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Treatment of relapsed acute leukemia by Ara C plus donor lymphocyte infusion using CD34+ cells reserved at the time of allogeneic transplantation

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Abstract

Currently, there is no standard therapy available for relapsed acute leukemia after allogeneic hematopoietic cell transplantation (allo-HCT). In this study, we evaluated the efficacy of cytoreduction with cytarabine followed by granulocyte colony-stimulating factor (G-CSF)-primed donor lymphocyte infusion (DLI) for patients with acute leukemia who relapsed after allo-HCT. We retrospectively reviewed 255 patients who had received allo-HCT for acute leukemia/myelodysplastic syndrome. Patients were divided into two groups based on the CD34+ cell dose they received during the initial transplantation; patients in the lower CD34+ group received a dose lower than 6×10^6 cells/kg and those in the higher CD34+ group received a dose higher than 6×10^6 cells/kg. No significant differences were noted between two groups with respect to overall survival, relapse-free survival, or graft-versus-host disease (GVHD)-free/relapse-free survival. Patients who relapsed after allo-HCT (n=93) were assigned into early or late relapse groups using the median time to relapse as the threshold. Among the 93 patients with relapse, 39 received G-CSF-primed DLI. The median dose of CD3+ cells was 2.82×10^7 cells/kg (range: 0.05-10.1). In the late relapse group, one-year overall survival was significantly higher in patients receiving DLI than that in patients not receiving DLI (53.4% ±7.4% vs. 26.7% ±7.4%; $P=0.039$), whereas no DLI effect was detected within the early relapse group. In addition, the incidence of DLI-induced GVHD did not differ between the two groups. In conclusion, treatment with G-CSF-primed DLI after allo-HCT with a limited CD34+ cell dose constitutes a feasible and effective option, which could replace second HCT in treatment of late-relapse patients.

Key words: Donor lymphocyte infusion, Hematopoietic stem cell transplantation, Acute leukemia, Recurrence, Granulocyte colony-stimulating factor

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Introduction

Allogeneic hematopoietic cell transplantation (allo-HCT) is a potentially curative therapy for acute leukemia[1]. However, patients with acute leukemia who relapse after allo-HCT show poor prognosis with a median survival of 34 months[2]. A second allo-HCT results in a long-term survival rate of only 10-35% with a higher treatment-related mortality. Currently, no standard treatment approach is available for relapsed acute leukemia[3,4].

Induction of graft-versus-leukemia (GVL) effects with donor lymphocyte infusions (DLIs) is an attractive option for patients with relapsed hematological malignancies. However, GVL efficacy depends on disease subtype and tumor burden at the time of DLI[3,5]. Schmid *et al*. demonstrated an overall survival (OS) benefit of DLI in patients with acute myelogenous leukemia (AML) who relapsed after allo-HCT (20% ±3% vs. 9% ±2%; $P<0.001$)[6]. Several different strategies have been explored to improve patient outcomes, such as dose-escalation of DLIs, addition of immunosuppressive agents to prevent graft-versus-host disease (GVHD), and modified DLI treatment with granulocyte colony-stimulating factor (G-CSF)[7-10]. A combination of chemotherapy and DLI showed promising results[11]. Here, we investigated the effect of cytoreduction with a high dose of cytarabine followed by DLI.

A pilot study performed at our institution reported a beneficial effect of cytarabine combined with G-CSF-primed DLIs using cryopreserved cells on patients with hematological malignancies who relapsed after allo-HCT[12]. This strategy was cost-effective and convenient for donors. In the current study, we aimed to determine the effectiveness of cytarabine in combination with G-CSF-primed DLI in treatment of patients with acute leukemia who relapsed after allo-HCT.

Materials and Methods

Data Collection

We conducted a retrospective review of the medical records of 255 patients who received allo-HCT for AML, myelodysplastic syndrome (MDS), or acute lymphoblastic leukemia (ALL) between December 1998 and August 2013 at the Department of Hematology/Oncology, Kyungpook National University Hospital (KNUH). Clinical and laboratory data were collected from electronic medical records according to protocol approved by the KNUH institutional review board.

