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동종조혈모세포이식술 후 재발한 급성 골수구성 백혈병 환자에서 세포원에 따른 공여자림프구주입술의 효과 비교

Comparison of the effect of donor lymphocyte infusion according to cell sources in relapsed acute myeloid leukemia after allogeneic stem cell transplantation

2020년 2월

서울대학교 대학원

의학과 중개의학전공

박우찬

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이 논문을 의학석사 학위논문으로 제출함

2019년 10월

서울대학교 대학원

의학과 중개의학전공

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박우찬의 의학석사 학위논문을 인준함

2020년 1월

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Abstract

Comparison of the effect of donor lymphocyte infusion according to cell sources in relapsed acute myeloid leukemia after allogeneic stem cell transplantation

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Patients with acute myeloid leukemia (AML) undergoing allogeneic hematopoietic stem cell transplantation (alloSCT) present relapse as the major cause of treatment failure. Donor lymphocyte infusion (DLI) is deemed as an effective therapy for relapsed AML after transplantation. Considering the efficacy of DLI, several centers cryopreserve an excess of cells that can be used later. However, the development of acute graft-versus-host disease (GVHD) after DLI hinders its success, and the DLI cell source remains a topic of debate. In this study, we aimed to compare the efficacy and safety of G-CSF mobilized cells (G-DLI) with conventionally collected DLI (C-DLI). A total of 81 patients (50 C-DLI vs. 31 G-DLI) were assessed for their clinical outcomes that were representative of the immunological results of DLI. The median overall survival was 106 days for C-DLI compared to 139 days for G-DLI ($p = 0.58$). No significant differences were observed in the CD3+ cell count (1.044×108/kg for C-DLI vs. 0.855×108/kg for G-DLI) and in the acute GVHD occurrence rates (44% in C-DLI vs. 41.9% in G-DLI) between the two groups. There was also no statistically significant difference in the severity of acute GVHD, the incidence of fatal GVHD and in post-DLI

engraftment rates. In conclusion, G-DLI appears to be a safe and equally efficacious substitute for C-DLI.

Keywords: Acute myeloid leukemia, stem cells, CD34, donor lymphocyte infusion
Student Number: 2018-26162

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1. Introduction

1.1. Study background

Acute myeloid leukemia (AML) patients undergoing allogeneic stem cell transplantation (alloSCT) present relapse as a major issue, which crucially impacts their survival. Although the optimal treatment in this setting remains undefined, the demonstration that infusion of lymphocytes from the original donor may eradicate recurrent leukemia via graft-versus-leukemia (GVL) effects has led to the consideration of donor lymphocyte infusion (DLI) as a therapeutic option (1-5). In general, DLI involves the collection of peripheral blood lymphocytes from the donor after the initial transplanted cell collection. The chief limitation of this conventional donor lymphocyte infusion (C-DLI) is the development of graft-versus-host disease (GVHD) with reported rates ranging from 40% to 60%, as well as the development of aplasia (5-7). Another issue is the donor's safety, as unexpected adverse events can occur during repeated cycles of collection. Such concerns have led researchers to investigate other cell sources for DLI, namely granulocyte-colony stimulating factor (G-CSF) mobilized donor lymphocytes. There is evidence that G-CSF mobilized peripheral blood progenitor cell infusion is associated with superior disease-free survival in patients who received unprimed lymphocytes for relapse after alloSCT without increased rates of GVHD occurrence (7, 8). Therefore, several centers prefer using the excess of cryopreserved cells from

the first alloSCT as the cell source of G-CSF mobilized DLI (G-DLI) (9, 10). Unfortunately, the best cell source is yet to be evaluated, specifically in Asian patients.

1.2. Purpose of research

We carried out this study to assess the safety and efficacies of G-DLI compared with C-DLI and to eventually determine whether G-DLI can safely substitute C-DLI.

2. Methods

2.1. Patients

This was a retrospective longitudinal cohort study of AML patients subjected to both G-DLI and C-DLI, between January 2001 and December 2017, at the Seoul National University Hospital. Patients aged 16 years or older and who received therapeutic DLI at relapse after alloSCT, were included in this study. Their medical records were reviewed and analyzed for demographics, baseline disease characteristics, details of alloSCT, information and outcomes of DLI, and survival rate. The data on CD3+ cell counts of the donor lymphocytes and $CD3^{+}/CD34^{+}$ cell counts of the residual stem cells were also collected. This study was conducted according to the Declaration of Helsinki, and was approved by the institutional review board of Seoul National University Hospital (IRB No. H-1903-020-1015). All authors had access to the study data and reviewed and approved this study.

2.2. Definitions and evaluations

Patients were diagnosed as AML according to the classification criteria used at the respective time of histological diagnosis (11-13). In this study, European LeukemiaNet classification was used for cytogenetic subgroup classification (14). However, because most patients were diagnosed with AML before the next-generation sequencing era, only cytogenetic results by G-banding and fluorescence in situ hybridization (FISH) were considered for the risk stratification.

