의학석사 학위논문

동종조혈모세포이식 후 재발한 급성골수성백혈병 환자에서 고강도 구제항암요법 후 과립구집락자극인자 가동 공여자백혈구주입 연구

Granulocyte-colony stimulating factor mobilized donor leukocyte infusion following intensive salvage chemotherapy for patients with relapsed acute myeloid leukemia after allogeneic hematopoietic cell transplantation

울산대학교 대학원

의 학 과

한 진 희

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이 논문을 의학석사 학위 논문으로 제출함

2024 년 8 월

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Abstract

Background: The prognosis for relapsed acute myeloid leukemia (AML) following allogeneic hematopoietic cell transplantation (HCT) remains remarkably poor, with no established standard therapy. Donor lymphocyte infusion or second transplantation are potential options for relapsed AML after HCT, providing long-term remission to a limited subset of patients. In this study, we aimed to evaluate the treatment outcomes of granulocyte-colony stimulating factor (G-CSF) mobilized door leukocyte infusion after intensive chemotherapy (chemo-mDLI).

Methods: We conducted a retrospective analysis of 55 patients with AML who experienced relapse after allogeneic HCT and received chemo-mDLI between 1997 and 2023. We evaluated treatment outcomes including complete remission (CR) rate, overall survival (OS), leukemia-free survival (LFS), cumulative incidence of relapse or progression (CIR), non-relapse mortality (NRM), engraftment, and graft-versus-host disease (GVHD).

Results: Thirty-six of 55 patients (65.5%) achieved CR/CR with incomplete hematologic recovery after chemo-mDLI. After a median follow-up period of 4.8 years for surviving patients, 31 patients (56.4%) experienced disease relapse or progression and 46 patients (83.6%) died. The 2-year CIR was 51.2%, while NRM was 27.3%, resulting in an estimated median OS of 8.4 months (95% confidence interval [CI], 5.8–11.1 months). Neutrophil and platelet engraftment were attained in 90.9% and 72.7% of patients at a median of 12 and 15 days, respectively. The incidence of all grade and grade II-IV acute GVHD were 43.6% and 40.0%, and the 2-year incidence of total and moderate-to-severe chronic GVHD were 38.2% and 20.0%, respectively. Patients who received chemo-mDLI as an initial treatment for relapse showed significantly higher CR rate (71.1% vs 41.2%; $P = 0.035$), longer OS (median 10.2 months vs 2.2 months, adjusted hazard ratio [HR], 4.12; 95% CI, 1.83–9.31; $P = 0.001$) and longer LFS (median 8.8 months vs 2.5 months, adjusted HR, 7.90; 95% CI, 2.09–29.87; $P = 0.002$) compared to patients who received the therapy as second-line or more. Clinical factors predicting longer OS and LFS after chemo-mDLI were lower bone marrow blast percentage (< 40%), favorable cytogenetics at relapse, higher CD34+ cell dose (≥ 3×10^6/kg) and receiving prior HCT in remission. Longer post-HCT remission duration (> 5months) was associated with a higher CR rate compared to shorter CR duration (71.1% vs. 41.2%; P = 0.035). Regarding salvage chemotherapy regimens, treatment with cytarabine, mitoxantrone, and etoposide or cytarabine, idarubicin, and etoposide was associated with lower CIR (44.7% vs 87.5%, adjusted HR, 5.11; 95% CI, 1.61–16.20; $P = 0.006$) compared to other regimens.

Conclusion: In patients with relapsed AML after allogeneic HCT, G-CSF mobilized donor leukocyte infusion following intensive salvage chemotherapy demonstrated a high CR rate and induced durable remission in a subset of patients. Our findings suggest that chemo-mDLI is an effective therapeutic approach as initial therapy for relapsed AML after HCT, particularly in patients who had achieved CR at prior HCT with a lower leukemic burden at relapse.

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1. Introduction

Allogeneic hematopoietic cell transplantation (HCT) offers the potential for achieving long-term remission in patients with acute myeloid leukemia (AML). However, a significant proportion of patients experience disease relapse following allogeneic HCT, and the prognosis for relapsed AML is remarkably poor (1). Despite various treatment options being attempted for relapsed patients, including salvage chemotherapy, donor lymphocyte infusion (DLI), and second allogeneic HCT, there is no standardized effective therapeutic strategy for relapsed AML post-transplantation (2).

DLI has been utilized as a therapeutic approach inducing a graft-versus-leukemia (GVL) effect (3). However, DLI alone has often been proven insufficient for achieving durable remission in patients with AML, possibly due to the aggressive nature of the disease, which may surpass a GVL effect and trigger diverse immune escape mechanisms (4). Therefore, several studies have attempted to enhance the efficacy of DLI, including incorporating pre-DLI chemotherapy for cytoreduction and adjusting the timing and dosage of DLI (5, 6). Some evidence indicates that combining chemotherapy with DLI promotes the GVL effect and induces more durable remission than DLI or chemotherapy alone for post-HCT relapse in AML (3, 7, 8). Second allogeneic HCT is also a valid treatment option for relapsed AML after allogeneic HCT. However, not all patients have available human leukocyte antigen (HLA)-matched donors. The likelihood of finding a matched available donor on registry ranges from 16% to 75%, depending on racial and ethnic groups (9). Besides, there is a concern that treatment-related mortality after second HCT might increase in patients who relapse early after the first HCT (10).

Given this background, we previously conducted a study regarding intensive chemotherapy followed by G-CSF-mobilized donor leukocyte infusion (chemo-mDLI) as a treatment for relapsed AML after allogeneic HCT. In that study, chemo-mDLI resulted in a high remission rate and acceptable survival outcomes, though the study had a limited number of patients (11). In this study, we aimed to analyze the treatment outcomes of chemo-mDLI in a larger cohort of patients with AML relapsed after allogeneic HCT. In addition, we evaluated prognostic factors associated with superior outcomes post-chemo-mDLI to identify patients who may benefit most from this treatment approach.

2. Methods

2.1. Study participants

In this retrospective study, we reviewed patients who underwent DLI for relapsed AML after allogeneic HCT at Asan Medical Center, a referral hospital in Seoul, Republic of Korea, between 1997 and 2023. Patients receiving DLI without G-CSF mobilization were excluded. Patients who did not receive salvage chemotherapy or who received low-intensity chemotherapy such as venetoclax/ hypomethylating agents prior to mobilized donor leukocyte infusion (mDLI) were also excluded from the study. Patients with an interval between chemotherapy and mDLI exceeding 1 month were excluded. Therefore, only patients who underwent mDLI within 3 days after the completion of salvage chemotherapy were included. Patients' data were collected from a retrospective chart review. The study protocol was approved by the Institutional Review Board (IRB) of Asan Medical Center (IRB No. 2024–0330). Due to the retrospective nature of the study, the requirement for informed consent was waived by the IRB.

2.2 Treatment procedures

The chemotherapeutic regimens prior to cell infusion were cytarabine, mitoxantrone, and etoposide (CME) in 29 patients; cytarabine, idarubicin, and etoposide (AIE) in 18 patients; cytarabine and daunorubicin (AD) in 3 patients, fludarabine, cytarabine, and idarubicin (FLAI) in 3 patients; cladribine, cytarabine, and mitoxantrone (CLAG-M) in 1 patient; and high-dose cytarabine and etoposide (EA) in 1 patient. The dosage of each chemotherapeutic agent is detailed in Table 1. Patients received intensive chemotherapy for 5 to 7 days depending on the regimen, and donor leukocytes were infused within 3 days of completion of chemotherapy. Donors received subcutaneous injection of G-CSF at a dose of 10 μg/kg daily starting from day -4 of cell infusion for 4 days. On day 0, fresh G-CSF-mobilized peripheral blood mononuclear cells from donors were administered without manipulation. For patients who received chemo-mDLI since 2008, cyclosporine has been given for GVHD prophylaxis, starting from day -1 and tapered beginning between 1 and 2 months after cell infusion if there were no signs of GVHD.