Definitions

The risk status at transplantation was determined based on previously published classification schemes[13]. Poor-risk cytogenetics were classified according to the revised Medical Research Council classification system for AML and the International Prognostic Scoring System for MDS[14,15]. Poor-risk cytogenetics for ALL were defined as MLL rearrangement, BCR/ABL1 translocation, hypoploidy, or complex karyotype. Graft failure was defined as the lack of myeloid engraftment in patients surviving in remission for at least 28 days after transplantation. The Keystone staging system was used to score acute GVHD (aGVHD) and chronic GVHD (cGVHD)[16,17]. Relapse was defined as the reappearance of leukemic cells in the peripheral blood, bone marrow, or extramedullary lesions after allo-HCT.

A novel composite end-point of refined GVHD-free/relapse-free survival (GRFS), where events included grade Ⅲ-Ⅳ aGVHD, systemic therapy requiring cGVHD, relapse, or death, was also[18]. OS was calculated from the date of the first allo-HCT to the date of death, or to the last follow-up. Relapse-free survival (RFS) was calculated from the date of the first allo-HCT to the date of disease recurrence or to the date of death. Post-relapse survival (PRS) was defined as the time from relapse post-transplantation to death or to the last follow-up[19].

Transplantation procedures

Preparative regimens for allogeneic peripheral blood stem cell transplantation (PBSCT) included busulfan (Bu, 4 mg/kg PO or 0.8 mg/kg IV for 4 days) and cytoxan (Cy, 60 mg/kg for 2 days) administered to 100 patients; Bu (3.2 mg/kg for 2-4 days) and fludarabine (Flu, 30 mg/m^2 for 6 days) administered to 135 patients; and total body irradiation and Cy (60 mg/kg for 2 days) administered to 20 patients. PBSCs were mobilized with 10 μg/kg per day G-CSF [filgrastim (Leukokine®; CJ, Co., Korea) or lenograstim (Neutrogin®; Chugai Co. Ltd, Tokyo, Japan)] alone (n = 183, 71.8%) or in combination with a concurrent regimen of 5 μg/kg per day G-CSF and 5 μg/kg per day granulocyte macrophage colony-stimulating factor (GM-CSF) (n = 72, 28.2%) from the donor. Administration of G-CSF with or without GM-CSF was continued, and apheresis was repeated every morning until the targeted cell dose (6×10^6 CD34+ cells/kg) was reached. GVHD prophylaxis consisted of methotrexate (MTX) and cyclosporine A (CyA) or MTX and tacrolimus (Tac).

Collection and infusion of donor lymphocytes

Collecting the targeted amount of PBSCs (more than 6×10^6 CD34+ cells/kg) allowed us to cryopreserve some PBSCs, including several CD3+ cells at the time of harvest for transplantation. The extra cells were cryopreserved with dimethyl sulfoxide in a nitrogen tank. DLI with cryopreserved cells was available only for patients who were able to receive more than 6×10^6 CD34+ cells/kg from donors. Other patients would receive salvage chemotherapy or second transplantation when they relapse.

For those patients who relapsed after allo-HCT, DLI was promptly performed using the cryopreserved cells. The CD3+ cell-count was determined by flow cytometry and used to calculate the DLI dose. Before DLI, immunosuppressive agents were discontinued and patients received pre-DLI chemotherapy with high-dose cytarabine (1 g/m^2; twice a day on days 1, 3, and 5). No patient received prophylactic immunosuppressive therapy after DLI.

The chimerism status, which was assessed by the number of tandem repeats or short tandem repeats, was compared before and after DLI. All patients underwent a bone marrow examination within 60 days after DLI or sooner (if clinically indicated) for the assessment of the response.

Statistical Analysis

Categorical data were analyzed using a chi-square test. Survival analysis was conducted using the Kaplan-Meier method, and groups were compared using a log-rank test. The cumulative incidence of GVHD was calculated using the Gray method considering treatment-related mortality and relapse as competing risks. The Cox proportional regression model was used to analyze potential risk factors affecting survival. Statistical analyses were per-

formed using the SPSS software version 18 (SPSS Inc., Chicago, IL, USA) and EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan)[20].

Results

Patient and transplant characteristics

A total of 255 patients were analyzed. The median infused cell doses were as follows: mononuclear cells, 7.94×10^8 cells/kg (range: 0.36-25.12); CD34+ cells, 5.13×10^6 cells/kg (range: 0.46-20.6); and CD3+ cells, 2.82×10^8 cells/kg (range: 0.05-10.0). Patients were reclassified into two groups according to the targeted CD34+ cell dose (6×10^6 cells/kg) according to the KNUH protocol. The lower CD34+ group (n=165; 64.7%) included patients who underwent allo-HCT with a CD34+ cell dose of lower than 6×10^6 cells/kg, and the higher CD34+ group (n=90; 35.3%) included patients who underwent allo-HCT with a CD34+ cell dose of at least 6×10^6 cells/kg. Patient characteristics are summarized in **Table 1**. No statistically significant differences in the transplantation outcomes, such as the incidence of aGVHD, cGVHD, and relapse rate, were found between the two groups (**Table 2**).

