditioning regimen was also associated with better outcome in the univariate analysis (*P*=0.002). Survival in more recent years has improved, but this difference has not reached statistical significance. Post-transplant maintenance therapy was given to a small group of patients so a meaningful evaluation of its role was not possible.

Table 3 outlines the multivariate analysis of factors predicting survival after a second HSCT. The analysis identified female gender as a factor predicting improved survival rate (HR=0.31, *P*=0.001). Short remission after first HSCT (HR=2.31, *P*=0.05), acute GvHD after first HSCT (HR=2.27, *P*=0.035) and HSCT2 from a haplo-identical donor (HR=13.04, *P*=0.001) or matched unrelated donor (HR=4.53, *P*=0.007) predicted lower survival rates. Earlier year of relapse after the first HSCT (in or before 2012) was associated with inferior survival (HR=2.46, *P*=0.012).

Table 3. Multivariate analysis of factors predicting survival after second hematopoietic stem cell transplantation.

	HR (95% CI)	*P* value
Gender (female)	0.35 (0.15-0.66)	0.001
Short remission	2.31 (1.00-5.34)	0.050
Prior acute GvHD	2.27 (1.06-4.87)	0.035
Donor for 2nd HSCT		
Haplo-identical	13.4 (3.52-51.3)	0.001
Matched unrelated	4.53 (1.52-13.5)	0.007
Year of relapse ≤ 2012	2.46 (1.22-4.96)	0.031

HR: hazard ratio; 95% CI: 95% confidence interval; GvHD: graft-*versus*-host disease; HSCT: hematopoietic stem cell transplantation.

Outcome of patients who did not have a second hematopoietic stem cell transplant

A total of 345 patients did not have second HSCT following relapse. The median follow-up for patients alive was 57 months (range, 8-223). At the last follow-up, 31 patients were alive. The median survival was 3.3 months. Figure 2A shows the Kaplan-Meier survival curves from relapse. The 2-year and 5-year overall survival rates were 13% (95% CI: 9-16%) and 7% (95% CI: 4-10%), respectively. The 5-year overall survival of patients given cellular therapy other than HSCT2 from a different donor was 14% (95% CI: 7-21) compared to 25% (95% CI: 14-36) in the HSCT2 group (*P*=0.05). In the comparative group of patients who had HSCT2, the survival was calculated from the time of HSCT2 in order not to overestimate the advantage of survival from relapse to HSCT2.

Factors predicting 2-year overall survival after relapse following hematopoietic stem cell transplantation

We analyzed data from 407 consecutive patients who relapsed after a first HSCT. The median follow-up of patients alive after relapse was 60 months (range, 8-222). At last follow-up, 47 patients were alive. The 2-year overall survival rate for the entire group was 18% (95% CI: 14-22). Univariate analysis of factors predicting 2-year overall survival after first relapse is presented in Table 4. Improved survival was seen in females (P=0.003), those with favorable ELN risk (P=0.008), patients in complete remission at HSCT (P=0.05), patients given myeloablative conditioning (P=0.006), those given methotrexate as GvHD prophylaxis (P=0.001), not a female donor to male recipient combination at first HSCT (P=0.04) and relapse in more recent years (after 2012, P=0.002). A significant advantage in 2-year overall survival rate was seen in patients with relapse occurring more than 6 months after HSCT (P<0.001). To reduce the bias of time to HSCT2 we analyzed only patients having HSCT2 within 6 months of relapse and found that having HSCT2 was a highly significant predictive factor in this univariate analysis (P=0.0002).

For multivariate analysis we used a Cox proportional hazard model with HSCT2 entered as a time-dependent variant. Patients who did not have HSCT2 had an inferior survival (HR=1.66, P=0.010). Other factors predicting an inferior outcome were relapse within the first 6 months after HSCT (HR=2.32, P<0.001) and acute GvHD before relapse (HR=1.47, P=0.005). Relapse in earlier years (before 2012) was associated with inferior overall survival (HR=1.33, P=0.031). Myeloablative conditioning after first HSCT and female gender predicted improved outcome (Table 5). Since only 15% had HSCT2, the performance of HSCT2 was the only post-HSCT2 factor included (as a time-dependent factor), while subgroups of second transplant characteristics could not be analyzed in this group.

