Optimizing transplantation procedures through identification of prognostic factors in second remission for children with acute myeloid leukemia with no prior history of transplant

Children with acute myeloid leukemia (AML) who present with a high likelihood of relapse are indicated for hematopoietic stem cell transplantation (HSCT) as a consolidation therapy in the first complete remission (CR1).[1] However, the proportion of patients undergoing HSCT in the CR1 in recently published clinical trials varied significantly, ranging from 8% to 29%.[1,2] Therefore, the patient population that would benefit from HSCT in the CR1 remains a topic of ongoing debate.[1,2]

In marked contrast to the CR1, nearly all research groups offer HSCT for all relapsed children in the second complete remission (CR2).[1,2] Previous studies have identified various prognostic indicators in pediatric patients with relapsed AML, including a history of HSCT, the year of HSCT, the duration of CR1, and achieving the CR2 prior to HSCT.[3,4] In addition, it is necessary to identify other modifiable prognostic factors associated with HSCT, such as donor type and conditioning regimen. Furthermore, given the inherent heterogeneity of relapsed AML, it is crucial to identify prognostic factors specifically in patients who underwent their first HSCT in their CR2, as this population is expected to benefit from HSCT.

In order to evaluate the characteristics and prognostic factors of children with AML undergoing their first allogenic HSCT in their CR2, data from 225 patients were collected through the Transplant Registry Unified Management Program (TRUMP).[5] The inclusion criteria for patients were as follows: (i) *de novo* non-M3 AML, (ii) age <16 years and in the CR2 at the time of undergoing allogeneic HSCT, (iii) no prior history of HSCT, and (iv) HSCT performed between 2000 and 2019. Patients with Down syndrome (DS) were excluded. Performance status was applied as defined by the Eastern Cooperative Oncology Group. The myeloablative conditioning (MAC) regimen was defined in a hierarchical manner as follows: (i) a total-body irradiation (TBI)-based regimen, which included >8 Gy TBI; (ii) a busulfan-based regimen, which included ≥9 mg/kg busulfan; or (iii) a melphalan-based regimen, which included >140 mg/m^2 melphalan. All other regimens were analyzed as reduced-intensity conditioning regimens.[6] High-risk cytogenetics/genetics were defined as previously described.[7] The patients or their parents provided written consent to undergo transplantation and for the use of medical records for research in accordance with the Declaration of Helsinki. The study was approved by the Data Management Committee of the TRUMP and the Institutional Ethics Committee of Okayama University (2205-004). Probabilities of overall survival (OS) and event-free survival (EFS) were calculated using Kaplan–Meier estimators. EFS was defined as survival in continuous CR after HSCT. Relapse was defined by morphological relapse of ≥ 5% blasts in bone marrow or extramedullary relapse. Non-relapse mortality (NRM) was defined as death from any cause other than relapse. Competing events were death without relapse for hematological relapse, and hematological relapse for NRM. Univariate analysis was performed using the log-rank test, and multivariate analysis was conducted using the Cox proportional hazard regression model. Factors that were known to affect patient outcomes after HSCT were entered into the multivariate analysis, including the age at HSCT, duration of CR1, year of HSCT, conditioning regimen, donor source, and human leukocyte antigen disparities. French-American-British (FAB) classifications and cytogenetic/genetic classifications were also entered based on the univariate analysis results. All statistical analyses were performed using EZR (Version 1.54. Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R (Version 4.2.2. The R Foundation for Statistical Computing, Vienna, Austria).[8] P<0.05 was considered significant for all analyses.

Table 1 includes the characteristics of the 225 patients in this study. The median age at HSCT was 8 years old (range, 1–15), and the median follow-up period for survivors was 7.95 years (range, 0.18–19.2).

The outcomes of the included patients are summarized in Figure 1A. The univariate analysis for EFS identified the duration of CR1, FAB classifications, cytogenetic/genetic classifications, and conditioning regimens as prognostic factors (the last one non-significant), while the duration of CR1 was unknown for 26.2% of patients (Table 1). We also performed univariate analyses for OS, the cumulative incidence of relapse, and the cumulative incidence of NRM (*Online Supplementary Table S1*). Among conditioning regimens, apart from “other MAC”, which was administered for only three patients, busulfan/melphalan-based MAC showed the best outcomes, with a 5-year-EFS (5y-EFS) of 75.8% (95% confidence interval [CI]: 58.5–86.7),

Table 1. Univariate analysis for event-free survival.