Impact of CD34+ cell dose on GRFS

The median follow-up duration was 18.1 months with a range of 0.2 to 209.7 months. The 1-year OS, RFS, and non-relapse mortality (NRM) were 55.3% ±3.1%, 66.0% ±3.2%, and 28.2% ±0.3%, respectively. The cumulative incidences of aGVHD and cGVHD were 40.7% ±0.3% and 41.6% ±0.3%, respectively. The unadjusted Kaplan-Meier estimate of 1-year GRFS was 32.9% ±3.1%. No significant difference was found in OS, RFS, NRM, or GRFS between the two groups classified according to the CD34+ cell dose (**Figure 1**). Moreover, there was no significant correlation between the number of infused CD3+ and CD34+ cells (Spearman correlation coefficient, $P=0.307$). However, a trend of more CD3+ cells (more than 3.1×10^8 cells/kg) was noted in the higher CD34+ group ($P=0.001$; **Table 2**). In the univariate analysis, patients transplanted with higher CD34+ and CD3+ cell doses did not show an improved GRFS (**Table 3**, $P=0.623$ and $P=0.158$, respectively). The risk status at transplantation was an independent factor associated with worse GRFS (hazard ratio (HR) = 1.782, 95% confidence interval (CI): 1.267-2.509, $P=0.001$; **Table 3**).

Post-Relapse Survival

Among the 255 patients, 93 (36.4%) relapsed after allo-HCT. The median time from allo-HCT to relapse was 4.6 months (range: 1.5-59.1). After relapse, 45 patients (48.4%) were treated with salvage chemotherapy with a regimen based on fludarabine or mitoxantrone (FLAG or MEC), 9 (9.7%) with a second allo-HCT because of unavailability of reserved cells for DLI, and 39 (41.9%) with G-CSF-primed DLI (4 patients received DLI only because of their refusal on the use of cytarabine). Thereafter, 13 patients (30.0%) achieved DLI-induced complete remission, 24 progressed, and 2 were not evaluable for response. DLI-induced aGVHD was observed in 24 patients (61.5%) with a median of 20 days after DLI (range: 3-98 days); ten patients with grade Ⅰ, six with grade Ⅱ, five with grade Ⅲ, and three with grade Ⅳ. As shown in **Table 4**, univariate analysis revealed that poor-risk cytogenetics (HR=2.512, $P=0.015$), risk status at transplantation (HR=4.406, $P<0.001$), myeloablative conditioning regimen (HR=0.567, $P=0.007$), cGVHD (HR=0.525, $P=0.006$), and longer post-transplantation remission duration (HR=0.297, $P<0.001$) were significantly associated with PRS. A longer post-transplantation remission duration was the only independent factor correlated with PRS (HR=0.297, 95% CI: 0.193-0.457, $P<0.001$).

G-CSF-primed DLI effect on PRS

Among the 39 patients (41.9%) who received DLIs, 34 received one infusion and 5 received two infusions. The median amount of CD3+ cells was 2.82×10^7 cells/kg (range: 0.05-10.1). The patient and transplant characteristics according to the post-transplantation remission duration are described in **Table 5**. Patients were classified into early and late relapse groups based on post-transplantation remission duration relative to the median RFS of 4.6 months (range: 1.5-59.1). For patients with early relapse (remission duration<4.6 months), one-year PRS rates were 7.7% ±5.3% and 5.3% ±4.3% in the DLI and non-DLI groups, respectively (**Figure 2A**; $P=0.667$). For patients with late relapse (remission duration≥4.6 months), one-year PRS rates were 40.0% ±7.4% and 17.9% ±7.2% in the DLI and non-DLI groups, respectively (**Figure 2B**; $P=0.039$). The number of second allo-HCT cases was small (n=9), they were, therefore, excluded from comparison analysis.