To further explore the role of HSCT2 we used a landmark analysis set at 60 days after relapse. At this landmark, 65 patients were in remission, 208 patients were alive with active disease, nine patients had already undergone HSCT2 due to relapse or graft failure, 112 patients had died, and four patients were lost to follow-up. A total of 33 patients who were alive at the 2-month landmark underwent HSCT2 within the following 4 months. The 2-year and 5-year overall survival rates were 35% (95% CI: 18-51%) and 22% (95% CI: 8-32%), respectively. Among all other patients alive at the 2-month landmark, 2-year and 5-year overall survival rates were 23% (95% CI: 18-29%) and 13% (95% CI: 8-18%), respectively (P=0.03) (Figure 2B). Of the latter, 24 patients underwent HSCT2 later in their disease course (>6 months).

Discussion

A second HSCT is a potentially curative treatment for patients with relapsed AML or MDS after a first transplant. The current single-center study shows that approximately 25% of HSCT2 recipients achieve long-term survival. A similar outcome has been found in several other retrospective studies[6-17] and is better than expected with no additional cellular therapy.[3,6,25] In the current study we analyzed the results of HSCT2 in the context of all relapsing AML or MDS patients with the intent to explore the independent effect of HSCT2. Despite our policy to offer a second transplant to patients relapsing after a first HSCT, only 15% of our patients did eventually undergo HSCT2. This group included patients who were younger and with a longer time to relapse, and as such with a better expected survival. The median time from relapse to HSCT2 was 4.5 months, while 28% of all patients had already died in the first 2 months after relapse and could not have been considered for HSCT2. After adjusting for these biases by multivariate analysis and by considering HSCT2 as a time-dependent variable, HSCT2 remained an independent positive prognostic factor for survival after relapse. In addition, we also performed a landmark analysis of patients alive 2 months after first relapse and treated with HSCT2 compared with other treatments and showed a similar survival advantage for HSCT2. These analyses are based on retrospective data and may not completely adjust for unknown considerations that led the attending physicians to select patients for HSCT2. A randomized study comparing HSCT2 to other treatments may be the only way to prove the advantage of the former but is unlikely to be performed because of the high probability of physicians' reluctance to include patients in such a study with the possibility of deferring a curative approach. In the absence of such studies the current analysis supports an independent advantage of HSCT2.

The definition of a second HSCT is not well established. HSCT2 with a different donor from the one used for the first transplant can obviously be defined as a second transplant. However, when using the same donor, peripheral blood stem cells left from the original HSCT or recollected can be used to support non-myeloablative salvage chemotherapy, with no or minimal immune suppression. This can be defined as a second transplant or, more appropriately, as donor lymphocyte infusion (mobilized donor lymphocyte infusion) or a form of cellular therapy.[26] To circumvent these differences in definition, we included in the subgroup of patients who underwent HSCT2 only those patients who received the second transplant from a different donor (another HLA-matched sibling, a matched unrelated donor, or a haplo-identical donor). The selection of a different donor is based on the assumption that this may provide a graft-*versus*-leukemia

Table 4. Univariate analysis of factors predicting 2-year overall survival after first relapse.