TABLE 1. Types and dosages of chemotherapeutic agents infused before donor leukocyte infusion.

Chemotherapy regimen		Number of patients
CME	Cytarabine 400-1000 mg/m^2/day (Day -7 to -3)	
	Mitoxantrone 12 mg/m^2/day (Day -7 to -5)	n = 29
	Etoposide 150 mg/m^2/day (Day -7 to -5)	
AIE	Cytarabine 1000 mg/m^2/day (Day -7 to -2)	
	Idarubicin 12 mg/m^2/day (Day -7 to -5)	n = 18
	Etoposide 150 mg/m^2/day (Day -7 to -5)	
FLAI	Fludarabine 30 mg/m^2/day (Day -7 to -3)	
	Cytarabine 2000 mg/m^2/day (Day -7 to -2)	n = 3
	Idarubicin 12 mg/m^2/day (Day -7 to -5)	
AD	Cytarabine 200 mg/m^2/day (Day -9 to -3)	n = 3
	Daunorubicin 90 mg/m^2/day (Day -7 to -5)	
EA	Cytarabine 3000 mg/m^2 Q12H (Day -7, -5, -3)	n = 1
	Etoposide 150 mg/m^2/day (Day -7 to -5)	
CLAG-M	Cytarabine 2000 mg/m^2/day (Day -7 to -3)	
	Cladribine 5 mg/m^2/day (Day -7 to -3)	n = 1
	G-CSF 300 μg/day (Day -7 to -3)	
	Mitoxantrone 10 mg/m^2/day (Day -7 to -5)	

Day 0 is the day of the day of cell infusion.

2.3 Outcomes and definitions

The treatment outcomes included complete remission (CR) rate, engraftment, overall survival (OS), leukemia-free survival (LFS), cumulative incidence of relapse or progression (CIR), non-relapse mortality (NRM), and cumulative incidence of acute and chronic GVHD. CR was defined by bone marrow (BM) blasts less than 5%, absence of circulating blast, and no evidence of extramedullary disease along with an absolute neutrophil count $\geq 1\times10^9$/L and a platelet count $\geq 100\times10^9$/L. If the count of either neutrophil or platelet remained below the above stated levels, it was classified as complete remission with incomplete hematologic recovery (CRi) (12). Neutrophil engraftment was defined as the first day of 3 consecutive days of an absolute neutrophil count $\geq 0.5\times10^9$/L, and platelet engraftment was defined as the first day of 7 consecutive days of a platelet count $\geq 20\times10^9$/L without transfusion. LFS was measured from the date of CR/CRi to the date of disease relapse, death from any cause, or last follow-up, whichever occurred first. Relapse was defined as an increase of BM blasts > 5%, or the reappearance of circulating blasts or extramedullary disease after achieving a morphologic remission, and progression was defined as persistence of active disease after chemo-mDLI without achieving a morphologic remission. Acute and chronic GVHD were graded according to Mount Sinai Acute GVHD International Consortium (13) and National Institutes of Health consensus criteria (14) respectively. The genetic analysis of patients at relapse was stratified by the European LeukemiaNet (ELN) 2022 classification system (15). In patients without molecular genetic testing data, risk was assessed only by cytogenetics.

2.4 Statistical analysis

OS and LFS were evaluated using the Kaplan-Meier method and differences between two groups were assessed by the log-rank test. Cox regression analyses were conducted for multivariate analysis of survivals and hazard ratios (HR) with a 95% confidence interval (CI) were reported. Variables with *P*-value less than 0.1 on univariate analysis were included in multivariate analysis. The incidence of engraftment, relapse or progression, NRM, and GVHD were analyzed using a cumulative incidence function with competing risks and compared by the Gray's test. The differences in CR rate according to the categorical variables were assessed by the chi-squared test or Fisher's exact test. Logistic regression analysis was used for evaluating association between the continuous variables and CR rate. All reported *P* values were two-sided with *P* value < 0.05 indicating statistical significance. The statistical analyses were conducted using SPSS version 23.0 (IBM Corp., Armonk, NY, USA) and R version 4.3.3 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1 Study participants and baseline characteristics

Between 1997 and 2023, a total of 174 patients received DLI for relapsed AML after allogeneic HCT from the same donors at Asan Medical Center. Of these patients, 119 patients were excluded: 109 underwent DLI without G-CSF mobilization, 6 received mDLI without prior conditioning chemotherapy, 1 received low-intensity salvage chemotherapy, and 3 underwent mDLI not immediately after chemotherapy. Finally, 55 patients were included in the final analysis (Figure 1).

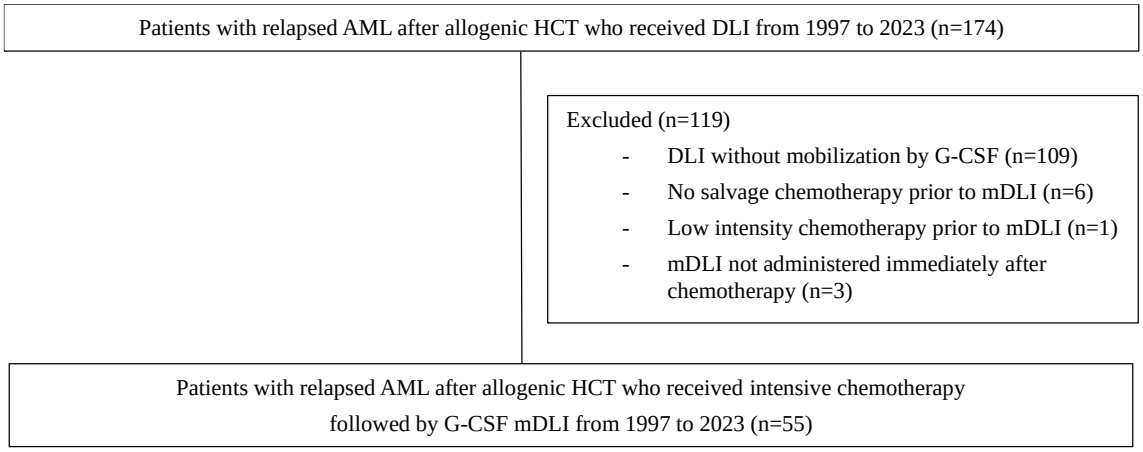


Figure 1. Flowchart of the screening and selection of the study population

Abbreviations: AML, acute myeloid leukemia; DLI, donor lymphocyte infusion; G-CSF, granulocyte-colony stimulating factor; HCT, hematopoietic cell transplantation; mDLI, mobilized donor leukocyte infusion

Table 2 presents the baseline characteristics of patients and donors. The median time interval between HCT and relapse was 10.5 months (range, 0.6–51.9 months), and the median time interval between relapse and mDLI was 20 days (range, 6–196 days). Among the 55 patients, 21 (38.2%) were male, and the median age was 46 years (range, 19–65 years). Forty-one patients (74.5%) received mDLI from matched sibling donors, 1 (1.8%) from a matched unrelated donor, and 13 (23.6%) from haploidentical familial donors. Among 54 evaluable patients, the ELN risk at the time of relapse was categorized into favorable in 6 patients, intermediate in 26 patients, and adverse in 22 patients. Forty-five patients (81.8%) received chemo-mDLI as a first systemic treatment for relapse after allogeneic HCT, whereas 10 patients (18.2%) had previously been treated with other systemic therapies prior to chemo-mDLI and subsequently experienced relapse or progression. Chronic GVHD was observed in 20 patients after allogeneic HCT but was well controlled at the time of chemo-mDLI. Only 3 patients were receiving systemic treatment for GVHD, which was being tapered at the time of relapse. The median infused dose of CD3+ cells was 2.82×10^8/kg (range, $1.23–8.25 \times 10^8$/kg), and the median CD34+ cell dose was 5.4×10^6/kg (range, $1.1–27.2 \times 10^6$/kg).