Discussion

The current study investigated the efficacy of cytarabine-based chemotherapy in combination with G-CSF-primed DLI in patients with acute leukemia who relapsed after allo-HCT. G-CSF-primed DLI treatment after allo-HCT with a limited CD34+ cell dose (lower than 6×10^6 cells/kg) constituted a feasible and effective option in terms of GRFS, donor convenience, and cost. Moreover, this treatment option may replace a second HCT for late-relapse patients. Although stem cell dose has already been explored in relation to the incidence of GVHD,

Table 1. Patient characteristics according to CD34+ cell dose

	Lower CD34+ dose ($<6\times10^6$/kg)	Higher CD34+ dose ($\geq6\times10^6$/kg)	p-value
Number of patients	165 (64.7)	90 (35.3)	
Median age, years (range)	39 (15-68)	38 (16-62)	0.908
Sex (male/female)	67 (40.6)/98 (59.4)	44 (48.9)/46 (51.1)	0.273
ECOG PS			0.102
0	57 (34.5)	43 (47.8)	
1	106 (64.2)	47 (52.2)	
Disease subtype or Diagnosis			
AML	93 (56.4)	56 (62.2)	0.072
MDS	18 (10.9)	13 (14.4)	
ALL	54 (32.7)	21 (23.3)	
Poor- risk cytogenetics	35 (23.0)	13 (16.7)	0.161
Disease status at transplantation			0.222
CR1	97 (58.8)	46 (51.1)	
Further CR	14 (8.5)	11 (12.2)	
Persistent disease	54 (32.7)	33 (36.7)	
Risk status at transplantation			0.512
Standard risk	89 (53.9)	44 (48.9)	
High risk	76 (46.1)	46 (51.1)	
Female donor to male recipient	42 (25.5)	6 (6.7%)	0.022
CMV status			0.230
Donor+/Recipient+	55 (33.3)	35 (38.9)	
Donor+/Recipient−	17 (10.3)	9 (10.0)	
Donor−/Recipient+	42 (25.5)	13 (14.4)	
Donor−/Recipient−	51 (30.9)	33 (36.7)	
Conditioning intensity			0.676
Myeloablative	113 (68.5)	59 (65.6)	
Reduced intensity conditioning	52 (31.5)	31 (34.4)	
Mobilization			0.246
G-CSF/GM-CSF	117 (70.9)/48 (29.1)	66 (73.3)/24 (26.7)	
Donor-Recipient HLA disparity			0.535
Matching sibling donor	96 (58.2)	47 (52.2)	
Matching unrelated donor	34 (20.6)	20 (22.2)	
Mismatched related donor	24 (14.5)	19 (21.1)	
Haploidentical-related donor	11 (6.7)	4 (4.4)	
GVHD prophylaxis			0.242
CsA/MTX	77 (46.7)	49 (54.4)	
Tacrolimus/MTX	88 (53.3)	41 (45.6)	
In vivo TCD			
ATG/alemtuzumab	73 (44.2)/12 (7.3)	32 (35.6)/7 (7.8)	0.414

ECOG PS, Eastern Cooperative Oncology Group performance status; AML, acute myelogenous leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukemia; CR, complete remission; CMV, cytomegalovirus; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte macrophage colony-stimulating factor; GVHD, graft-versus-host disease; CsA, cyclosporine; MTX, methotrexate; TCD, T-cell depletion; ATG, anti-thymoglobulin.

relapse, and survival, no consensus has been reached[21-23].

Regarding CD 34+ cell dose for allogeneic PBSCT, higher CD34+ dose (higher than 8×10^6 cells/kg) resulted in better neutrophil and platelet recovery, and was associated with development of chronic GVHD, but was not correlated with improved survival. Optimal CD34+ dose seemed to be dependent on donor type and stem cell source[24].

Preliminary results from our institution demonstrated that CD34+ cell transplantation with a dose of higher than 6×10^6 cells/kg did not improve refined GRFS (median survival 5.5 months vs. 6 months, $P=0.245$; **Figure 1D**). Moreover, a higher CD34+ cell dose did not increase the neutrophil or platelet engraftment rate. As the current study found no correlation between the CD3+ and CD34+ cell numbers in the harvested cells (Spearman correlation coefficient, $P=0.307$), it is planned to limit the CD34+ cell dose (6×10^6 CD34+