Factor	Group	N (%)	5y-EFS (95% CI)	*P*
Age in years at HSCT	0-4	73 (32.4)	58.4 (45.8-69.0)	0.238
	5-9	62 (27.6)	67.7 (53.6-78.4)	
	10-15	90 (40.0)	61.1 (49.8-70.6)	
PS	0-1	189 (84.0)	63.1 (55.5-69.8)	0.785
	2-4	8 (3.6)	62.5 (22.9-86.1)	
	NA	28 (12.4)	55.8 (35.5-72.0)	
Duration of the first CR	≤1 year	23 (10.2)	36.0 (17.0-55.6)	0.001
	>1 year	143 (63.6)	68.9 (60.1-76.1)	
	NA	59 (26.2)	55.9 (42.0-67.7)	
Interval from the CR2 to HSCT	≤79 days	87 (38.7)	57.2 (45.3-67.4)	0.320
	>79 days	82 (36.4)	69.2 (57.6-78.3)	
	NA	56 (24.9)	59.0 (44.6-70.8)	
FAB classification	M0	9 (4.0)	60.0 (19.5-85.2)	0.005
	M1	31 (13.8)	64.2 (44.7-78.4)	
	M2	92 (40.9)	66.4 (55.3-75.4)	
	M4	32 (14.2)	71.7 (52.7-84.2)	
	M5	34 (15.1)	59.2 (38.8-74.8)	
	M6	5 (2.2)	53.3 (6.8-86.3)	
	M7	16 (7.1)	18.8 (4.6-40.2)	
	Others	2 (0.9)	50.0 (0.6-91.0)	
	NA	4 (1.8)	NA	
Year of HSCT	2000-2009	126 (56.0)	60.4 (51.2-68.4)	0.455
	2010-2019	99 (44.0)	64.0 (52.6-73.4)	
Cytogenetics/genetics	CBF	78 (34.7)	72.2 (60.6-80.9)	0.003
	11q23*	22 (9.8)	43.8 (21.4-64.3)	
	HR cytogenetics**	25 (11.1)	36.4 (17.8-55.3)	
	Others	100 (44.4)	63.8 (52.9-72.9)	
Conditioning regimen	TBI/Cy based MAC	81 (36.0)	49.5 (38.0-59.9)	0.088
	Bu/Cy based MAC	11 (4.9)	60.0 (25.3-82.7)	
	TBI/Mel based MAC	48 (21.3)	66.2 (50.8-77.7)	
	Bu/Mel based MAC	40 (17.8)	75.8 (58.5-86.7)	
	Mel based MAC	27 (12.0)	75.3 (48.7-89.4)	
	other MAC	3 (1.3)	100.0 (NA)	
	RIC	15 (6.7)	52.4 (22.0-75.9)	
GvHD prophylaxis	CSA ± MTX	71 (31.6)	56.0 (43.3-66.9)	0.642
	TAC ± MTX	146 (64.9)	64.3 (55.5-71.9)	
	NA	8 (3.6)	75.0 (31.5-93.1)	
HLA disparities	0	78 (34.7)	60.7 (48.5-70.9)	0.848
	1	71 (31.6)	64.1 (51.0-74.6)	
	2	47 (20.9)	62.3 (45.5-75.2)	
	NA	29 (12.9)	58.2 (38.3-73.8)	
Donor source	Rel-BM	55 (24.4)	54.2 (39.5-66.8)	0.391
	Rel-PB	21 (9.3)	61.1 (34.5-79.6)	
	UR-BM	70 (31.1)	67.7 (54.8-77.7)	
	UR-CB	79 (35.1)	62.0 (49.7-72.1)	
Donor-recipient sex match	Match	104 (46.2)	59.4 (49.0-68.5)	0.204
	Male to Female	60 (26.7)	59.7 (45.2-71.4)	
	Female to Male	44 (19.6)	59.5 (42.1-73.2)	
	NA	17 (7.6)	88.2 (60.6-96.9)	