The treatment response was analyzed according to the definition of the international working group (15): The complete remission (CR) was defined as normal values for absolute neutrophil (>1,000/μL) and platelet (>100,000/μL) counts, and as independence from red cell transfusion. A bone marrow biopsy revealed that less than 5 percent of blast cells are present in the bone marrow. Extramedullary leukemia must be absent (15, 16). Hematologic relapse was defined as more than 5% bone marrow blasts, or blasts in the blood, or development of extramedullary disease such as chloroma. Extramedullary relapse is a recurrence of AML in other organs, while bone marrow remains in remission.

A chimerism study was performed by analyzing the short tandem repeats (STRs) using PCR amplification (17). Complete chimerism (CC) was defined as a complete changeover to donor cells (18, 19). The coexistence of donor cells and recipient cells (>1%) was defined as mixed chimerism (MC), and

when only recipient cells were present, they were defined as recipient cells only. The CC conversion was defined as the change to CC after DLI in recipient cell only and MC.

DLI is defined as the infusion of lymphocyte concentrations obtained from the original stem cell donor without immunosuppressive conditioning (4). In contrast, the prophylactic immunosuppression conditioning for preventing GVHD is termed as secondary transplantation. In this study, C-DLI was defined as the infusion of lymphocytes collected from previous alloSCT donors without prophylactic immunosuppression conditioning, and G-DLI was defined as the infusion of remnant stem cells that were cryopreserved after previous alloSCT. Peripheral blood stem cells remained after alloSCT were cryopreserved with 10% DMSO solution and stored in −190°C liquid nitrogen. Cryopreserved peripheral blood stem cells were thawed at 37°C before infusion.

The DLI used for analysis in this study included only the first DLI post relapse after alloSCT. Patients who received additional treatment, such as consecutive DLI after first DLI, were also used for analysis only in response to first DLI after relapse. Patients with and without reinduction chemotherapy before DLI, were analyzed. Preemptive DLI, prophylactic DLI, DLI for treatment of engraftment failure, and DLI for hematologic malignancies other than AML were excluded. If immunosuppressants such as a calcineurin inhibitor or steroids were used within one month prior to DLI, patients were included in the analysis.

The graft-versus-host disease (GVHD) included both newly developed

acute GVHD after DLI and deterioration of known GVHD after DLI. The diagnosis of GVHD included both histologic confirmation and clinical evaluation with typical symptoms such as skin rash, elevated serum bilirubin, and diarrhea (20). The Mount Sinai Acute GVHD International Consortium (MAGIC) criteria were used for grading acute GVHD (21). Fatal GVHD was defined as the death of a patient with uncontrolled GVHD.

Similar to the criteria for defining long term survivors in the previous study, patients who survived for more than two years were defined as long-term survivors (22).

2.3. Statistical analysis

Differences between groups were assessed using a Student's t-test or one-way analysis of variance for continuous variables, and Pearson chi-square test for categorical variables, as indicated. The survival analysis was performed using the Kaplan-Meier method. Overall survival (OS) was defined as the time from the first DLI to death from any cause. If patients survived without death or progression, survival was censored at the latest date of follow-up when no death or progression was confirmed, and data available up to July 2019 were used. Univariate and multivariate proportional hazards regression models were used to identify independent risk factors for OS by means of Cox proportional hazards models. A stepwise backward procedure was used to construct a set of independent predictors at each endpoint. All predictors achieving a P value below 0.10 were considered, and sequentially removed if the P value in the multiple model was above 0.05. All data were analyzed using the Rstudio (Rstudio, version 1.2.1335). P values of <0.05 were considered statistically significant.

3. Results

3.1. Patient characteristics

A total of 244 consecutive cases of DLI diagnosed between January 2001 and December 2017 at the Seoul National University Hospital were included in this study. Of these cases, 147 cases were the first DLI cases. Among them, 91 DLIs were performed for AML and 56 for diseases other than AML. Of the 91 cases of DLI , 81 cases were used for the analysis except 1 prophylactic DLI and 9 preemptive DLI (Figure 1). Of these, 76 received chemotherapy before C-DLI or G-DLI, and 5 only DLI without chemotherapy. About fifty (25 males and 25 females) and 31 (11 males and 20 females) patients received C-DLI and G-DLI, respectively, after the reinduction therapy. The median ages at the time of AML diagnosis were 43.8 and 46.9 years, respectively. The median time interval from alloSCT to relapse was 153 (range, 14–4960 days) and 161 (range, 18–1030 days) days. The clinical characteristics of the patients divided into two groups are presented in Table 1.

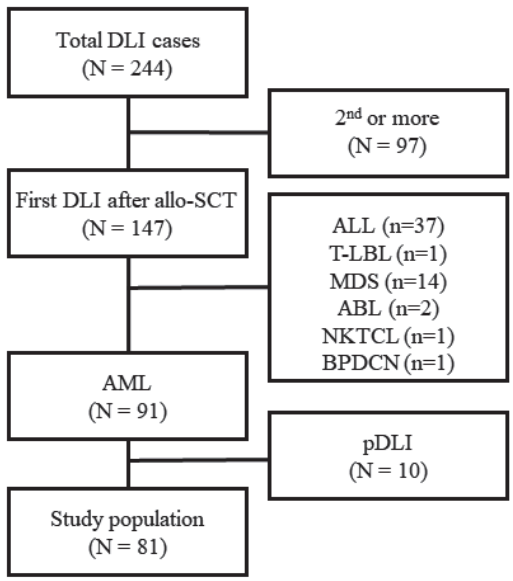

Figure 1. Consort diagram.
A total of 244 cases treated with DLI between January 2001 and December 2017, at the Seoul National University Hospital were included. Of these cases, 147 cases were the first DLI cases after relapse. Ninety DLI cases remained when excluding any disease other than AML. After excluding 10 patients who received either preemptive or prophylactic DLI, a total of 81 patients were deemed eligible for analysis.