	N	Alive (N)	2-year OS, percent (95% CI)	*P* value
All patients	407	47	18 (14-22)	
Age				
<55 years	187	27	22 (16-28)	0.06
≥55 years	220	20	15 (10-19)	
Gender				
Female	172	25	25 (19-32)	0.003
Male	235	22	13 (9-17)	
Diagnosis				
AML	338	37	18 (14-22)	0.22
MDS	69	10	20 (11-29)	
ELN risk (AML only)				
Good	35	10	29 (14-45)	0.008
Intermediate	136	16	20 (14-27)	
Poor	123	5	12 (6-17)	
Missing	44	6		
Status at 1st HSCT				
CR1/MDS no blasts	154	18	21 (14-27)	0.05
CR2	46	8	26 (13-39)	
Active disease	207	21	15 (10-20)	
Conditioning for 1st HSCT				
MAC	153	26	24 (17-31)	0.006
RIC	108	11	16 (9-22)	
RTC	136	10	14 (8-20)	
GvHD prophylaxis				
CSA/MTX	321	42	21 (17-26)	0.001
Other	86	5	6 (1-12)	
Stem cell source				
Peripheral blood stem cells	399	46	18 (14-22)	0.89
Bone marrow	8	1	13 (0-35)	
Donor for 1st HSCT				
Sibling	200	23	17 (12-23)	0.34
Matched unrelated	207	24	19 (14-24)	
F to M				
Yes	79	5	20 (16-24)	0.04
No	328	42	11 (4-18)	
Prior acute GvHD				
Yes	126	11	13 (7-19)	0.13
No	281	36	21 (16-25)	
Prior chronic GvHD				
Yes	82	11	24 (15-33)	0.10
No	325	36	17 (13-20)	
Time from HSCT to relapse				
<6 months	243	17	11 (8-16)	< 0.001
>6 months	164	30	29 (22-36)	
2nd HSCT within 6 months of relapse				
Yes	38	9	36 (20-51)	0.0002
No	329	38	16 (13-20)	
Median year of relapse				
≤ 2012	213	17	14 (9-18)	0.0002
> 2012	192	30	23 (17-29)	

OS: overall survival; 95% CI: 95% confidence interval; AML: acute myeloid leukemia; MDS: myelodysplastic syndrome; ELN: European Leukemia Network; HSCT: hematopoietic stem cell transplantation; CR1: first complete remission; CR2: second complete remission; MAC: myeloablative conditioning; RIC: reduced intensity conditioning; RTC: reduced toxicity myeloablative conditioning; GvHD: graft-*versus*-host disease; CSA: cyclosporine A; MTX: methotrexate; F to M: female donor to male recipient.

effect that was not induced by the first transplant or that may overcome resistance mechanisms against the first given immune system. However, there are no data to support HSCT2 from a different donor being more effective.[2,8,10,11,13] Thus, our data may underestimate the advantage of HSCT2. In addition, other forms of cellular therapies, such as donor lymphocyte infusion or mobilized donor lymphocyte infusion can also be associated with long-term survival and there are currently no data to support HSCT2 over these other forms of cellular therapy.[7,15] The other poor prognostic factors for survival after relapse identified by the multivariate analysis were short duration of prior remission and prior acute GvHD. Female patients and patients given myeloablative conditioning at the first HSCT had better outcomes. In line with most previous studies,[6-10,25] our study showed that relapse within 6 months after the first HSCT was consistently associated with poor outcome, also among patients who were able to proceed with HSCT2. This reflects an aggressive biology of the underlying leukemia and, in our series, overrode the role of other prognostic factors such as cytogenetics and ELN classification. The results of all therapies in these patients are dismal and such patients are often offered palliative care alone. However, a small fraction of these patients (approximately 10%) did enjoy long survival and they should not be automatically deferred from an intensive treatment approach. The better outcome of patients given myeloablative conditioning is not consistent in all studies.[15] Patients initially given myeloablative conditioning may be fitter for additional treatments even after relapse. In addition, the anti-leukemia effect following reduced intensity condition is more dependent on the graft-*versus*-leukemia effects.[27] Patients relapsing after reduced intensity conditioning may, therefore, respond less to immune manipulations. This may also explain the worse prognosis of patients with acute GvHD before relapse. We previously reported better outcomes in female recipients.[5] This was also seen in the current study but not in other large series. There is no definite biological explanation for this observation and it merits further study. The prognostic factors for poor survival in the group of patients who had a HSCT2 included male gender, short duration from first HSCT to relapse and prior acute GvHD. We also found that HSCT2 from a second unrelated donor and in particular from a haplo-identical donor was associated with inferior survival. An inferior outcome of HSCT from a second unrelated donor has been described in earlier reports.[9] A recent EBMT report showed that haplo-identical second transplants may be associated with lower survival due to increased non-relapse mortality.[2] However, a subsequent EBMT report found no difference between outcomes following transplants from haplo-identical or unrelated HSCT2 donors.[28] The group of second haplo-identical transplants in the current study was too small to enable definite conclusions. In addition, about half of the transplants were T-cell-depleted while the recent EBMT studies included patients conditioned with post-transplant cyclophosphamide, which may be much safer. Due to the small numbers and in order to create a less heterogeneous group, we did not include patients with a first HSCT from a haplo-identical donor in our study. In these patients a different haplo-identical donor is usually required for a second haplo-identical HSCT to overcome the possibility of leukemia immune escape by loss of the unshared haplotype.[29] We did include HSCT2 from a haplo-identical donor as we wanted to explore all HSCT2 options and the potential role of the graft-*versus*-leukemia effect from a mismatched donor transplant after failure of a matched donor transplant. In all, it seems there is no graft-*versus*-leukemia advantage from haplo-identical transplantation that could justify preferential switching to a haplo-identical donor in a second transplant.