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*The 11q23 group included those with no information on the rearrangement partner of *KMT2A* or those who did not meet the criteria of high-risk (HR) cytogenetics; 2 patients with t(11;19)(q23;p13.1) and 1 patient with t(9;11)(p22:q23). **HR cytogenetics found in the current study included 20 patients with complex karyotypes, 2 patients each with t(6;11)(q27;q23) or 7-/7q-, and 1 patient each with t(9;22)(q34;q11.2), t(7;12)(q36;p13), or *FLT3*-internal tandem duplication. Among them, 1 patient had both a complex karyotype and deletion of chromosome 7, and another patient had both a complex karyotype and t(7;12)(q36;p13). 5y-EFS: 5-year event-free survival; CI: confidence interval; HSCT: hematopoietic stem cell transplantation; PS: performance status; CR: complete remission; FAB: French-American-British; CBF: core-binding factor; TBI: total-body irradiation; Cy: cyclophosphamide; Mel: melphalan; MAC: myeloablative conditioning; RIC: reduced-intensity conditioning; CSA: cyclosporine A; TAC: tacrolimus; MTX: methotrexate; HLA: human leukocyte antigen; Rel: related; UR: unrelated; BM: bone marrow; PB: peripheral blood; CB: cord blood; GvHD: graft-*versus*-host disease; NA: not available.

Table 2. Multivariate analysis for event-free survival.

Factor		Hazard ratio (95% CI)	*P*
Age in years at HSCT	0-4	ref	
	5-9	0.83 (0.37-1.85)	0.652
	10-15	0.98 (0.42-2.31)	0.963
Duration of the first CR	≤1 year	ref	
	>1 year	0.35 (0.18-0.70)	0.003
Year of HSCT	2000-2009	ref	
	2010-2019	1.09 (0.61-1.96)	0.769
Conditioning regimen	Others	ref	
	Mel-containing non-TBI MAC	0.42 (0.20-0.86)	0.018
Donor source	Rel-BM	ref	
	Rel-PB	1.27 (0.47-3.44)	0.643
	UR-BM	0.56 (0.22-1.41)	0.219
	UR-CB	0.64 (0.32-1.28)	0.207
HLA disparities	0	ref	
	1	0.80 (0.39-1.63)	0.533
	2	0.66 (0.30-1.47)	0.312
FAB	Others	ref	
	M7	2.57 (1.06-6.20)	0.036
Cytogenetics	Others	ref	
	CBF	0.66 (0.30-1.41)	0.278
	11q23*	1.62 (0.67-3.93)	0.286
	HR cytogenetics**	1.81 (0.75-4.35)	0.186

*The 11q23 group included those with no information on the rearrangement partner of *KMT2A* or those who did not meet the criteria of high-risk (HR) cytogenetics; 2 patients with t(11;19)(q23;p13.1) and 1 patient with t(9;11)(p22:q23). **HR cytogenetics found in the current study included 20 patients with complex karyotypes, 2 patients each with t(6;11)(q27;q23) or 7-/7q-, and 1 patient each with t(9;22)(q34;q11.2), t(7;12)(q36;p13), or *FLT3*-internal tandem duplication. Among them, 1 patient had both a complex karyotype and deletion of chromosome 7, and another patient had both a complex karyotype and t(7;12)(q36;p13). CI: confidence interval; HSCT: hematopoietic stem cell transplantation; CR: complete remission; Mel: melphalan; TBI: total-body irradiation; MAC: myeloablative conditioning; Rel: related; UR: unrelated; BM: bone marrow; PB: peripheral blood; CB: cord blood; HLA: human leukocyte antigen; FAB: French-American-British; CBF: core-binding factor; NA: not available; ref: reference.

followed by melphalan-based non-TBI MAC, with a 5y-EFS of 75.3% (95% CI: 48.7–89.4; Table 1). The detailed distributions among melphalan-based non-TBI MAC and busulfan/melphalan-based MAC regimens are presented in the *Online Supplementary Table S2*. The 5y-EFS of the patients receiving chemotherapy-based MAC was significantly superior to that of patients receiving TBI-based MAC (74.4% *vs*. 56.2%, respectively; *P*=0.010).