Table 1. Baseline characteristics

Characteristics (*N*, %)	All (*N* = 81)	C-DLI (*N* = 50)	G-DLI (*N* = 31)	*P* value
Age, years (median, range)[1]	45.6 (16.2–65.5)	43.8 (16.2–65.5)	46.9 (16.4–62.9)	0.096
Sex (male, %)	36 (44.4%)	25 (50%)	11 (35.5%)	0.201
Cytogenetic risk				0.823
Low	13 (16.0%)	9 (18.0%)	4 (12.9%)	
Intermediate	46 (56.8%)	28 (56.0%)	18 (58.1%)	
High	22 (27.2%)	13 (26.0%)	9 (29.0%)	
Conditioning regimen				0.684
MAC	23 (28.4%)	15 (30.0%)	8 (25.8%)	
NMC or RIC	58 (71.6%)	35 (70.0%)	23 (74.2%)	
Donor type				0.841
Sibling, FM	53 (65.4%)	34 (68.0%)	19 (61.3%)	
Unrelated, FM	16 (19.8%)	10 (20.0%)	6 (19.4%)	
Unrelated, PM	6 (7.4%)	3 (6.0%)	3 (9.7%)	
Haploidentical	6 (7.4%)	3 (6.0%)	3 (9.7%)	
Status at alloSCT				0.052
CR1	42 (51.9%)	21 (42.0%)	21 (67.7%)	
CR>1	17 (21.0%)	14 (28.0%)	3 (9.7%)	
Non CR	22 (27.2%)	15 (30.0%)	7 (22.6%)	
Chimerism				0.268
Recipient only	3 (3.7%)	3 (6.0%)	0 (0%)	
Mixed chimerism	67 (82.7%)	42 (84.0%)	25 (80.6)	
Complete chimerism	6 (7.4%)	2 (4.0%)	4 (12.9%)	
Missing	5 (6.2%)	3 (6.0%)	2 (6.5%)	
Recipient DNA, % (median, range)	50.3 (0.0 – 100)	61.8 (0.0 – 100)	45.7 (0.0 – 91.2)	0.34
Interval from alloSCT to relapse, days (median, range)	156 (14.0–4960)	153 (14.0–4960)	161 (18.0–1030)	0.370
Interval from relapse to DLI, days (median, range)	183 (16.0–5000)	186 (16.0–5000)	183 (35.0–1050)	0.355
GVHD at relapse[2]	18 (22.2%)	11 (22.0%)	7 (22.6%)	0.951
ISA at DLI				0.791
Cyclosporin	52 (64.2%)	32 (64.0%)	20 (64.5%)	
Tacrolimus	6 (7.4%)	3 (6.0%)	3 (9.7%)	
No use	23 (28.4%)	15 (30.0%)	8 (25.8%)	
Chemotherapy before DLI	76 (93.8%)	47 (94.0%)	29 (93.5%)	0.935

[1]Age at diagnosis.

[2] Includes both chronic and acute graft-versus-host disease.

C-DLI, conventional donor lymphocyte infusion; G-DLI, G-CSF mobilized lymphocyte

infusion; MAC, myeloablative conditioning; NMC, non-myeloablative conditioning; RIC, reduced intensity conditioning; FM, fully HLA matched donor; PM, partially HLA mismatched donor; alloSCT, allogeneic stem cell transplantation; CR1, first complete remission; CR>1, second or more complete remission; non CR, non complete remission; GVHD, graft-versus-host disease; ISA, immunosuppressant.

Fifty-nine patients (72.9%) received alloSCT under the disease status of complete remission (CR; Table 1). Two patients with favorable cytogenetic risk underwent allogeneic hematopoietic stem cell transplantation at CR1. One of them received the second induction treatment as the first did not result in remission. One patient underwent follow-up bone marrow examination after induction treatment, and morphologic and cytogenetic remission was confirmed and progressed to consolidation. Nevertheless, $CD34^+$ and $CD117^+$ cells were observed in a few compartments during the bone marrow examination, followed by hematopoietic stem cell transplantation.

About 82.7% of patients showed MC before DLI. Five of 6 patients with CC were treated with DLI due to extramedullary relapse. The other patient maintained CC, but recurrence was confirmed with an increase in blast counts of 8% on bone marrow biopsy. There was no statistically significant difference in chimerism between the two groups.

Regarding the baseline characteristics of patients with initial alloSCT donors, the HLA full-matched sibling donors were the most common, followed by full-matched unrelated donors, partially matched unrelated donors, and haplo-related donor order.

