

A Phase I/II Study of Chemotherapy Followed by Donor Lymphocyte Infusion plus Interleukin-2 for Relapsed Acute Leukemia after Allogeneic Hematopoietic Cell Transplantation

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The efficacy of donor lymphocyte infusion (DLI) for treatment of relapsed acute leukemia after allogeneic hematopoietic cell transplantation is limited. We hypothesized that interleukin-2 (IL-2) combined with DLI after chemotherapy might augment graft-versus-leukemia effects. To identify a safe and effective IL-2 regimen, a phase I/II study of DLI plus IL-2 therapy was performed for such patients. After chemotherapy, 17 patients received DLI (1×10^8 CD3/kg for patients with related donors, and 0.1×10^8 CD3/kg for those with unrelated donors) and an escalating dose of induction IL-2 ($1.0, 2.0,$ or 3.0×10^6 IU/m²/day representing levels I [$n = 7$], Ia [$n = 9$], and II [$n = 1$]) for 5 days followed by maintenance (1.0×10^6 IU/m²/day) for 10 days as a continuous intravenous infusion. Unacceptable IL-2-related toxicities developed in 1 patient at level I, 2 at level Ia, and 1 at level II. Grades III-IV acute graft-versus-host disease (aGVHD) developed in 5 patients, and extensive chronic GVHD (cGVHD) developed in 8. Eight patients had a complete remission after chemotherapy prior to DLI, and 2 additional patients had a complete remission after DLI plus IL-2 therapy. In conclusion, the maximal tolerated induction dose of IL-2 combined with DLI appears to be 1.0×10^6 IU/m²/day. IL-2 administration after DLI might increase the incidence of cGVHD.

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INTRODUCTION

Recurrent acute leukemia after allogeneic hematopoietic cell transplantation (HCT) poses a challenging clinical problem. Relapse tends to occur within 1 year after HCT, and the prognosis is poor [1]. Although remission can be induced in some patients, the median survival for these patients is usually 6 months or less [2]. Efficacy of a second transplantation is limited by refractory disease and high transplant-related mortal-

ity, especially in adults [3,4]. Donor lymphocyte infusion (DLI) has been used to treat recurrent malignancy after HCT. Although the rate of response after DLI is >70% in patients with relapsed or persistent chronic myeloid leukemia, it is only 15% to 29% in those with acute leukemia after HCT [5-8]. Chemotherapy followed by DLI appears to improve response rates and disease-free survival (DFS) in patients with relapsed acute leukemia [9,10].

Interleukin-2 (IL-2), a 15-kDa protein secreted by antigen-activated T cells, is critical for the generation and maintenance of an effective immune response. IL-2 stimulates the proliferation of antigen-specific or activated T cells and B cells, induces expansion of natural killer (NK) cells, induces the secretion of other lymphokines such as interferon gamma (INF- γ), granulocyte macrophage colony-stimulating factor (GM-CSF), and tumor necrosis factor (TNF), and augments the cytolytic function of cytotoxic T cells and NK cells [11,12]. Moreover, lymphocytes incubated with pharmacological concentrations of IL-2 can become nonspecific cytolytic effector cells, termed

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lymphokine-activated killer cells, which are largely activated NK cells capable of killing fresh and cultured tumor targets in a major histocompatibility complex (MHC) nonrestricted fashion [13].

The failure of DLI in most patients with acute leukemia may reflect an absent or inadequate graft-versus-leukemia (GVL) effect. The potential effector mechanisms of GVL, which may not be the same for all tumors, include cytotoxic and noncytotoxic T cells, NK cells, and secreted cytokines [14,15]. Because these mechanisms may be enhanced by IL-2, it is postulated that IL-2 therapy in combination with DLI might augment a GVL effect, especially after cytoreduction by chemotherapy. The major concern, however, is that IL-2 therapy might also increase the incidence and severity of graft-versus-host disease (GVHD) associated with DLI, because GVL and GVHD might share similar effector mechanisms.

Several studies explored the use of IL-2 therapy after allogeneic HCT [16-18]. The results of a phase I study in children indicated that the maximum tolerated dose (MTD) of IL-2 for induction was $3.0 \times 10^6/\text{m}^2/\text{day}$ by continuous intravenous infusion for 5 days, which could be administered to prevent leukemic relapse after allogeneic HCT [18]. Results from 2 encouraging reports suggested that the combination of IL-2 with DLI was effective for treatment of diseases refractory to DLI alone [7,19]. The feasibility and efficacy of an escalating dose of IL-2 regimen combined with a standardized dose of DLI have not yet been determined. Here, we report the results of a prospective phase I/II study of chemotherapy followed by donor lymphocyte infusion plus IL-2 therapy for adult patients with relapsed acute leukemia after a T cell-replete allogeneic HCT.