The status of disease at HSCT2 has been shown in multiple studies to be an important predictive factor for outcome, with patients transplanted in remission having significantly better outcome. We did not find such an association in the current study possibly because of the small number of patients. However, some of the patients with active disease at HSCT2 had not been previously treated at the time of the HSCT2. When these patients were excluded the 2-year overall survival of this group was much lower at 14%, but still some were salvaged with HSCT2. We used treosulfan-based conditioning in the majority of HSCT2. Treosulfan has shown some advantages compared to other regimens in patients with active disease.[30] In the current series, treosulfan was indeed associated with a survival advantage in the HSCT2 setting in the univariate analysis, but not in the multivariate analysis. Other studies have not shown an advantage for any conditioning regimen in HSCT2.[2] It seems that a small subset of patients who do not achieve a stringent re-

Table 5. Multivariate analysis of factors predicting survival after relapse.

	HR (95% CI)	*P* value
No 2nd HSCT	1.66 (1.13-2.45)	0.010
Short remission	2.32 (1.75-3.06)	< 0.001
Female gender	0.71 (0.54-0.91)	0.007
MAC for 1st HSCT	0.67 (0.49-0.91)	0.011
Prior acute GvHD	1.47 (1.11-1.93)	0.005
Year of relapse ≤ 2012	1.33 (1.03-1.74)	0.031

HR: hazard ratio; 95% CI: 95% confidence interval; HSCT: hematopoietic stem cell transplantation; MAC: myeloablative conditioning; GvHD: graft-*versus*-host disease.

mission with salvage chemotherapy prior to HSCT2 can still benefit from a second transplant, but this subgroup should be defined better in larger studies.

With modern transplantation techniques, non-relapse mortality after HSCT2 is relatively acceptable. The current study, in line with other studies, has shown that the outcomes following post-transplant relapse and HSCT have improved in recent years.[4,7] However, the major obstacle to cure remains a very high incidence of relapse, with a 2-year cumulative incidence of 50% in the current series. The chances of prolonged survival after a post-HSCT2 relapse are very low.[31] Novel maintenance therapies and immune therapies need to be explored in an attempt to improve the survival.

In conclusion, relapse of AML or MDS following HSCT is associated with a relatively poor outcome. A second HSCT can be curative in a subset of patients, in particular those with longer remission after the first HSCT.