TABLE 2. Clinical characteristics of the study population

Characteristics	Total (n = 55)
Follow-up of survivors, months, median (range)	57.3 (6.4–134.3)
Age, years, median (range)	46 (19–65)
Sex	
Male/Female	21 (38.2%)/34 (62.8%)
Donor relationship	
Matched sibling donor	41 (74.5%)
Matched unrelated donor	1 (1.8%)
Haploidentical familial donor	13 (23.6%)
Donor age, years, median (range)	44 (17–64)
Donor sex	
Male/Female	38 (69.1%)/ 17 (31.9%)
Disease status at the time of prior HCT	
Complete remission	39 (70.9%)
Active leukemia (primary induction failure, relapse or untreated)	16 (29.1%)
Time interval between HCT and relapse, months, median (range)	10.5 (0.6–81.0)
Time interval between relapse and mDLI, days, median (range)	20 (6–196)
BM blast at relapse, %, median (range)	38 (0–94.0)
Extramedullary disease at mDLI	11 (20.0%)
HCT-CI at mDLI, median (range)	3 (0-7)
ELN risk at relapse	
Favorable	6 (10.9%)
Intermediate	26 (47.3%)
Adverse	22 (40.0%)
Not evaluable	1 (1.8%)
Chronic GVHD after HCT	20 (36.4%)
mDLI as the first systemic treatment for relapse	45 (81.8%)
Conditioning chemotherapy regimen	
CME	29 (52.7%)
AIE	18 (32.7%)
Others: FLA-I/AD/CLAG-M/EA	3 (5.5%)/3 (5.5%)/1 (1.8%)/1 (1.8%)
Infused CD3+ cells dose, $\times 10^8$/kg, median (range)	2.82 (1.23–8.25)
Infused CD34+ cells dose, $\times 10^6$/kg, median (range)	5.4 (1.1–27.2)
Cyclosporine for GVHD prophylaxis	39 (70.9%)

Data are presented as the median (range) or frequency (proportion).

Abbreviation: AD, cytarabine and daunorubicin; AIE, cytarabine, idarubicin, and etoposide; BM, bone marrow; CLAG-M, cladribine, cytarabine, and mitoxantrone; CME, cytarabine, mitoxantrone, and etoposide; EA, high-dose cytarabine and etoposide; ELN, European LeukemiaNet; FLAI, fludarabine, cytarabine, and idarubicin; GVHD, graft-versus-host disease; HCT, hematopoietic cell transplantation; HCT-CI, HCT specific comorbidity index; mDLI, mobilized donor leukocyte infusion;

3.2 Treatment response and engraftment

Following chemo-mDLI, CR/CRi was achieved in 36 patients (65.5%), including 2 patients who remained in CRi. Eleven patients were unevaluable due to early death within 2 months after chemo-mDLI (Table 3). Among the 36 patients achieving CR/CRi, 23 experienced disease relapse, and 5 patients died from non-relapse causes. Patients who received chemo-mDLI as the first systemic treatment for relapse showed a significantly higher CR rate than those receiving chemo-mDLI after other systemic therapies (71.1% vs 20.0%; P = 0.004). Post-HCT remission duration longer than 5 months showed a higher CR rate than shorter CR duration before chemo-mDLI (71.1% vs 41.2%; P = 0.035).

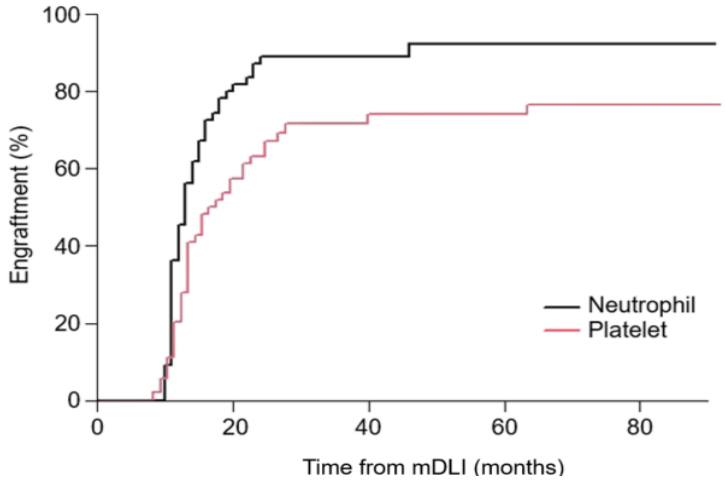
Neutrophil was engrafted in 50 out of 55 patients at a median of 12 days, with a cumulative incidence at day 28 of 89.1% (Figure 2). Platelet engraftment was attained in 40 of 55 patients at a median of 15 days, with the cumulative incidence of platelet engraftment at day 90 being 75.0%. There were no significant factors associated with the incidence of neutrophil and platelet engraftment.

Table 3. Treatment outcomes of the study population (N=55)

Outcome	% (95% CI)
Response, No (%)	
CR	34 (61.8)
CRi	2 (3.6)
No response	8 (14.5)
Early death (within 2 months)	11 (20.0)
OS, 2-year	27.9 (21.7–34.1)
OS, months, median (95% CI)	8.4 (5.8–11.1)
LFS in CR/CRi, 2-year	32.9 (25.0–40.8)
LFS in CR/CRi, months, median (95% CI)	7.6 (5.4–9.9)
CIR, 2-year	51.2 (44.3–58.1)
NRM, 2-year	27.3 (21.2–33.36)

Abbreviations: CI, confidence interval; CIR, cumulative incidence of relapse or progression; CR, complete remission; CRi, complete remission with incomplete count recovery; LFS, leukemia-free survival; NRM, non-relapse mortality; OS, overall survival

Figure 2. Cumulative incidence of neutrophil and platelet engraftment until day 90 after mobilized donor leukocyte infusion (mDLI).



3.3 Relapse, non-relapse mortality, and survival outcomes

After a median follow-up duration of 4.8 years for surviving patients, 31 patients (56.4%) experienced disease relapse or progression, with 11 exhibiting extramedullary relapse. A total of 46 patients (83.6%) died, of which 16 died from non-relapse causes. The estimated 2-year CIR was 51.2% (95% CI, 44.3–58.1%), and the 2-year NRM was 27.3% (95% CI, 21.2–33.4; Figure 3). The estimated median OS and 2-year OS rate of total patients were 8.4 months (95% CI, 5.8–11.1 months) and 27.9% (95% CI, 21.7–34.1%; Figure 4-A), and the median LFS and 2-year LFS rate of 36 patients achieving CR/CRi after chemo-mDLI were 7.6 months (95% CI, 5.4–9.9 months) and 32.9% (95% CI, 25.0–40.8%; Figure 4-B), respectively.