3.2. Outcomes of DLI

Clinical outcomes of DLI are summarized in Table 2. No difference was observed in the $CD3^+$ cell count between the two groups (Figure 2, median value of 1.044×10^8/kg and 0.855×10^8/kg, respectively, p=0.356). There were two missing values in the G-DLI group and one in the C-DLI group. Median $CD34^+$ cell count was 2.34×10^6/kg in the G-DLI group.

Among the 81 patients assessed, 30 (37%) obtained bone marrow remission after cell therapy (14 patients in the C-DLI group, 16 patients in the G-DLI group). In the chimerism study, 22 patients had a chimerism conversion (11 patients in the C-DLI group, 11 patients in the G-DLI group, respectively). There were 16 missing values regarding chimerism after the first DLI (11 cases in C-DLI, 5 cases in G-DLI, respectively).

About 35 patients (43.2%) developed acute GVHD after cell therapy (22 patients in the C-DLI group, 13 patients in the G-DLI group). Ten patients showed grade IV acute GVHD according to the MAGIC criteria (7 patients in the C-DLI group, 3 patients in the G-DLI). Among them, 6 cases of fatal GVHD, in which acute GVHD was the direct cause of death in one patient, were included (4 cases in the C-DLI group, 2 cases in the G-DLI).

No significant difference was observed in OS between the two groups (Figure 3). The median overall survival was 106 and 139 days for C-DLI and G-DLI, respectively. The 1-year survival rate from DLI was 21.0%; 20.0% for the C-DLI group, and 22.6% for the G-DLI group.

Furthermore, two factors were identified as prognostic factors (Table 3, whether bone marrow remission was obtained after DLI and CC in the chimerism study). The median OS of patients who obtained bone marrow remission (BM CR group) was 360 days, and the median OS of patients who did not obtain bone marrow remission was 60 days (non-BM CR group). The 1-year survival rate was 50.0% in the BM CR group and 5.90% in the non-BM CR group (p<0.001). The median OS of patients with complete chimerism (CC group) was 223.0 days and 62.5 days in the other patients (non-CC group). The 1-year survival rate was 37.0% in the CC group and 14.8% in the non-CC group (p=0.012).

Table 2. Clinical outcomes of donor lymphocyte infusion

Characteristics	**All**	**C-DLI**	**G-DLI**	***p* value**
(median, range)	(*N* = 81)	(*N* = 50)	(*N* = 31)	
$CD3^+$ cells in DLI ($\times 10^6$/kg, range)	0.974 (0.1–6.9)	1.044 (0.1–6.9)	0.855 (0.18–2.2)	0.356
$CD34^+$ cells in DLI ($\times 10^8$/kg, range)	2.340 (0.1–6.42)	-	2.340 (0.1–6.42)	
Complete remission (*N*, %)	30 (37.0%)	14 (28.0%)	16 (51.6%)	0.057
Chimerism conversion (*N*, %)	22 (27.2%)	11 (22.0%)	11 (35.5%)	0.285
Newly developed or aggravated GVHD after DLI (*N*, %)	35 (43.2%)	22 (44.0%)	13 (41.9%)	0.855
Acute GVHD grading				0.23
Grade I	12 (14.8%)	8 (16.0%)	4 (12.9%)	
Grade II	10 (12.3%)	4 (8.0%)	6 (19.4%)	
Grade III	3 (3.7%)	3 (6.0%)	0 (0%)	
Grade IV	10 (12.3%)	7 (14.0%)	3 (9.7%)	
Fatal GVHD	6 (7.4%)	4 (8.0%)	2 (6.5%)	0.414
Median overall survival (days, range)	115 (3–2933)	106 (3–2476)	139 (8–2933)	0.58

C-DLI, conventional donor lymphocyte infusion; G-DLI, G-CSF mobilized lymphocyte infusion; CR, complete remission; GVHD, graft-versus-host disease.