Table 2. Transplantation outcomes according to CD34+ cell dose

	Lower CD34+ dose ($<6\times10^6$/kg)	Higher CD34+ dose ($\geq6\times10^6$/kg)	p-value
Number of patients	165 (64.7)	90 (35.3)	
Median follow-up, days (range)	534 (8-4962)	636 (6-6381)	0.168
Median stem cell infusion, (range)			
CD34+ $\times10^6$/kg	3.94 (0.46-6.00)	7.54 (6.01-20.6)	<0.001
MNC $\times10^8$/kg	6.91 (0.36-12.70)	9.65 (3.68-25.12)	<0.001
CD3+ $\times10^8$/kg	2.70 (0.05-7.45)	3.10 (1.25-10.01)	0.001
Engraftment			
Neutrophil>500 mm^3	157 (95.2)	84 (94.4)	0.810
Platelet>20,000/mm^3	150 (90.9)	77 (85.6)	0.159
Median time to engraftment, days			
Neutrophil>500/mm^3	13 (8-30)	12 (9-24)	0.791
Platelet>20,000/mm^3	13 (8-121)	12 (7-161)	0.672
aGVHD			0.594
Ⅱ-Ⅳ	65 (39.4)	39 (43.0)	
Ⅲ-Ⅳ	16 (9.7)	12 (13.3)	
cGVHD, Seattle			0.786
Classic chronic	44 (26.7)	26 (28.9)	
Overlap	17 (10.3)	10 (11.1)	
cGVHD, NIH 2005			0.821
mild	36 (21.8)	20 (22.2)	
moderate	30 (18.2)	20 (22.2)	
severe	1 (0.6)	0	
Cause of Death	100 (60.1)	58 (64.4)	0.712
Relapse	37 (22.4)	24 (26.7)	
Infection	29 (17.6)	12 (13.3)	
GVHD	18 (10.9)	11 (12.2)	
VOD	10 (6.1)	9 (10.0)	
Others	6 (3.6)	2 (2.2)	

Others: 3 patients died of cardiac arrest, 2 patients died of brain hemorrhage, 1 patient died of pulmonary hemorrhage, 1 patient died of malnutrition, 1 patient died of acute renal failure.
MNC, mononuclear cell; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; VOD, veno-occlusive disease.

cells/kg) for transplantation and cryopreserve the rest of the harvested cells for relapse or prophylactic use.

Cryopreservation for the purpose of future DLI was not performed in patient group who received a dose of lower than 6×10^6 CD34+ cells/kg in the initial allo-HCT.

This retrospective study has several limitations, including the heterogeneity of patients and transplant characteristics. Furthermore, DLI treatment has a minimal effect in the case of a rapidly advancing disease, as evidenced in patients with early relapse who experienced no benefits from DLIs. However, patients with longer post-transplantation remission duration showed better PRS in the DLI group (**Figure 2B**; 1-year OS: 46.7% ±12.9% vs. 21.7% ±8.9%, $P=0.039$).

A second allo-HCT is regarded as the optimal option for patients who relapse after the first transplantation. Yet, it is only available for a limited number of patients due to concerns of high mortality and unavailability of donors. Thus, for the late relapse group, DLI treatment may replace second HCT. A faster recovery can also be expected in the case of chemotherapy followed by G-CSF-primed DLI treatment with a sufficient number of CD34+ cells. Regarding GRFS, allo-HCT with a limited CD34+ cell dose (lower than 6×10^6 cells/kg) is not inferior to allo-HCT with a higher CD34+ cell dose. Moreover, the surplus cells from the harvest can be cryopreserved at the time of the first transplantation. Then, DLI treatment using these cryopreserved cells can be promptly performed at the time of relapse without a need for a new harvest. From the perspective of donor convenience and cost-effectiveness, this strategy constitutes an attractive option for patients with dismal prognosis after post-transplantation relapse.

Well-designed prospective clinical trials are required to answer such DLI-related questions as to when, how, and to whom. Previous studies have shown multiple biological effects of G-CSF on peripheral blood stem cells, including the ability to polarize T cells from Th1 to Th2, promotion of regulatory T cells, and tolerogenic dendritic cell differentiation[25,26]. In addition, this study indicated