Figure 3. The plot of cumulative incidence of relapse or progression (CIR) and non-relapse mortality (NRM) after mobilized donor leukocyte infusion (mDLI).

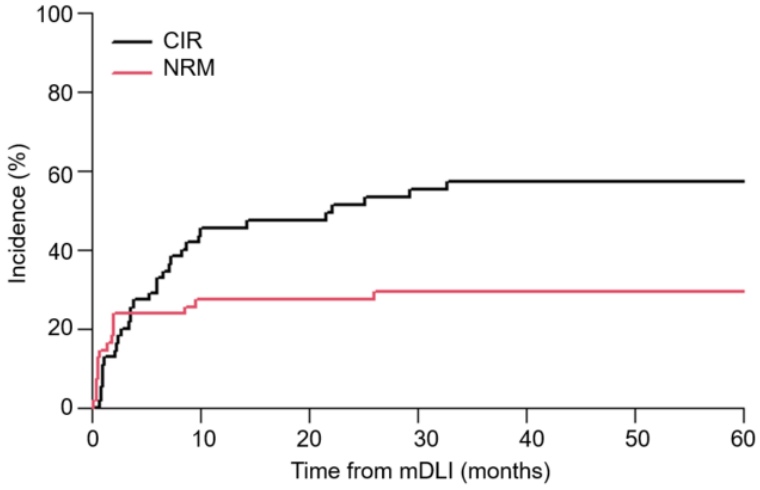


Figure 4-A. Kaplan–Meier plot of overall survival after mobilized donor leukocyte infusion (mDLI)

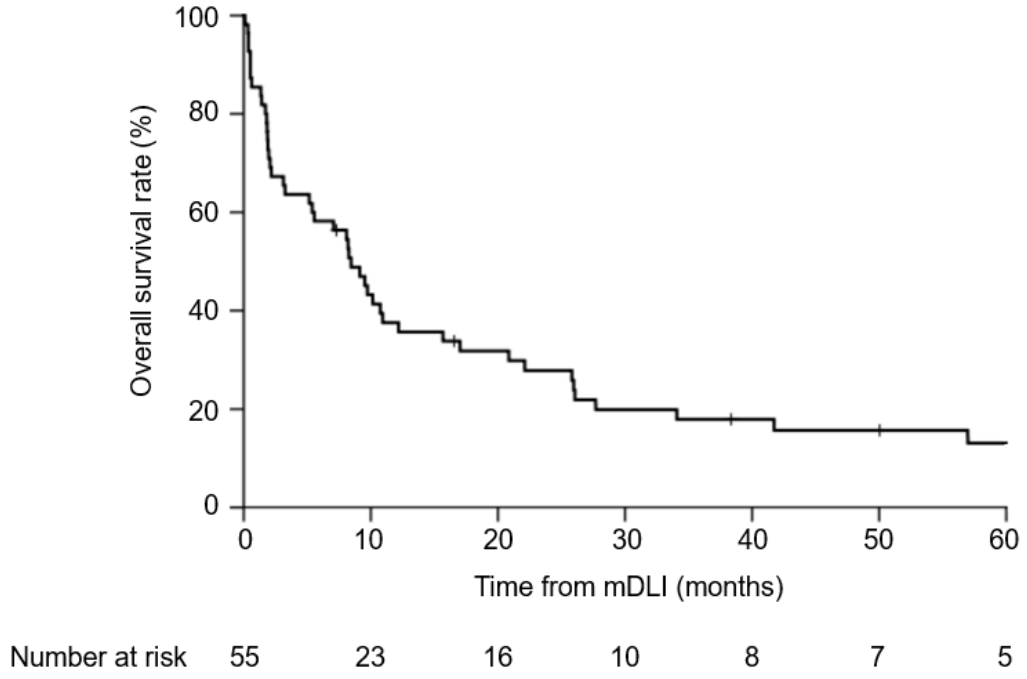


Figure 4-B. Kaplan–Meier plot of leukemia-free survival from the time of achieving complete remission or incomplete hematologic recovery (CR/CRi)

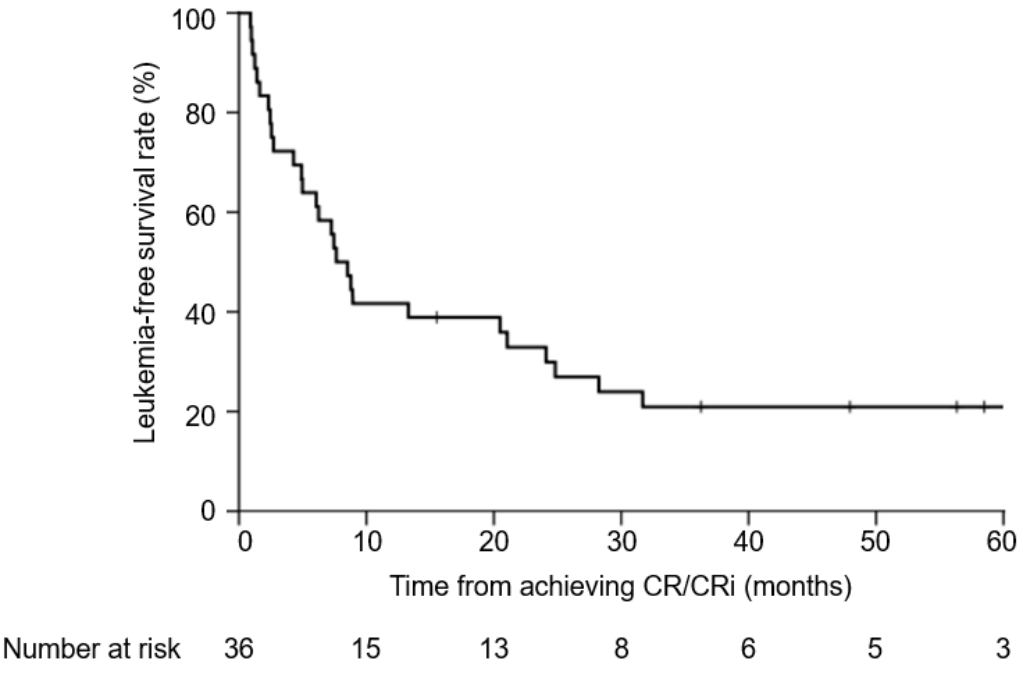
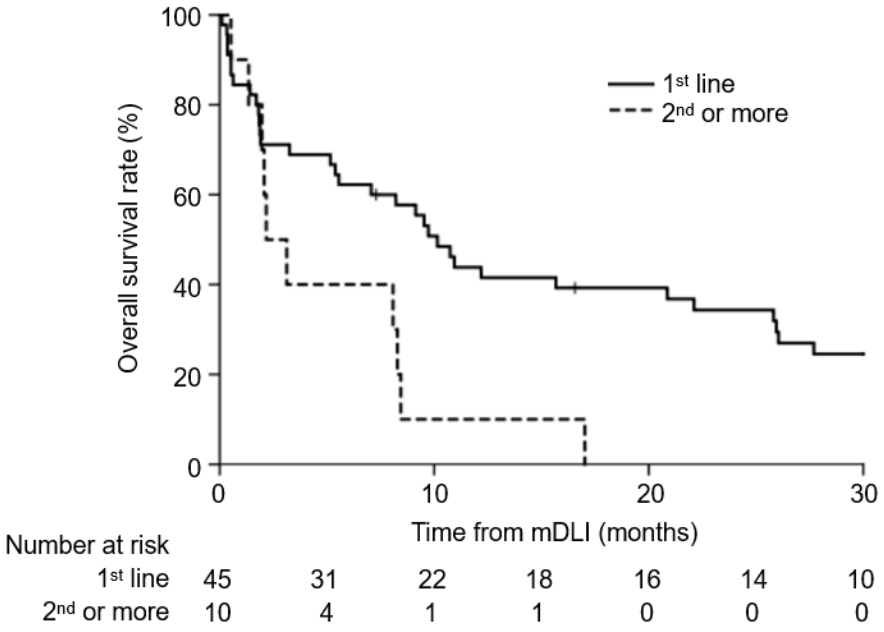