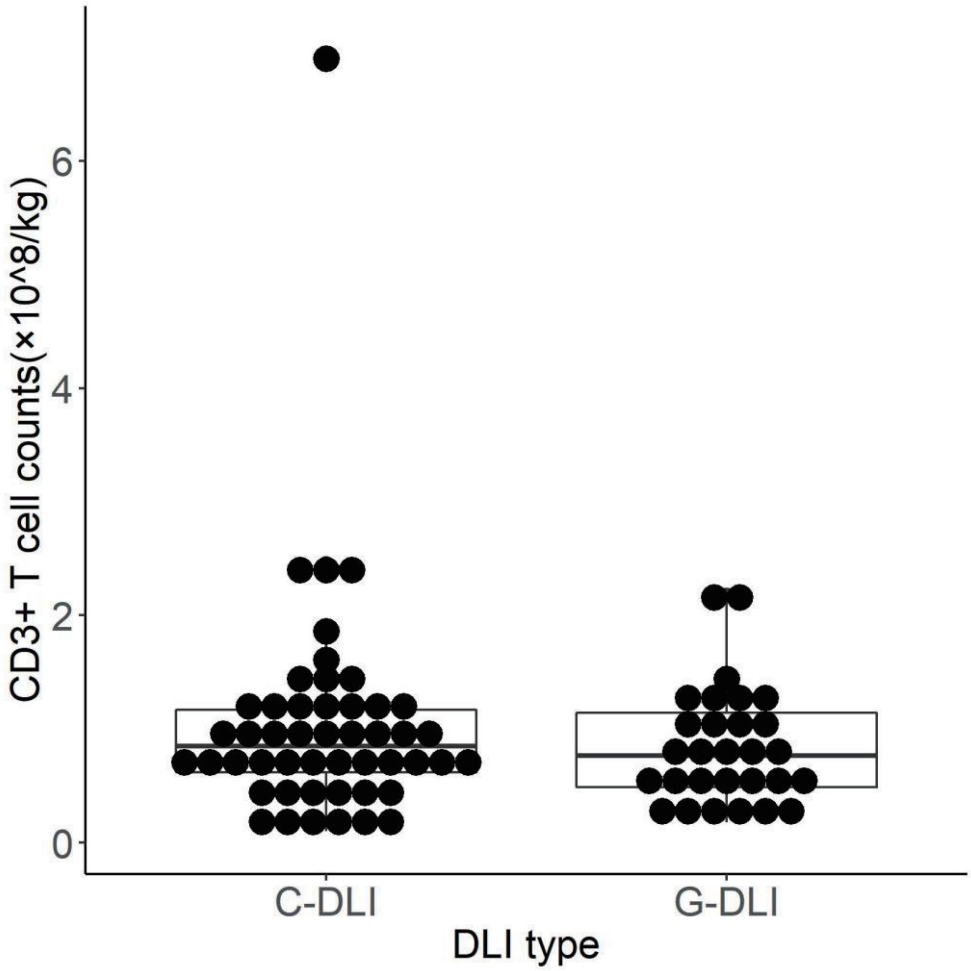

Figure 2. CD3+ T cell counts of the C-DLI and G-DLI groups.
There was no statistically significant difference in the CD3+ T cell count between the two groups (median value of 1.044×10^8/kg for C-DLI group and 0.855×10^8/kg for G-DLI group, p=0.356).

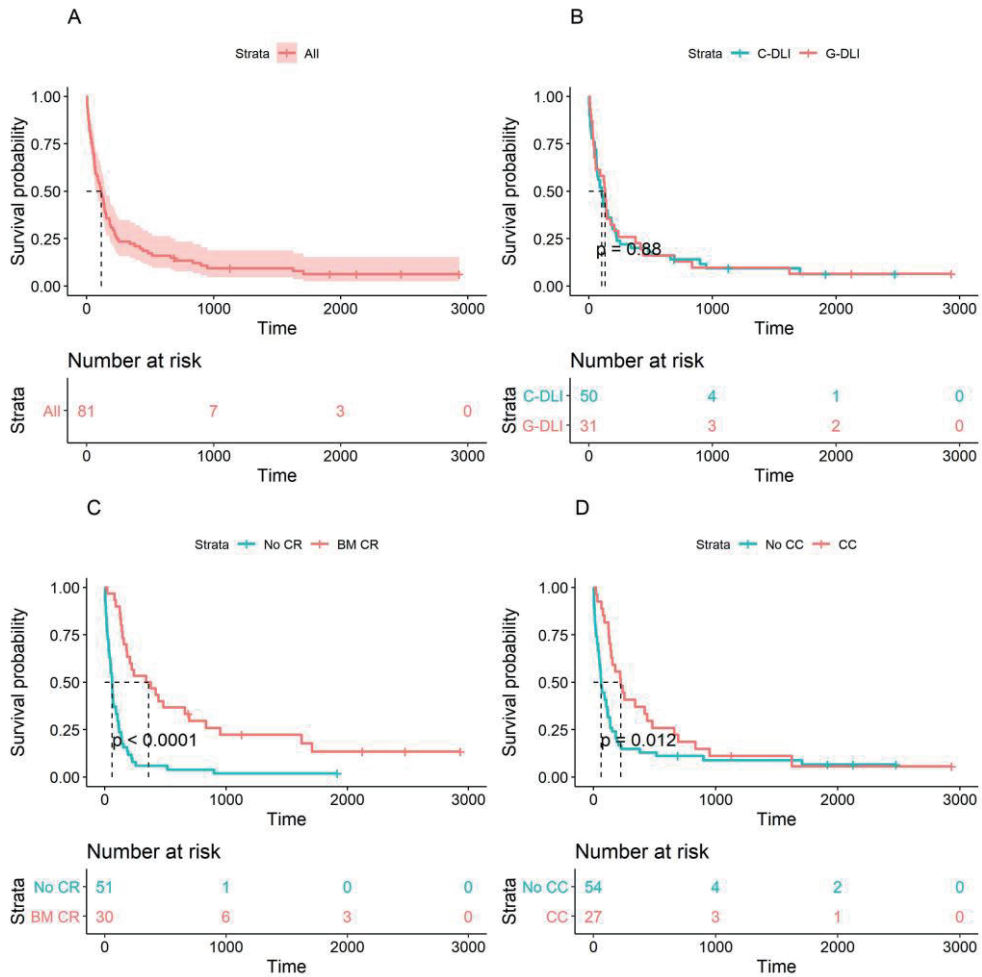

Figure 3. Survival outcomes of 81 patients subjected to donor lymphocyte infusion (DLI).