MATERIALS AND METHODS

Patient and Donor Eligibility

Relapse in the current study was defined by morphological, flow cytometric, cytogenetic, or molecular relapse. Patients with relapsed acute myeloid leukemia (AML) or acute lymphoblastic leukemia (ALL) after allogeneic HCT were eligible for this study. Other eligibility criteria at study registration included a Southwest Oncology Group (SWOG) performance status ≤ 2 , serum creatinine concentration ≤ 2 mg/dL, and serum total bilirubin concentration ≤ 2 mg/dL. Exclusion criteria included the absence of complete remission after HCT, the presence of preexisting congestive heart failure or arrhythmia not manageable with medical therapy, pulmonary dysfunction requiring O_2 therapy, pneumonia, or severe obstruction ($\text{FEV}_1 < 50\%$ predicted value or 50% decline from baseline), or severe restrictive lung disease (total lung capacity [TLC] $< 60\%$ or 50% declined from baseline) not because of leukemia,

and the presence of sepsis, aspergillosis, or other active infections likely to cause death within 3 months. A prior history of DLI was not an exclusion criterion if other study eligibility criteria were met.

The transplant donor used for DLI had to meet the eligibility criteria for apheresis. HLA-A or B allele mismatch was allowed for unrelated donors. Other mismatching was not allowed. The treatment protocol was approved by the institutional review board (IRB) of the Fred Hutchinson Cancer Research Center. Written informed consent was obtained from all study participants in accordance with the Declaration of Helsinki.

Treatment Plan

Chemotherapy

Before DLI, patients received a chemotherapy regimen that was appropriate for type of leukemia, prior treatment, and response history. Patients with extramedullary lesions also received local irradiation, and those with ALL or central nervous system (CNS) relapse were treated with intrathecal chemotherapy.

Immunosuppressive drugs

Administration of systemic immunosuppressive medications was withdrawn when the chemotherapy induction regimen was started. If no flare of GVHD was observed after 1 week from discontinuation of immunosuppressive medications, steroid treatment was also tapered within 5 weeks as tolerated. Treatment with prednisone at doses < 0.5 mg/kg/day could be continued, but the protocol required complete withdrawal of all other systemic immunosuppressive medications without GVHD exacerbation for at least 1 week before DLI. If GVHD flared, patients were treated with high-dose prednisone (1-2 mg/kg/day), and if the response was inadequate, other systemic immunosuppressive medications were added.

DLI

Patients could not have active grades II-IV acute GVHD (aGVHD) or extensive chronic GVHD (cGVHD) at the time of DLI. Patients were treated with a single DLI between 27 and 180 days after chemotherapy regardless of remission status. The cell dose was standardized at 1.0×10^8 $\text{CD}3^+$ cells/kg for patients with related donors and 0.1×10^8 $\text{CD}3^+$ cells/kg for those with unrelated donors.

IL-2 administration

Recombinant IL-2 (aldesleukin, Chiron, Emeryville, CA) was mixed in 500 mL of 5% dextrose in water containing 0.1% human serum albumin and administered by 24-hour continuous intravenous infusion (CIV) daily for 5 days in the induction phase, using an ambulatory infusion pump starting immediately

after DLI or on the following day. The induction phase was followed by maintenance treatment with IL-2 at 1.0×10^6 IU/m²/day by CIV daily for 10 days beginning on day 7. The original protocol defined escalating dose levels of IL-2 at 1.0 (level I) and 3.0 (level II) $\times 10^6$ IU/m²/day for 5 days. After the unexpected death of the first patient treated at dose level II, the protocol was amended to treat subsequent patients at an intermediate induction dose of IL-2 (2.0×10^6 IU/m²/day; level Ia). Patients received acetaminophen 15 mg/kg and diphenhydramine 25 to 50 mg orally before daily doses of IL-2 during induction. Prophylactic ciprofloxacin 500 mg orally was given throughout the entire course of IL-2 therapy. Toxicity during the administration of IL-2 was graded according to World Health Organization criteria. Treatment with IL-2 was discontinued when grade 3 toxicity occurred in any organ or when grade 2 neurological or cardiac toxicity occurred. If toxicity returned to baseline or grade 1 severity within 48 hours, administration of IL-2 was resumed at 50% of the previous dose. Any doses missed because of toxicity were not administered, and total duration of therapy was not extended beyond 19 days.