Disclosures

No conflicts of interest to disclose.

Contributions

YY and AS designed the study, analyzed the data and wrote the manuscript. NS, ID, JC, AA, RY, AN, and AS collected patients' data. YY and AS performed the statistical analysis. All authors revised the manuscript and approved the last version.

Data-sharing statement

The data are available from the corresponding author upon reasonable request.

References

1. Gooley TA, Chien JW, Pergam SA, et al. Reduced mortality after allogeneic hematopoietic-cell transplantation. N Engl J Med. 2010;363(22):2091-2101.
2. Shimoni A, Labopin M, Finke J, et al. Donor selection for a second allogeneic stem cell transplantation on AML patients relapsing after a first transplant: a study of the Acute Leukemia Working Party of EBMT. Blood Cancer J. 2019;9(12):88.
3. Bejanyan N, Weisdorf DJ, Logan BR, et al. Survival of patients with acute myeloid leukemia relapsing after allogeneic hematopoietic cell transplantation: a Center for International Blood and Marrow Transplant Research study. Biol Blood Marrow Transplant. 2015;21(3):454-459.
4. Bazarbachi A, Schmid C, Labopin M, et al. Evaluation of trends and prognosis over time in patients with AML relapsing after allogeneic hematopoietic cell transplant reveals improved survival for young patients in recent years. Clin Cancer Res. 2020;26(24):6475-6482.
5. Shem-Tov N, Saraceni F, Danylesko I, et al. Isolated extramedullary relapse of acute leukemia after allogeneic stem cell transplantation: different kinetics and better prognosis than systemic relapse. Biol Blood Marrow Transplant. 2017;23(7):1087-1094.
6. Schmid, C, Labopin M, Nagler A, et al. Treatment, risk factors, and outcome of adults with relapsed AML after reduced intensity conditioning for allogeneic stem cell transplantation. Blood. 2012;119(6):1599-1606.
7. Zuanelli Brambilla C, Lobaugh SM, Ruiz JD, et al. Relapse after allogeneic stem cell transplantation of acute myelogenous leukemia and myelodysplastic syndrome and the importance of second cellular therapy. Transplant Cell Ther. 2021;27(9):771.
8. Eapen, M, Giralt SA, Horowitz MM, et al. Second transplant for acute and chronic leukemia relapsing after first HLA-identical sibling transplant. Bone Marrow Transplant. 2004;34(8):721-727.
9. Shaw BE, Mufti GJ, Mackinnon S, et al. Outcome of second allogeneic transplants using reduced-intensity conditioning following relapse of hematological malignancy after an initial allogeneic transplant. Bone Marrow Transplant. 2008;42(12):783-789.
10. Orti G, Sanz J, Bermudez A, et al. Outcome of second allogeneic hematopoietic cell transplantation after relapse of myeloid malignancies following allogeneic hematopoietic cell transplantation: a retrospective cohort on behalf of the Grupo Español de Trasplante Hematopoyetico. Biol Blood Marrow Transplant. 2016;22(3):584-588.
11. Christopeit M, Kuss O, Finke J, et al. Second allograft for hematologic relapse of acute leukemia after first allogeneic stem-cell transplantation from related and unrelated donors: the role of donor change. J Clin Oncol. 2013;31(26):3259-3271.
12. Yaniv I, Krauss AC, Beohou E, et al. Second hematopoietic stem cell transplantation for post-transplantation relapsed acute leukemia in children: a retrospective EBMT-PDWP study. Biol Blood Marrow Transplant. 2018;24(8):1629-1642.
13. Ruutu T, de Wreede LC, van Biezen A, et al. Second allogeneic transplantation for relapse of malignant disease: retrospective analysis of outcome and predictive factors by the EBMT. Bone Marrow Transplant. 2015;50(12):1542-1550.
14. Michallet M, Tanguy ML, Socié G, et al. Second allogeneic haematopoietic stem cell transplantation in relapsed acute and chronic leukaemias for patients who underwent a first allogeneic bone marrow transplantation: a survey of the Société Française de Greffe de Moelle (SFGM). Br J Haematol. 2000;108(2):400-407.
15. Kharfan-Dabaja MA, Labopin M, Polge E, et al. Association of second allogeneic hematopoietic cell transplant vs donor lymphocyte infusion with overall survival in patients with acute myeloid leukemia relapse. JAMA Oncol. 2018;4(9):1245-1253.
16. Vrhovac R, Labopin M, Ciceri F, et al. Second reduced intensity conditioning allogeneic transplant as a rescue strategy for acute leukaemia patients who relapse after an initial RIC allogeneic transplantation: analysis of risk factors and treatment outcomes. Bone Marrow Transplant. 2016;51(2):186-193.
17. Kedmi M, Resnick IB, Dray L, et al. A retrospective review of the outcome after second or subsequent allogeneic transplantation. Biol Blood Marrow Transplant. 2009;15(4):483-489.
18. Bacigalupo A, Ballen K, Rizzo D, et al. Defining the intensity of conditioning regimens: working definitions. Biol Blood Marrow Transplant. 2009;15(12):1628-1633.
19. Spyridonidis A, Labopin M, Savani BN, et al. Redefining and measuring transplant conditioning intensity in current era: a study in acute myeloid leukemia patients. Bone Marrow Transplant. 2020;55(6):1114-1125.
20. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus

Conference on acute GVHD grading. Bone Marrow Transplant. 1995;15(6):825-828.
21. Jagasia MH, Greinix HT, Arora M, et al. National Institutes of Health Consensus Development Project on criteria for clinical trials in chronic graft-versus-host disease: I. The 2014 Diagnosis and Staging Working Group report. Biol Blood Marrow Transplant. 2015;21(3):389-401.
22. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc. 1958;53(282):457-481.
23. Gooley TA, Leisenring W, Crowley J, Storer BE. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. Stat Med. 1999;18(6):695-706.
24. 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(4):424-447.
25. Thanarajasingam G, Kim HT, Cutler C, et al. Outcome and prognostic factors for patients who relapse after allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2013;19(12):1713-1718.
26. Abbi KK, Zhu J, Ehmann WC, et al. G-CSF mobilized vs conventional donor lymphocytes for therapy of relapse or incomplete engraftment after allogeneic hematopoietic transplantation. Bone Marrow Transplant. 2013;48(3):357-362.
27. Baron F, Labopin M, Niederwieser D, et al. Impact of graft-versus-host disease after reduced-intensity conditioning allogeneic stem cell transplantation for acute myeloid leukemia: a report from the Acute Leukemia Working Party of the European Group for Blood and Marrow Transplantation. Leukemia. 2012;26(12):2462-2468.
28. Kharfan-Dabaja MA, Labopin M, Bazarbachi A, et al. Comparing outcomes of a second allogeneic hematopoietic cell transplant using HLA-matched unrelated versus T-cell replete haploidentical donors in relapsed acute lymphoblastic leukemia: a study of the Acute Leukemia Working Party of EBMT. Bone Marrow Transplant. 2021;56(9):2194-2202.
29. Vago L, Perna SK, Zanussi M, et al. Loss of mismatched HLA in leukemia after stem-cell transplantation. N Engl J Med. 2009;361(5):478-488.
30. Shimoni A, Labopin M, Savani B, et al. Intravenous busulfan compared with treosulfan-based conditioning for allogeneic stem cell transplantation in acute myeloid leukemia: a study on behalf of the Acute Leukemia Working Party of European Society for Blood and Marrow Transplantation. Biol Blood Marrow Transplant. 2018;24(4):751-757.
31. Shimoni A. Relapse of acute leukemia after a second allogeneic stem-cell transplantation; is there any hope for cure? Bone Marrow Transplant. 2022;57(3):336-337.