A. Survival curves for all patients. The median survival of all patients was 115 days. **B**. Overall survival according to DLI type. There was no significant difference in OS between the patients receiving C-DLI and those receiving G-DLI. The median overall survival was 106 and 139 days for C-DLI and G-DLI, respectively. **C**. Overall survival according to bone marrow remission after the first DLI. The median OS of patients who obtained bone marrow remission after the first DLI was significantly longer than that in those who did not (360 days vs. 60 days, $p<0.001$). **D**. Overall survival according to chimerisms after first DLI. The median OS of patients who had complete chimerism after the first DLI was significantly longer than those who did not (223 days vs. 62.5 days, $p=0.012$).

To identify the risk factors for death after DLI, univariate analysis was performed using the Cox proportional hazard model (Table 3). Cytogenetic risk (Good vs. Poor, HR 2.09, 95% CI 1.00-4.38, p=0.049), donors of alloSCT (sibling, fully HLA matched donor (FM) vs. Haplo-identical, HR 3.47, 95% CI 1.45–8.30, p=0.005), persistent disease after first DLI (BM CR vs. non-BM CR, HR 3.64, 95% CI 2.20–6.04, p<0.001), and no CC (CC vs. non-CC, HR 1.85, 95% CI 1.14-3.02, p=0.013) were significant factors. Shorter interval from transplantation to relapse (over 5 months vs. within 5 months, HR 1.53, 95% CI 0.97–2.42, p=0.069) was not statistically significant in the univariate analysis, but was also present in the multivariate analysis. In multivariate analysis, donors of alloSCT (sibling, FM vs. Haplo-identical, HR 2.68, 95% CI 1.10–6.48, p=0.036), persistent disease after first DLI (BM CR vs. persistent, HR 4.85, 95% CI 2.40–9.80, p<0.001) and shorter interval from transplantation to relapse (over 5 months vs. within 5 months, HR 1.68, 95% CI 1.03–2.74, p=0.016), were still significant.

Table 3. Univariate and multivariate analyses for overall survival

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value
OS				
Cell source				
C-DLI	1			
G-DLI	0.97 (0.61–1.54)	0.881		
Cytogenetic risk				
Good	1		1	
Intermediate	1.23 (0.63–2.38)	0.551	0.96 (0.48-1.93)	0.920
Poor	2.09 (1.00–4.38)	**0.049**	1.93 (0.92-4.05)	0.083
Treatment				
DLI once only	1			
Additional treatment	0.76 (0.48–1.21)	0.244		
Donor				
Sibling, FM	1		1	
Unrelated, FM	0.83 (0.45–1.54)	0.555	1.14 (0.59–2.20)	0.457
Unrelated, PM	0.54 (0.21–1.37)	0.193	0.51 (0.20–1.34)	0.294
Haploidentical	3.47 (1.45–8.30)	**0.005**	2.68 (1.10–6.48)	**0.036**
Bone marrow status after first DLI				
CR	1		1	
Persistent	3.64 (2.20–6.04)	**<0.001**	4.85 (2.40-9.80)	**<0.001**
Chimerisms after first DLI				
Complete chimerism	1		1	
Others	1.85 (1.14-3.02)	**0.013**	0.94 (0.48-1.82)	0.850
GVHD after DLI				
Newly developed or aggravated	1			
None	1.33 (0.84-2.12)	0.227		
Interval from alloSCT to relapse				
>5months	1		1	
<5months	1.53 (0.97-2.42)	0.069	1.68 (1.03-2.74)	**0.037**

FM, fully HLA matched donor; PM, partially HLA mismatched donor; CR, complete remission

3.3. Long-term survivors

Table 4 summarizes the characteristics of patients who survived longer than 2 years (long-term survivors). Most of them obtained bone marrow remission after the first DLI. Four patients did not relapse until the last follow up after DLI. Two patients (patient numbers 3 and 6) had a long-term survival with complete bone marrow remission; however, they experienced extramedullary relapse (central nervous system, CNS). Three patients (patient numbers 4, 8, 9) achieved complete remission of the bone marrow after DLI and then additionally received secondary alloSCT from another donor.