Figure 5. Kaplan–Meier plot of overall survival according to the line of treatment of mobilized donor leukocyte infusion (mDLI) for relapse after hematopoietic cell transplantation



The results of univariate and multivariate analyses of risk factors influencing OS and LFS are summarized in Table 4. Patients who received chemo-mDLI as a first-line systemic treatment for relapse demonstrated a higher survival rate than those who received it as a second-line or more (2-year OS of 34.3% vs. 0%; adjusted HR, 4.12; 95% CI, 1.83–9.31; P = 0.001; Figure 5). Higher BM blasts percentage (> 40%) (adjusted HR, 2.43; 95% CI, 1.28–4.63; P = 0.007) and unfavorable cytogenetic risk at relapse (adjusted HR, 4.70; 95% CI, 1.32–16.67; P = 0.017 for intermediate risk; adjusted HR, 7.73; 95% CI, 2.06–29.05; P = 0.002 for adverse risk) were also found to be significant risk factors for OS.

Higher BM blast percentages (adjusted HR, 2.36; 95% CI, 1.06–5.26; P = 0.036) and whether chemo-mDLI was the initial treatment for relapse (adjusted HR, 7.90; 95% CI, 2.09–29.87; P = 0.002) were both identified as predictive factors for LFS. In addition, patients who achieved CR at the time of prior HCT showed longer LFS after chemo-mDLI than those who underwent transplantation with active leukemia (adjusted HR, 5.00; 95% CI, 1.91–13.09; P = 0.001). Furthermore, infused CD34+ cell dose exceeding 3×10^6/kg was associated with longer LFS after chemo-mDLI compared to infused CD34+ cells less than 3×10^6/kg (adjusted HR, 5.45; 95% CI, 1.72–17.29; P = 0.004).

A higher CD34+ cell dose ($\geq 3\times10^6$/kg) was also associated with a lower CIR (adjusted HR, 2.82; 95% CI, 1.15–6.94; P = 0.024) (Table 5). Regarding salvage chemotherapy regimens, CME or AIE regimens were associated with a lower CIR (adjusted HR, 5.11; 95% CI, 1.61–16.20; P = 0.006) compared to other regimens. There were no significant factors associated with NRM.

TABLE 4. Univariate and multivariate analyses of prognostic factors for survival outcomes

Characteristics	Overall survival				Leukemia free survival			
	Univariate analysis		Multivariate analysis†		Univariate analysis		Multivariate analysis†	
	Months, median (95% CI)	*P*	HR (95% CI)	*P*	Months, median (95% CI)	*P*	HR (95% CI)	*P*
Age		0.516				0.176		
< 50	7.1 (0–15.4)				8.9 (0–30.2)			
≥ 50	8.4 (6.4–10.4)				7.4 (4.4–10.5)			
Sex		0.170				0.156		
Male	8.3 (0–19.9)				5.0 (0.9–9.1)			
Female	8.4 (6.5–10.4)				8.8 (6.8–10.8)			
Donor type		0.813				0.381		
Matched	8.1 (2.4–13.7)				8.9 (0–18.8)			
Haploidentical	9.1 (7.7–10.6)				7.4 (4.5–10.4)			
Donor age		0.887				1.00		
< 50	8.3 (3.2–13.3)				7.4 (3.9–11.0)			
≥ 50	9.1 (6.2–12.1)				7.6 (5.2–10.0)			
Donor sex		0.361				0.435		
Male	8.2 (3.6–12.8)				7.4 (4.6–10.3)			
Female	10.9 (5.8–16.1)				8.8 (5.4–9.9)			
Disease status at the time of prior HCT		0.335				0.030		0.001
Complete remission	8.4 (5.5–11.3)				8.8 (0–24.9)		1	
Active leukemia	8.2 (0–16.4)				4.9 (1.9–7.9)		5.00 (1.91–13.09)	
Time interval between HCT and relapse		0.154				0.962		
< 5 months	2.2 (0.5–3.8)				1.4 (0–3.3)			
≥ 5 months	10.7 (7.6–13.9)				8.5 (6.6–10.4)			
BM blast percentage at relapse		0.044		0.007		0.034		0.036
< 40%	10.9 (0–31.7)		1		21.0 (14.1–28.0)		1	
≥ 40%	5.6 (1.8–9.3)		2.43 (1.28–4.63)		5.0 (1.3–8.7)		2.36 (1.06–5.26)	

Characteristics	Overall survival				Leukemia free survival			
	Univariate analysis		Multivariate analysis†		Univariate analysis		Multivariate analysis†	
	Months, median (95% CI)	*P*	HR (95% CI)	*P*	Months, median (95% CI)	*P*	HR (95% CI)	*P*
Extramedullary disease at mDLI		0.523				0.848		
No	8.4 (3.9–13.0)				7.6 (5.6–9.7)			
Yes	8.2 (0–17.8)				6.3 (1.5–11.1)			
HCT-CI at mDLI		0.103				0.041		
< 4	9.5 (6.1–12.9)				21.0 (0–44.3)			
≥ 4	5.6 (5.8–11.1)				6.1 (0.3–11.8)			
ELN risk at relapse		0.084		0.002		0.476		
Favorable	17.0		1		7.2			
Intermediate	10.2 (6.1–14.2)		4.70 (1.32–16.67)	0.017	8.8 (5.2–12.4)			
Adverse	3.1 (0–9.0)		7.73 (2.06–29.05)	0.002	7.4 (2.5–12.4)			
First systemic treatment for relapse		0.012		0.001		0.038		0.002
Yes	10.2 (7.8–12.5)		1		8.8 (1.0–16.6)		1	
No (second or more)	2.2 (0.5–3.8)		4.12 (1.83–9.31)		2.5 (0–8.7)		7.90 (2.09–29.87)	
Conditioning chemotherapy regimen		0.179				0.632		
CME	12.2 (4.4–20.0)				8.8 (0–17.5)			
AIE	5.2 (0–15.5)				6.3 (0–13.2)			
Others: FLA-I/AD/CLAG-M/EA	3.1 (1.6–4.6)				2.5 (0–8.8)			
Infused CD34+ cells dose		0.444				0.013		0.004
< 3×10^6/kg	7.1 (0–17.6)				2.7 (2.1–3.3)		5.45 (1.72–17.29)	
≥ 3×10^6/kg	8.4 (6.5–10.4)				8.9 (5.4–9.9)		1	

† Variables with P-value less than 0.1 on univariate analysis were included in multivariate analysis.