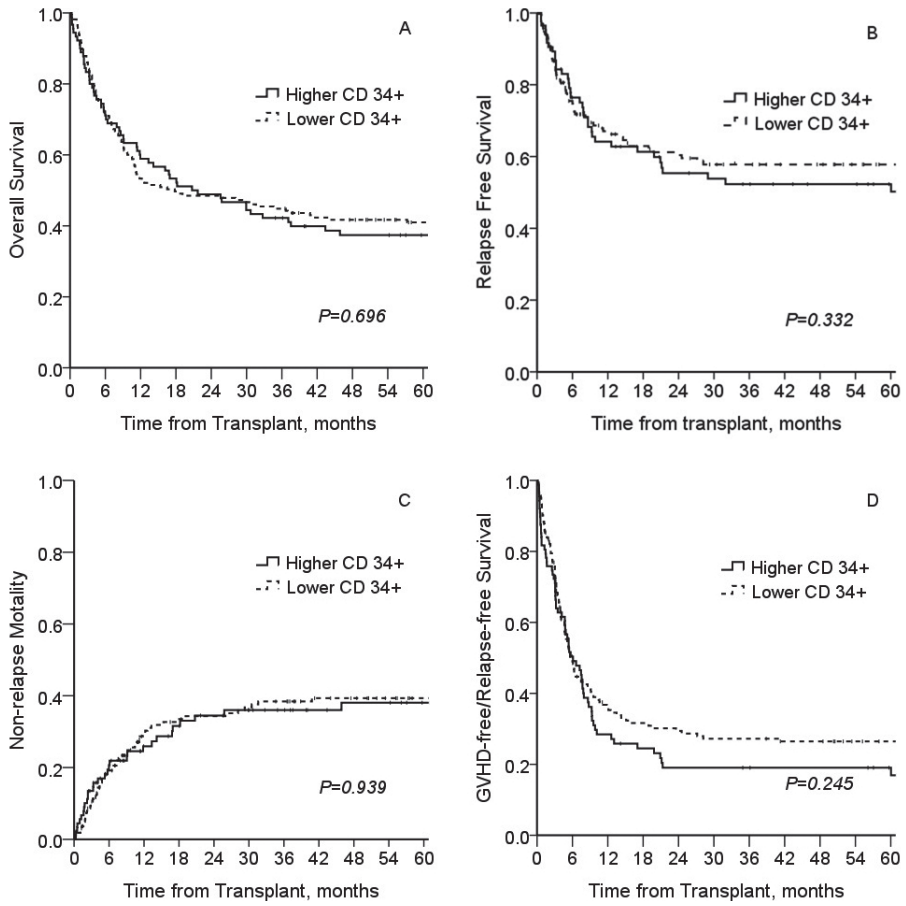

Figure 1. Survival curves according to infused CD34+ cell dose
Patients were divided into lower or higher CD34+ dose group based on cut-off dose (6×10^6/kg) of CD 34+ cells.(A) OS: 1-year OS rates were 53.3% ±3.9% and 58.9% ±5.2% for the lower and higher groups, respectively (P=0.696).(B) RFS: 1-year RFS were 66.7% ±4.0% and 63.2% ±5.5% for the lower and higher groups, respectively (P=0.332).(C) NRM: 1-year NRM were 29.5% ±3.8% and 25.6% ±4.7% for the lower and higher groups, respectively (P=0.939).(D) GRFS: 1-year GRFS were 35.3% ±4.0% and 28.4% ±5.1% for the lower and higher groups, respectively (P=0.245).

that G-CSF-primed DLI, rather than unstimulated DLI, included more CD34+ cells and led to early recovery. Moreover, interestingly, low mortality was associated with DLI-induced GVHD, and most of the mortality resulted from disease relapse or refractory disease rather than GVHD.

According to a study from Japan, in the case of non-primed DLI[27], complete remission (CR) rate was 38% and the probability of remaining in CR at 3 years was 7% in AML. Acute GVHD (grade 2 or higher) developed in 31 out of 89 (35%) patients with HLA-identical related donors and was fatal for seven (8%). The incidence of aGVHD (cumulative incidence of aGVHD; 40%) was similar to that in the current report. Chronic GVHD developed in 24 of 73 (33%) patients who received DLI from HLA-identical relatives.