The multivariate analysis for EFS identified a duration of CR1 of more than 1 year (hazard ratio [HR] =0.35; *P*=0.003) and a melphalan-containing non-TBI MAC regimen (HR=0.42; *P*=0.018) as independent favorable prognostic factors, while M7 was identified as an independent adverse prognostic factor (HR=2.57; *P*=0.036; Table 2). These results were similar when conditioning regimens were classified into three groups (TBI-MAC, chemotherapy-based MAC, and reduced intensity conditioning; *Online Supplementary Table S3*).

This study focused specifically on patients who had received chemotherapy alone in their CR1, and all included patients underwent HSCT for the first time after achieving their CR2. This finding implies that patients in this cohort

had a viable opportunity for treatment intensification with HSCT in their CR1. Additionally by identifying favorable prognostic factors related to HSCT, we can further optimize HSCT parameters in the CR2 as well.

Our analysis revealed that the outcomes for patients diagnosed with AML M7 who relapsed after chemotherapy alone were particularly poor, even after achieving CR2. Recently, Hama *et al.*[9] demonstrated that among those with non-DS M7, patients who underwent HSCT in their CR1 showed significantly better survival than those who underwent HSCT in their CR2 or those who did not achieve a CR. In the current study, the poor outcomes for those with M7 were reproduced in a more recent cohort (Figure 1B). Furthermore, non-DS M7 is a heterogeneous disease, and HSCT in the CR1 is recommended at least for high-risk groups in non-DS M7, such as those with *CBFA2T3::GLIS2* or *NUP98::KDM5A*.[10] A prospective trial to evaluate the efficacy of HSCT in the CR1 for this population is currently underway in Japan (*JCCG AML-20 study: jRCTs041210015*).

In pediatric patients with AML, chemotherapy-based MAC regimens have been reported to be as effective, or even more effective, than TBI-MAC regimens.[11] Our analysis further demonstrated that chemotherapy-based MAC showed comparable rates of relapse and reduced rates of NRM, resulting in improved EFS in the univariate and multivariate analyses compared with TBI-based MAC (*Online Supplementary Tables S1* and *S3*).

Excellent outcomes of chemotherapy-based MAC regimens containing melphalan have been demonstrated in previous studies,[12–14] and these regimens have been associated with a superior outcome compared with other chemotherapy-based regimens[11,15] for children with AML. In fact, European groups have adopted the use of busulfan, cyclophosphamide, and melphalan as their standard MAC regimen for pediatric AML.[12] Our results also demonstrated that patients receiving the melphalan-containing non-TBI MAC regimen achieved superior outcomes compared with those of patients receiving other regimens in their CR2 (Table 2); however, none of these patients received cyclophosphamide (*Online Supplementary Table S2*). Further research is needed to determine the optimal combination of agents to add to melphalan in the treatment of relapsed AML.

Along with adverse prognostic factors, we also identified several factors that did not alter outcomes. The year of HSCT was previously suggested to be a prognostic factor,[4] but this was not observed in our study. Furthermore, as in previous reports,[3,12] alternative-donor HSCT was feasible for these populations (Table 1; *Online Supplementary Table S1*). This information is also valuable for performing HSCT in a timely manner after achieving the CR2.