Abbreviations: AD, cytarabine and daunorubicin; AIE, cytarabine, idarubicin, and etoposide; BM, bone marrow; CI, confidence interval; CLAG-M, cladribine, cytarabine, and mitoxantrone; CME, cytarabine, mitoxantrone, and etoposide; EA, high-dose cytarabine and etoposide; ELN, European LeukemiaNet; FLAI, fludarabine, cytarabine, and idarubicin; HCT, hematopoietic cell transplantation; HCT-CI, HCT specific comorbidity index; HR, hazard ratio; mDLI, mobilized donor leukocyte infusion

TABLE 5. Univariate and multivariate analyses of prognostic factors for relapse or progression

Characteristics	Univariate analysis		Multivariate analysis†	
	CIR, 2-year	*P*-value	Adjusted HR (95% CI)	*P*-value
Age		0.386		
< 50	45.2%			
≥ 50	59.4%			
Sex		0.375		
Male	58.7%			
Female	47.1%			
Donor type		0.937		
Matched	52.4%			
Haploidentical	46.2%			
Disease status at the time of prior HCT		0.038		0.064
Complete remission	43.6%		1	
Active leukemia	68.8%		2.03 (0.96–4.30)	
Time interval between HCT and relapse		0.454		
< 5 months	41.2%			
≥ 5 months	55.8%			
BM blast percentage at relapse		0.468		
< 40%	45.2%			
≥ 40%	57.1%			
Extramedullary disease at mDLI		0.257		
No	45.7%			
Yes	72.7%			
ELN risk at relapse		0.256		
Favorable	50.0%			
Intermediate	65.4%			
Adverse	36.4%			
First systemic treatment for relapse		0.106		
Yes	49.2%			
No (second or more)	60.0%			
Conditioning chemotherapy regimen		<0.001		0.006
CME or AIE	44.7%		1	
Others: FLA-I/AD/CLAG-M/EA	87.5%		5.11 (1.61–16.20)	
Infused CD34+ cells dose		0.096		0.024
< 3×10^6/kg	62.5%		1	
≥ 3×10^6/kg	47.1%		2.82 (1.15–6.94)	
Immunosuppression		0.210		
No	37.5%			
Cyclosporine	56.7%			

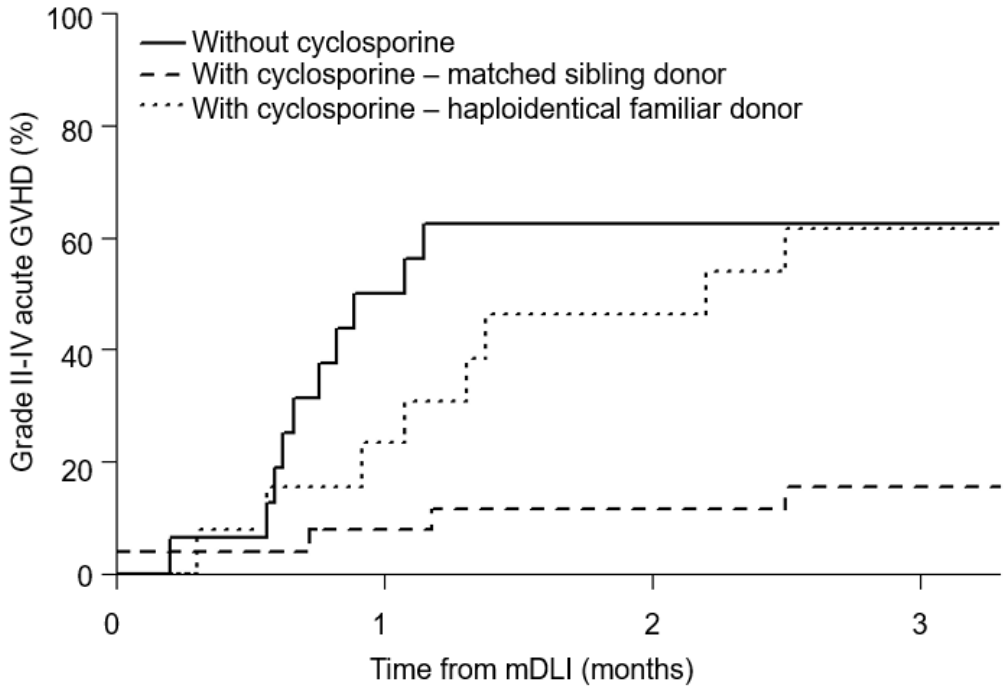
† Variables with P-value less than 0.1 on univariate analysis were included in multivariate analysis.

Abbreviation: CI, confidence interval; CIR, cumulative incidence of relapse or progression; ELN, European LeukemiaNet; HCT, hematopoietic cell transplantation; HR, hazard ratio; mDLI, mobilized donor leukocyte infusion

3.4 Graft-versus-host disease

The incidences of all grade and grade II-IV acute GVHD were 43.6% and 40.0% at 4 months, and those of total and moderate-to-severe chronic GVHD at 2 years were 38.2% and 20.0%, respectively. The incidence of grade II-IV acute GVHD was significantly lower in patients who received prophylactic cyclosporine after chemo-mDLI compared to those not receiving immunosuppressants (30.8% vs. 62.5%, $P = 0.008$; Figure 6). There was no significant difference in the 2-year incidence of moderate-to-severe chronic GVHD according to immunosuppression following chemo-mDLI. Whether patients had GVHD after prior HCT was not a significant predictive factor for the incidence of acute or chronic GVHD after chemo-mDLI. In a subgroup of patients who received cyclosporine after mDLI, haploidentical donors were associated with a higher incidence of grade II-IV acute GVHD than matched sibling donors (15.4% with 95% CI of 8.1–22.6 vs. 61.5% with 95% CI of 46.9–76.1; $P = 0.004$).

Figure 6. The cumulative incidence of grade II-IV acute graft-versus-host disease according to the administration of cyclosporine and donor type.



4. Discussion

In this retrospective study, G-CSF mobilized donor leukocyte infusion following intensive salvage chemotherapy in patients with AML relapsed after allogeneic HCT demonstrated a considerably high CR rate, leading to long-term remission in a subset of patients. The treatment outcomes of chemo-mDLI are comparable to those observed in second allogeneic HCT conducted at our center which demonstrated 2-year OS rate of 21.0% and 2-year CIR of 60.2% (16). Notably, receiving chemo-mDLI as an initial treatment for relapse, a lower BM blasts percentage at relapse, favorable cytogenetics at DLI, a higher dose of CD34+ cells, and achieving CR at prior HCT were identified as favorable prognostic factors in our study. These findings are in line with results from a retrospective study conducted by the European Society for Blood and Marrow Transplantation (EBMT), which demonstrated that BM blast percentage lower than 35% at relapse, remission prior to DLI, and favorable cytogenetics were predictive factors for longer survival in AML patients experiencing their first relapse after HCT (7).

We observed that an administered CD34+ cell dose higher than 3×10^6/kg was associated with a significantly lower CIR and prolonged LFS compared to a CD34+ cell dose less than 3×10^6/kg. Several reports have demonstrated a relationship between a higher CD34+ cell dose and favorable outcomes in the allogeneic HCT setting. Remberger et al. observed that CD34+ cells dose lower than 5×10^6/kg was associated with a lower incidence of chronic GVHD and a higher relapse rate, while CD34+ cell dose between 6 and 7×10^6/kg was related to a longer OS and a lower transplant-related mortality (17). Data from the EBMT registry on T-cell replete haploidentical HCT also demonstrated that patients receiving a higher dose of CD34+ cells experienced faster engraftment, less NRM, and longer OS and LFS (18).