Table 4. Long-term survivors

Patient number, age, sex	Cytogenetic risk	Disease state at alloSCT	Donor specifics	Time to relapse/Time to DLI (days)	Type/DLI once or additional treatment	CD3+ cell count ($\times 10^8$/kg)/CD34+ cell count ($\times 10^6$/kg)	Response to DLI (bone marrow)	Chimerism	Acute GVHD	OS (days)	Status at last follow up
1, 16/F	Intermediate	CR1	Unrelated, FM	100/183	G-DLI/once	NA/6.35	CR	CC	Present	2933	Alive
2. 36/F	Good	CR2	Sibling, FM	278/302	C-DLI/additional	1.27/NA	CR	MC	None	2476	Expired
3. 40/F	Good	CR1	Sibling, FM	466/665	G-DLI/additional	0.8/1.16	CR	MC	Present	2126	Expired
4. 33/M	Intermediate	CR1	Unrelated, PM	265/308	C-DLI/additional	0.43/NA	CR	MC	Present	1916	Alive
5. 16/F	Intermediate	CR2	Unrelated, PM	197/225	C-DLI/once	0.7/NA	CR	MC	Present	1708	Expired
6. 43/M	Intermediate	CR1	Sibling, FM	684/721	G-DLI/additional	NA/2.63	CR	CC	None	1624	Expired
7. 33/F	Intermediate	CR4	Unrelated, FM	414/595	C-DLI/once	0.74/NA	Persistent (delayed CR)	CC	Present	1129	Alive
8. 46/M	Poor	CR1	Sibling, FM	51/70	C-DLI/additional	0.38/NA	CR	CC	Present	951	Expired
9. 20/M	Intermediate	CR1	Sibling, FM	4960/5001	C-DLI/additional	0.13/NA	Persistent	MC	None	900	Expired
10. 40/M	Poor	Induction failure	Sibling, FM	170/225	G-DLI/once	NA/3.73	CR	CC	None	835	Alive

CR, complete remission; FM, fully HLA matched donor; PM, partially HLA mismatched donor; alloSCT, allogeneic stem cell transplantation; CR1, first complete remission; CR2, second complete remission; CC, complete chimerism; MC, mixed chimerism; GVHD, graft-versus-host disease.

4. Discussion

This study did not reveal a significant difference in the survival rate, based on the origin of cells used for DLI (C-DLI or G-DLI). In previous studies, no difference was observed between G-DLI and C-DLI after incomplete engraftment or relapse (23). In this study, no significant difference was seen in the survival outcome between C-DLI and G-DLI in overt relapse. The 1-year survival rate of the present study was 20.0% and 22.6% in the C-DLI and G-DLI groups, respectively, which was similar to a previous study (24). C-DLI requires the donor to take the risk of inserting a large-bore catheter and spending additional time in the clinic (25). G-DLI is advantageous as no additional time and resource consumption is required. G-DLI uses the remnant peripheral stem cells collected during initial alloSCT.

Univariate and multivariate analyses for OS revealed that the patients whose initial alloSCT donor was haplo-related donor have a worse prognosis than the patients whose donor was a full-matched sibling donors. All 6 patients who received haploidentical donor transplants received alloSCT after 2010. Patients receiving these haploidentical donor transplants and DLI were more likely to develop acute GVHD after DLI. Acute GVHD occurred in 4 out of 6 patients (66.7%) and in 31 out of 75 patients (41.3%), who received transplants from the haploidentical donor and other donors, respectively. This suggests that the acute GVHD incidence is increased while transplantation from the haploidentical donor and DLI, as described in previous studies (9, 26). Four of the 6 patients who received haploidentical donor transplants had

maintained bone marrow remission for at least 5 months after alloSCT, which is considered as a favorable prognostic factor. However, 5 of these 6 patients died within 30 days of receiving DLI. The OS was shorter compared to other studies (27, 28). There were two fatal GVHD cases among these 6 patients, one of whom received C-DLI, whose major symptom was massive diarrhea and serum bilirubin elevation. The other one had received G-DLI and died rapidly due to hepatitic variant GVHD. This hepatitic variant GVHD is known to occur especially after DLI, and is thought to be due to a mechanism different from that of GVHD after alloSCT. The longer interval from alloSCT to relapse indicated a better prognosis in both univariate and multivariate analyses. This result is similar to that of a retrospective study conducted earlier (29). The most important prognostic factor was obtaining bone marrow remission after the treatment. This trend was also observed in long-term survivors. In addition, maintaining bone marrow remission with appropriate additional treatment was essential for long-term survival. In particular, solitary extramedullary (central nervous system) relapse was observed in two of the patients treated with G-DLI and had long-term survival. Similar results have been reported in previous studies (9, 30). The reason of this solitary relapse pattern remains unknown, and thus, future studies are warranted.

In this study, 6 patients had CC at the baseline chimerism study. One of them had AML relapse while maintaining CC. The patient underwent alloSCT from a matched sibling donor. It appears that some genetic abnormality triggered donor cell origin AML. After reinduction chemotherapy and the first DLI, he received one more chemotherapy and DLI and obtained bone marrow

CR. However, cord compression occurred due to chloroma in the spine, and after surgery, he died of pneumonia. The remaining five patients with CC were extramedullary relapses. Two patients died of GVHD and combined sepsis after DLI. One patient had a slight decrease in chloroma size, and chloroma was surgically removed. However, the patient died of pneumonia. The remaining two patients experienced extramedullary relapses several times but survived longer than two years. One patient maintained complete remission after reinduction chemotherapy and DLI to treat chloroma in the left hip joint. However, chloroma recurred repeatedly in the CNS, spine and kidney. The other patient developed chloroma in the scalp and left adnexa. After chemotherapy and DLI, the lesions improved, but chloroma recurred at the pelvis and the patient received additional treatment. Then, the patient received 2nd alloSCT from the same donor. The treatment of these extramedullary relapses has not yet established. The DLI is considered as one of the treatments (31-33). However, in sites where immune escape is possible, the GVL effect is known to be diminished, which results in less impact of the DLI in these sites (34). Besides, extramedullary relapse after DLI has been reported in other studies (9, 30, 35).