Recently, Claiborne *et al*.[28] reported that treatment of relapsed AML/MDS (n=28) after allo-HCT with acombination of azacytidine and DLI resulted in a two-year OS rate of 35%, and noted a trend towards higher absolute CD4+ cell count in the patient group achieving remission. Hypomethylating agents may induce synergistic effect by promoting cytotoxic effects against leukemic cells when combined with DLI[28]. Schroeder *et al*. also reported complete and partial remission rates of 27% and 6%, respectively, which correspond to an overall response rate of 33%, following the treatment of relapsed AML/MDS after allo-HCT with a combination of azacytidine and DLI. Two-year OS rate was higher in MDS patients and correlated with disease burden in patients with AML. Overall incidence of aGVHD after treatment with Aza and DLI was 23% among all patients included in the study (n=154) and 31% among patients who had received DLI (n=105). The authors concluded that the combination of azacytidine and DLI was an effective and well-tolerated treatment option for patients with relapse after allo-HCT, in particular for those with MDS or AML with low disease burden[29]. Another group, which tested a

Table 3. Factors affecting GRFS

	Univariate			Multivariate		
	HR	95% CI	p-value	HR	95% CI	p-value
CD34+ cell doses, high	1.197	0.822-1.533	0.623	—	—	—
CD3+ median, high	1.300	0.903-1.807	0.158	—	—	—
HCT risk, high	1.898	1.411-2.554	<0.001	1.782	1.267-2.509	0.001
In vivo TCD, yes	0.915	0.675-1.240	0.568	—	—	—
Conditioning intensity				—	—	—
RIC/MAC	1.003	0.731-1.375	0.986	—	—	—
Donor disparity				—	—	—
MUD/MSD	0.909	0.616-1.343	0.633	—	—	—
Haploidentical/MSD	4.062	2.243-7.355	<0.001	4.024	1.870-8.658	<0.001
FD to MR	0.895	0.631-1.269	0.533	—	—	—
Donor CMV positivity	0.757	0.562-1.020	0.066	—	—	—

GRFS, graft-versus-host disease-free, relapse-free survival; HCT, hematopoietic cell transplantation; TCD, T-cell depletion; RIC, reduced-intensity conditioning; NMA, non-myeloablative; MAC, myeloablative conditioning; MUD, matched unrelated donor; MSD, matched sibling donor; FD, female donor; MR, male recipient; CMV, cytomegalovirus.

Table 4. Factors affecting Post-Relapse Survival (PRS)*

	Univariate			Multivariate		
	HR	95% CI	p-value	HR	95% CI	p-value
Age	1.161	0.753-1.789	0.499	—	—	—
ECOG 0 vs 1	1.273	0.833-1.945	0.266	—	—	—
CD34+ cell doses**	1.162	0.764-1.767	0.483	—	—	—
Median CD3+ cell dose***	0.867	0.577-1.303	0.492	—	—	—
Poor-risk cytogenetics	2.512	1.195-5.227	0.015	—	—	0.671
Transplantation risk, high	4.406	2.592-6.317	<0.001	—	—	0.192
In vivo TCD, yes	1.197	0.795-1.802	0.389	—	—	—
Myeloablative conditioning regimen	0.567	0.736-0.855	0.007	—	—	0.079
Donor disparity	0.828	0.505-1.359	0.455	—	—	—
MUD vs. MSD	0.997	0.580-1.715	0.992	—	—	—
MMSD vs. MSD	3.182	1.422-7.121	0.005	—	—	—
FD to MR	1.208	0.736-1.981	0.455	—	—	—
Donor CMV positivity	1.371	0.902-2.084	0.140	—	—	—
aGVHD	0.908	0.600-1.374	0.647	—	—	—
cGVHD	0.525	0.333-0.829	0.006	—	—	0.140
Delayed PLT engraftment	1.385	0.731-2.623	0.318	—	—	—
Use of DLI	0.803	0.527-1.222	0.306	—	—	—
Median post-transplantation remission duration****	0.297	0.193-0.457	<0.001	0.569	0.375-0.865	0.008

*Post-relapse survival (PRS) was defined as the time from relapse post-transplantation to death or last follow-up. **Patients were reclassified into two groups according to the targeted CD34+ cell doses (6×10^6/kg) by the KNUH protocol. ***The median CD3+ cell dose was 2.82×10^7/kg (range: 0.05-10.1). ****Post-transplantation remission duration was divided by the median RFS 4.6 months (range: 1.5-59.1).
ECOG PS, Eastern Cooperative Oncology Group performance status; TCD, T-cell depletion; MUD, matched unrelated donor; MSD, matched sibling donor; MMSD, mis-matched sibling donor; FD, female donor; MR, male recipient; CMV, cytomegalovirus; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; PLT, platelet; DLI, donor lymphocyte infusion.

combination of decitabine and DLI, reported that the effectiveness of this regimen was not restricted to patients with low leukemic burden[30].