Post-transplantation remission duration has been well established as a prognostic indicator in patients with relapsed AML after allogeneic HCT through previous studies. Even in the case of DLI, post-transplant remission duration longer than 6 months was associated with better response and survival (19, 20). In our study, longer post-transplant remission duration was associated with a higher CR rate but not with survival outcomes, possibly due to a small number of patients and a relatively high rate of early death from non-relapse causes in our study population, which need to be confirmed in a larger prospective study.

While chemotherapy combined with DLI has consistently shown better outcomes than DLI alone (7, 8, 21), the optimal chemotherapy regimens prior to DLI has not been well established. In our study, CME or AIE regimen resulted in a significantly lower CIR compared to other regimens in multivariate analysis, although these results should be interpreted cautiously due to the limited number of study participants.

Previous studies about chemo-mDLI conducted at our center reported a similar response and long-term survival rate as observed in the current study, but it notably exhibited a high frequency of extramedullary relapse at 80-100% (11, 20). High frequency of extramedullary relapse after allogeneic HCT may imply uneven GVL effect between BM and extramedullary sites (22, 23). However, in this study, the presence of extramedullary disease at chemo-mDLI was not associated with response rate or prognosis, and extramedullary relapses represented 35% of all relapses after chemo-mDLI. Whether the potency of GVL effect varies depending on the disease site is unclear, and further studies are required to assess the efficacy of chemo-mDLI for relapsed AML with the extramedullary disease.

The incidence of acute GVHD in our study is comparable with that reported in previous studies using mDLI (19, 24) and DLI with conventional doses without intensive chemotherapy (7, 25). Notably, patients who received chemo-mDLI from HLA-matched sibling donors with a short course of immunosuppression exhibited a low incidence of grade II-IV acute GVHD at 15.4%, without an increase in disease relapse. Challenges persist in chemo-mDLI from HLA-haploidentical donors, which showed a high incidence (61.5%) of acute GVHD despite the use of cyclosporine. These results align with findings from the previous study, which observed a cumulative incidence of 62.7% for grade II-IV acute GVHD (8). Since donor types were not associated with survival outcomes or relapse incidence after chemo-mDLI, strategies such as augmenting immunosuppression with post-transplantation cyclophosphamide, antithymocyte globulin, or other immunosuppressive agents could be considered to reduce GVHD after chemo-mDLI, particularly in HLA-haploidentical settings.

There are several limitations in our study. Firstly, the limited cohort size may produce selection bias and result in the oversight of significant factors due to low statistical power. In addition, due to the retrospective nature, the study population was heterogeneous, and treatment strategies were not unified. Moreover, since our study included patients over a long period of time, factors affecting survival may have been influenced by advances in medical technology and treatment modalities. Despite these limitations, we suggest that chemo-mDLI is effective as the first line treatment in patients with relapsed AML after allogeneic HCT.

5. Conclusion

We observed that the infusion of G-CSF-mobilized donor leukocytes following intensive salvage chemotherapy demonstrated a high CR rate and induced durable remission in a subset of patients with relapsed AML after allogeneic HCT. This therapeutic approach may particularly benefit patients receiving chemo-mDLI as an initial therapy for relapse, exhibiting a lower BM blasts percentage at relapse, and achieving remission at prior HCT. However, challenges remain regarding the relatively high rates of acute GVHD in haploidentical settings and the high incidence of disease relapse in the entire cohort. Further prospective studies involving larger patient cohorts and varying GVHD prophylactic approaches according to donor types are warranted to define optimal therapeutic strategies for chemo-mDLI in patients with relapsed AML after HCT.

List of abbreviations

AD	Cytarabine and daunorubicin
AIE	Cytarabine, idarubicin, and etoposide
AML	Acute myeloid leukemia
BM	Bone marrow
CI	Confidence interval
CIR	Cumulative incidence of relapse or progression
CLAG-M	Cladribine, cytarabine, and mitoxantrone
CME	Cytarabine, mitoxantrone, and etoposide
CR	Complete remission
DLI	Donor lymphocye infusion
EA	High dose cytarabine and etoposide
EBMT	European Society for Blood and Marrow Transplantation
ELN	European LeukemiaNet
FLAI	Fludarabine, cytarabine, and idarubicin
G-CSF	Granulocyte-colony stimulating factor
GVHD	Graft-versus-host disease
GVL	Graft-versus-leukemia
HCT	Hematopoietic cell transplantation
HCT-CI	Hematopoietic cell transplantation specific comorbidity index
HLA	Heuman leukocyte antigen
HR	Hazard ratio
LFS	Leukemia-free survival
mDLI	Mobilized donor leukocyte infusion
NRM	Non-relapse mortality
OS	Overall survival

6. References

1. Devillier R, Crocchiolo R, Etienne A, Prebet T, Charbonnier A, Furst S, et al. Outcome of relapse after allogeneic stem cell transplant in patients with acute myeloid leukemia. Leuk Lymphoma. 2013;54(6):1228-34.
2. Bejanyan N, Weisdorf DJ, Logan BR, Wang HL, Devine SM, de Lima M, 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):454-9.
3. Kolb HJ. Graft-versus-leukemia effects of transplantation and donor lymphocytes. Blood. 2008;112(12):4371-83.
4. Porter DL, Roth MS, Lee SJ, McGarigle C, Ferrara JL, Antin JH. Adoptive immunotherapy with donor mononuclear cell infusions to treat relapse of acute leukemia or myelodysplasia after allogeneic bone marrow transplantation. Bone Marrow Transplant. 1996;18(5):975-80.
5. Ye Y, Yang L, Yuan X, Huang H, Luo Y. Optimization of Donor Lymphocyte Infusion for AML Relapse After Allo-HCT in the Era of New Drugs and Cell Engineering. Front Oncol. 2021;11:790299.
6. Wang Y, Xu L, Yan C, Huang X. Modification of donor lymphocyte infusion: how to improve the outcome? Sci China Life Sci. 2019;62(9):1253-6.
7. Schmid C, Labopin M, Nagler A, Bornhauser M, Finke J, Fassas A, et al. Donor lymphocyte infusion in the treatment of first hematological relapse after allogeneic stem-cell transplantation in adults with acute myeloid leukemia: a retrospective risk factors analysis and comparison with other strategies by the EBMT Acute Leukemia Working Party. J Clin Oncol. 2007;25(31):4938-45.
8. Yan CH, Wang JZ, Liu DH, Xu LP, Chen H, Liu KY, et al. Chemotherapy followed by modified donor lymphocyte infusion as a treatment for relapsed acute leukemia after haploidentical hematopoietic stem cell transplantation without in vitro T-cell depletion: superior outcomes compared with chemotherapy alone and an analysis of prognostic factors. Eur J Haematol. 2013;91(4):304-14.
9. Gragert L, Eapen M, Williams E, Freeman J, Spellman S, Baitty R, et al. HLA match likelihoods for hematopoietic stem-cell grafts in the U.S. registry. N Engl J Med. 2014;371(4):339-48.
10. Shaw BE, Mufti GJ, Mackinnon S, Cavenagh JD, Pearce RM, Towlson KE, et al. Outcome of second allogeneic transplants using reduced-intensity conditioning following relapse of haematological malignancy after an initial allogeneic transplant. Bone Marrow Transplant. 2008;42(12):783-9.
11. Lee JH, Lee KH, Kim S, Seol M, Kim SH, Kim WK, et al. Combination chemotherapy of intermediate-dose cytarabine, idarubicin, plus etoposide and subsequent mobilized donor leukocyte infusion for relapsed acute leukemia after allogeneic bone marrow transplantation. Leuk Res. 2001;25(4):305-12.