This study has several limitations. First, we identified non-DS M7 as an unfavorable prognostic factor, but we did not have information on the underlying genetic aberrations,[10] and further studies including genetic information are warranted. Second, as a retrospective study, there may be selection bias among groups. We performed a multivariate analysis to adjust for the effects of known prognostic factors, including the age at diagnosis, duration of CR1, year of HSCT, donor sources, and cytogen-

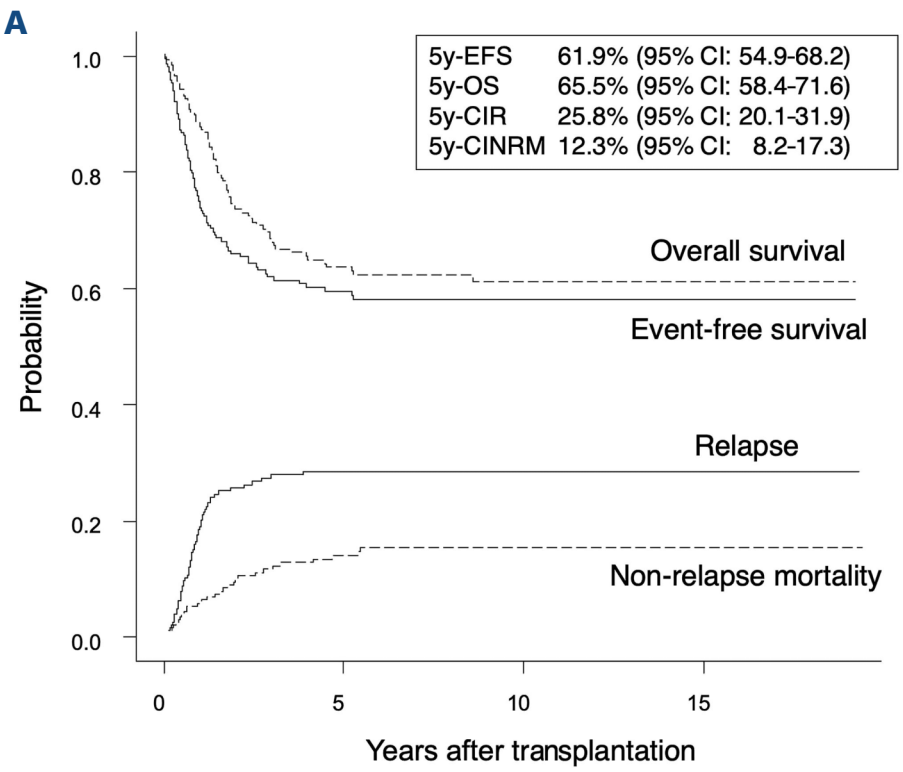

Figure 1. The survival outcomes of the included patients. (A) The outcomes of all included patients. (B) Event-free survival of patients with French-American-British (FAB) M7 disease and patients with other types of acute myeloid leukemia (AML). 5y-EFS: 5-year event-free survival; OS: overall survival; CIR: cumulative incidence of relapse; CINRM: cumulative incidence of non-relapse mortality.

etic/genetic abnormalities. We identified melphalan-containing non-TBI MAC as being superior to other regimens (Table 2), and chemotherapy-based MAC was superior to TBI-based MAC (*Online Supplementary Table S3*). However, other potential prognostic factors, such as the details of pre-HSCT treatment or pre-HSCT minimal residual disease status, were not included due to a lack of information. Third, the current practice in Japan differs from those in other countries in several ways, such as more common usage of cord blood or busulfan in the context of chemotherapy-based regimens. As such, careful consideration should be given to apply these results in the practices of other countries. Fourth, it should be noted that the duration of CR1 was unknown for 26.2% of patients.

In summary, patients with relapsed non-DS M7 exhibited poor outcomes after HSCT in their CR2, underscoring the need to identify high-risk subpopulations within this group and to consider offering HSCT in the CR1. Furthermore, melphalan-containing non-TBI MAC regimens demonstrated superior outcomes compared with other conditioning regimens, and alternative-donor HSCT was a viable option for the population included in this study. These findings can aid in the optimization of HSCT strategies for children with AML in both the CR1 and CR2.

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Disclosures

No conflicts of interest to disclose.

Contributions

HI designed the study and analyzed the data. HI, ST, DH, HS, SY and YO wrote the manuscript. MY, KaK, AW, AH, YC, KeW, MN, MT, JT, KaW, KeK, TK, YH, KT and MH collected patient data. HI and YA performed the statistical analysis. All authors revised the manuscript and approved the final version.

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Data-sharing statement

Original data presented in this manuscript are available upon request addressed to the corresponding author.

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