In conclusion, our results indicate that G-CSF-primed DLI treatment after allo-HCT with a limited CD34 + cell dose (lower than 6×10^6 cells/kg) is a feasible and effective option in terms of GRFS, donor convenience, and cost. Therefore, it has potential to replace the second HCT in treatment of late relapse patients.

Author's Contribution

As the first author, Y. J. L. collected and interpreted the data, drafted the article, and critically revised the impor-

Table 5. Outcomes of DLI treatment according to post-transplantation remission duration

Number of patients	Early relapse 19 (48.7%)	Late relapse 20 (51.3%)	
Age, median	38 (19-58)	41 (24-56)	0.341
Sex, F/M	10/9	9/11	0.634
ECOG PS, 0 vs 1	7/12	8/12	0.839
Diagnosis			0.634
AML	12 (63.2)	14 (70.0)	
MDS	4 (21.1)	3 (15.0)	
ALL	3 (15.8)	3 (15.0)	
Poor-risk cytogenetics	7 (36.8)	7 (35.0)	0.841
HCT risk, high	16 (84.2)	9 (45.0)	0.011
Pre-DLI chemotherapy	16 (84.2)	19 (95.0)	0.449
Median CD3+ cell (10^8/kg)	2.32 (1.94-4.66)	2.57 (0.11-7.91)	
Median CD34+ cell (10^6/kg)	3.21 (1.26-4.85)	3.28 (0.98-5.0)	
Response			0.365
CR achieved	5 (26.3)	8 (40.0)	
Persistent disease	12 (63.2)	12 (60.0)	
Not available (TRM)	2 (10.5)	0	
DLI induced GVHD	10 (52.6%)	14 (70.0)	0.265

Post-transplantation remission duration was divided by the median RFS of 4.6 months (range: 1.5-59.1).
DLI, donor lymphocyte infusion; F/M, female/male; ECOG PS, Eastern Cooperative Oncology Group performance status; AML, acute myelogenous leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukemia; HCT, hematopoietic cell transplantation; CR, complete remission; TRM, transplant related mortality; GVHD, graft-versus-host disease.

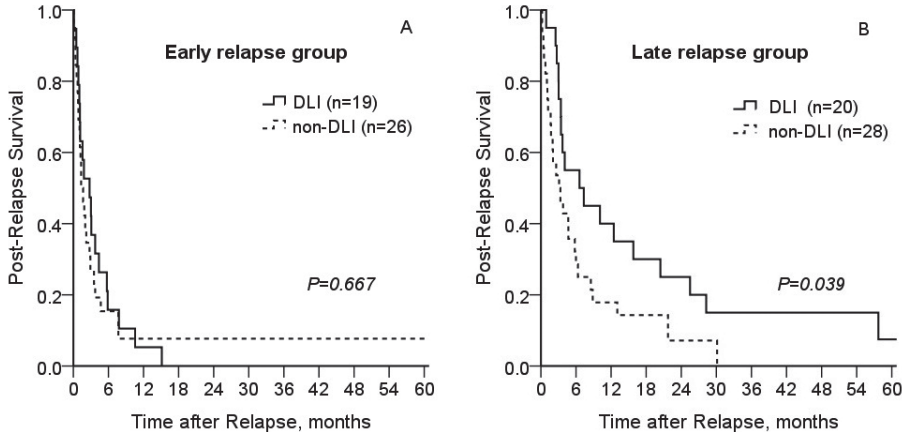

Figure 2. Post-Relapse Survival (PRS) according to the use of DLI
Early relapse and late relapse groups were determined based on median post-transplantation remission duration. (A) In the early relapse group, 1-year PRS rates were 7.7% ±5.3% and 5.3% ±4.3% for DLI and non-DLI groups, respectively (P=0.667). (B) In the late relapse group, 1-year PRS rates were 40.0% ±7.4% and 17.9% ±7.2% for DLI and non-DLI groups, respectively (P=0.039).

tant intellectual content; Y. J. L., D. W. B., H. J. J., J. H. M. and S. K. S. performed the treatment, supplied the acquisition of data, and revised the manuscript; S. K. S. provided the conception and design of the study, critically revised the article for important intellectual content, and gave the final approval of the version to be submitted.

Conflict of Interest

The authors declar no conflict of interest. Disclosure forms provided by the authors are available here.

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