12. Cheson BD, Bennett JM, Kopecky KJ, Buchner T, Willman CL, Estey EH, et al. Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. J Clin Oncol. 2003;21(24):4642-9.

13. Harris AC, Young R, Devine S, Hogan WJ, Ayuk F, Bunworasate U, et al. International, Multicenter Standardization of Acute Graft-versus-Host Disease Clinical Data Collection: A Report from the Mount Sinai Acute GVHD International Consortium. Biol Blood Marrow Transplant. 2016;22(1):4-10.

14. Jagasia MH, Greinix HT, Arora M, Williams KM, Wolff D, Cowen EW, 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 e1.

15. Dohner H, Wei AH, Appelbaum FR, Craddock C, DiNardo CD, Dombret H, et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood. 2022;140(12):1345-77.

16. Choi Y, Choi EJ, Lee JH, Lee KH, Jo JC, Park HS, et al. Second allogeneic hematopoietic stem cell transplantation in patients with acute leukemia relapsed after allogeneic hematopoietic stem cell transplantation. Clin Transplant. 2021;35(3):e14199.

17. Remberger M, Gronvold B, Ali M, Mattsson J, Egeland T, Lundin KU, et al. The CD34(+) Cell Dose Matters in Hematopoietic Stem Cell Transplantation with Peripheral Blood Stem Cells from Sibling Donors. Clin Hematol Int. 2020;2(2):74-81.

18. Maffini E, Labopin M, Blaise D, Ciceri F, Gulbas Z, Deconinck E, et al. CD34+ cell dose effects on clinical outcomes after T-cell replete haploidentical allogeneic hematopoietic stem cell transplantation for acute myeloid leukemia using peripheral blood stem cells. A study from the acute leukemia working Party of the European Society for blood and marrow transplantation (EBMT). Am J Hematol. 2020;95(8):892-9.

19. Levine JE, Braun T, Penza SL, Beatty P, Cornetta K, Martino R, et al. Prospective trial of chemotherapy and donor leukocyte infusions for relapse of advanced myeloid malignancies after allogeneic stem-cell transplantation. J Clin Oncol. 2002;20(2):405-12.

20. Choi SJ, Lee JH, Lee JH, Kim S, Seol M, Lee YS, et al. Treatment of relapsed acute myeloid leukemia after allogeneic bone marrow transplantation with chemotherapy followed by G-CSF-primed donor leukocyte infusion: a high incidence of isolated extramedullary relapse. Leukemia. 2004;18(11):1789-97.

21. Zeidan AM, Forde PM, Symons H, Chen A, Smith BD, Pratz K, et al. HLA-haploidentical donor lymphocyte infusions for patients with relapsed hematologic malignancies after related HLA-haploidentical bone marrow transplantation. Biol Blood Marrow Transplant. 2014;20(3):314-8.

22. Lee KH, Lee JH, Kim S, Lee JS, Kim SH, Kim WK. High frequency of extramedullary relapse of acute leukemia after allogeneic bone marrow transplantation. Bone Marrow Transplant. 2000;26(2):147-52.

23. Lee KH, Lee JH, Choi SJ, Lee JH, Kim S, Seol M, et al. Bone marrow vs extramedullary relapse of acute leukemia after allogeneic hematopoietic cell transplantation: risk factors and clinical course. Bone Marrow Transplant. 2003;32(8):835-42.

24. Chandy M, Mathews V, Rajasekar T, Viswabandya A, Lakshmi KM, John JM, et al. Treatment of Relapsed and Refractory Acute Myeloid Leukemia with a Salvage FLAG-IDA Chemotherapy Regimen Followed by a HLA Matched Related Allogeneic PBSC Infusion without Additional Conditioning. Blood. 2007;110(11):5050-.

25. Collins RH, Jr., Shpilberg O, Drobyski WR, Porter DL, Giralt S, Champlin R, et al. Donor leukocyte infusions in 140 patients with relapsed malignancy after allogeneic bone marrow transplantation. J Clin Oncol. 1997;15(2):433-44.

7. 국문요약

연구배경: 동종 조혈모세포 이식 후 재발한 급성 골수성 백혈병의 경우 예후가 불량하며 현재까지 정립된 치료가 없다. 공여자 백혈구 주입술의 이식편 대 종양효과를 극대화하기 위해 항암요법 병합 등 다양한 처치가 시도되어왔다. 이에 본 연구에서는 동종 조혈모세포 이식 후 재발한 급성 골수성 백혈병 환자에서 '고강도 구제 항암요법 후 과립구 집락 자극인자 가동 공여자 백혈구 주입 (chemo-mDLI)'의 효과와 해당 치료에 반응이 좋은 환자군을 구별하기 위한 예후인자를 분석하고자 하였다.

연구 방법: 1997 년부터 2023 년 사이에 chemo-mDLI 를 시행받은 동종 조혈모세포 이식 후 재발한 55 명의 급성 골수성 백혈병 환자를 후향적으로 분석하였다. 완전 관해율, 생착률, 전체생존 및 무병생존율, 재발률과 비재발사망률, 이식편 대 숙주 반응의 발생과 정도를 분석하였고 각 결과에 영향을 미치는 예후 인자들을 분석하였다.

연구결과: 평가 가능한 44 명의 환자 중 36 명에서 완전 관해를 획득하였고 전체 환자 중 호중구와 혈소판 생착률은 각각 90.9%와 72.7%였다. 생존자의 추적기간의 중앙값은 4.8 년으로, 31 명의 환자에서 질병 재발 혹은 진행을 보였고 16 명의 비재발사망을 포함하여 총 46 명이 사망하였다. 2 년간의 누적재발율은 51.2%, 비재발사망율은 27.3%, 전체 생존율은 27.9%였다. 전체 및 2-4 등급 급성 이식편 대 숙주 질환 (GVHD)의 발생율은 각각 43.6%와 40%였으며 2 년간 누적 만성 GVHD 발생율은 전체 38.2%, 중등도 이상은 20%로 확인되었다. Chemo-mDLI 를 재발 후 초치료로 시행 받은 경우 이차 혹은 그 이상의 치료로 받은 경우보다 유의하게 높은 완전 관해율과 (71.1% vs 41.2%; P = 0.035) 긴 생존 (위험비 4.12, 95% 신뢰구간 1.83-9.31; P = 0.001) 및 무병생존기간 (위험비 7.90, 95% 신뢰구간 2.09-29.87; P = 0.002)을 보였다. 이 외에도 재발 당시 낮은 골수내 아세포 비율 (<40%), 예후가 좋은 세포유전형, 3×10^6/kg 이상의 CD34+ 세포용량, 이식 전 완전 관해 획득여부가 긴 생존을 예측하는 예후인자로 확인되었다. 이식과 재발 사이의 기간이 길 경우 완전 관해율이 더 높은 경향을 보였다. 항암화학 요법의 경우 다른 요법에 비해 CME 혹은 AIE 로 치료받은 군에서 낮은 재발율을 보였다.

연구결론: 동종 조혈모세포 이식 후 재발한 급성 골수성 백혈병에서 고강도 항암요법 후 과립구 집락 자극인자 가동 공여자 백혈구 주입은 높은 완전 관해율과 허용가능한 수준의 이식편 대 숙주반응 및 비재발사망률을 보였다. 특히 재발 이후 첫 치료일 때,

재발 시 질병 부담이 적을 때, 주입된 CD34+ 세포 용량이 높은 경우, 이식 전 완전 관해를 이룬 경우, 재발 당시 유전자형이 좋은 경우 더 양호한 결과를 보였다.