A factor as important as efficacy is safety. There were no significant differences in the incidence of acute GVHD, grades, and fatal GVHD according to the cell sources.

This study was a single center, retrospective study, with certain limitations: it does not include further treatments such as additional DLI or second alloSCT. In particular, our institution cryopreserves the remnant stem

cells at −190°C liquid nitrogen, which may differ from the other institution's protocols. In the case of G-DLI, the cryopreserved $CD34^+$ cell count is not based on thawing and remeasurement, but is estimated based on the amount collected during the initial alloSCT. Cell viability could not be confirmed as well. Moreover, there were missing values of the chimerism after DLI. This is because the patients often died before reassessing chimerism.

In this study, we investigated the difference in prognosis according to the cell source of DLI in patients with relapsed AML after alloSCT. As a result, G-DLI, which uses remnant stem cells collected from a previous alloSCT, was as effective as C-DLI, and there was a trend of a better effect. The use of remnant cryopreserved stem cells without additional donor lymphocyte collection from the initial donor provides further economic, time, and human resources savings. In addition, by identifying the prognostic factors affecting survival in relapsed AML patients after alloSCT, it was confirmed that acquiring sustainable bone marrow remission is important for the prognosis of AML patients.

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초 록

동종조혈모세포이식술(allogeneic stem cell transplantation)을 시행받은 급성 골수구성 백혈병 환자에서 재발은 치료 실패의 주요 원인 중 하나이다. 공여자림프구주입술(donor lymphocyte infusion, DLI)은 동종조혈모세포이식술 시행 후 재발한 급성 골수구성 백혈병(acute myeloid leukemia, AML) 환자에서 사용가능한 치료로 알려져있다. 여러 기관에서는 동종조혈모세포이식 진행시 공여자림프구주입술을 시행할 것을 고려하여 조혈모세포를 동결 보존해두었다가 재발한 환자의 구제요법으로 사용한다. 하지만 공여자림프구주입술을 시행할 때 이전 동결보존한 조혈모세포를 세포원(cell source)으로 사용하는 것에 대해서는 아직까지 그 효과와 안정성에 대한 연구가 부족하다. 특히, 공여자림프구주입술의 부작용 중 하나인 이식편대숙주병(graft-versus-host disease, GVHD)이 생길 우려가 있고, 세포원에 따라 그 발생 빈도의 차이가 어떤지는 명확히 알려져 있지 않다. 이 연구에서는 동종조혈모세포이식술 시행 후 재발한 급성 골수구성 백혈병 환자들에서 과립구집락자극인자 주입 후 채집된 세포를 이용한 공여자림프구주입술(granulocyte colony-stimulating factor mobilized donor lymphocyte infusion, G-DLI) 와 전통적인 방식으로 채집된 세포를 이용한 공여자 림프구 주입술(conventional donor lymphocyte infusion, C-DLI) 의 효능과 안정성을 비교하고자 하였다. 2001년 1월부터 2017년 12월까지 총 244례의 공여자림프구주입술 중 분석에 적합한 81례를 대상으로 분석하였다. 50명의 환자가 전통적인 방식으로 채집된 세포를 이용하였고 31명의 환자가 과립구집락자극인자 주입 후 채집된 세포를 이용하였다. 두 군 간에 $CD3^+$ T 세포의 수는 통계적으로 유의한 차이를 보이지 않았다 (C-DLI: 1.044×10^8/kg vs. G-DLI: 0.855×10^8/kg). 전통적인 방식으로 채집된 세포를 이용한 공여자림프구주입술을 시행받은 환자들의 생존기간의 중위값은 106일이었고, 과립구집락자극인자 주입 후 채집된 세포를 이용한

환자들의 생존기간의 중위값은 139일로 양 군 간에 통계적으로 유의한 차이를 보이지 않았다(p=0.58). 또, 양 군 간에 이식편대숙주반응의 발생률에서도 통계적으로 유의한 차이를 보이지 않았다(44% in C-DLI vs. 41.9% in G-DLI). 발생한 이식편대숙주반응의 중증도에서도 차이를 보이지 않았고, 치명적인 이식편대숙주반응의 발생 빈도에서도 차이를 보이지 않았다. 따라서, 동종조혈모세포이식시 채집하여 냉동보존 해두었던 과립구집락자극인자 주입 후 채집된 세포들을 동종조혈모세포이식 후 재발한 급성 백혈병 환자들의 공여자림프구주입술에 사용하는 것은 전통적인 방식으로 채집된 세포를 이용한 공여자림프구주입술 대신 사용할 수 있을만큼 효과적이고 안전하다.

주요어 : 동종조혈모세포이식술, 급성 골수구성 백혈병, CD34, 공여자림프구주입술
학 번 : 2018-26162