Statistical Considerations

The primary objective of this study was to test the hypothesis that an IL-2 regimen similar to our previous children's study as prophylaxis for relapse after allogeneic HCT [18] was also tolerable when given to adult patients who have received chemotherapy and DLI for treatment of recurrent acute leukemia. Study toxicity was defined as any of the following: (1) death related to aGVHD or cGVHD, (2) development of aGVHD or cGVHD with inability to decrease the dose of prednisone to less than 1 mg/kg/day within 1 month after initiating treatment for GVHD, or (3) life-threatening or fatal organ toxicity attributable to IL-2. An excess study toxicity rate was defined as any incidence where the lower limit of the 80% one sided confidence interval exceeded 25%. Acute GVHD was graded according to consensus criteria [20], and cGVHD was classified by traditional criteria [21]. IL-2 may produce side effects of skin rash, diarrhea, and cholestasis, which are similar clinically and pathologically to GVHD, but these side effects were expected to resolve soon after discontinuation of treatment with IL-2 [18,22]. Therefore, the diagnosis of GVHD was based on clinical characteristics such as persistence or worsening of signs or symptoms despite cessation of IL-2 therapy.

The secondary objective of this study was to examine the potential efficacy of DLI plus IL-2 at the highest acceptable dose of IL-2. However, all patients with all dose levels were analyzed together, because the total number of patients within each stratum was too small

for meaningful analysis. DFS was utilized for this purpose. Based on the prespecified study design, patients were analyzed separately according to diagnosis and remission status at time of DLI: patients with AML in complete remission (CR) at the time of DLI (group 1), and those with AML not in CR or ALL in any stage at the time of DLI (group 2). DFS was calculated according to the Kaplan-Meier method, beginning from the date of DLI in patients with disease in remission at the time of DLI or from the date of remission after DLI in patients with disease not in remission at the time of DLI, ending at the date of relapse or death from any cause, and censoring at the end of follow-up for patients who survived without relapse. Patients who never achieved complete remission were considered to have a relapse event at the date of DLI.

RESULTS

Patient Characteristics

Between June 1999 and February 2005, a total of 17 patients (median age 33 years, range, 19-61) were enrolled in this study. Patient characteristics are summarized in Table 1. The underlying disease was AML in 11 (65%) patients and ALL in 6 (35%). Two (12%) patients had biphenotypic AML, and 3 AML cases (18%) had emerged from myelodysplastic syndrome. The ALL in 2 patients (12%) had a Philadelphia chromosome. Sixteen patients (94%) had HCT with high-intensity conditioning, and 1 patient (6%) had HCT with reduced-intensity conditioning (case #17). Six (35%) (cases #1, 2, 3, 7, 8, and 15) had extramedullary involvement at relapse. Four (24%) received bone marrow grafts, and 13 (76%) received growth-factor-mobilized blood cell grafts. Donors were HLA-identical relatives for 13 (76%) patients, HLA-identical unrelated volunteers for 3 (18%), and an HLA-C allele-mismatched unrelated volunteer for 1 (6%) (case #3). Two (12%) patients had received DLI previously (cases #7 and 14). All patients received chemotherapy before DLI plus IL-2 therapy. The median time from HCT to chemotherapy was 11.8 months (range: 3.2-121.3). The median time from chemotherapy to DLI was 61 days (range: 36-91). Chemotherapies for each patient are summarized in Table 1. All patients had systemic immunosuppression discontinued at the time of chemotherapy. The shortest interval between discontinuation of immunosuppression and the first dose of IL-2 was 36 days.

Although 16 (94%) patients had a prior history of grades II-IV aGVHD or extensive cGVHD, no patients had active GVHD at the time of DLI. Two patients (cases #2 and 3) were receiving low-dose prednisone at the time of DLI, and the remaining 15 patients were not receiving any immunosuppressive medications at the time of DLI. At the time of DLI,

Table 1. Patient Characteristics

Study Case #	Age at DLI/Sex	Donor	HCT Graft	HCT to Chemotherapy (Months)	Chemotherapy to DLI (Days)	Chemotherapy	Remission at DLI
Acute myeloid leukemia							
2	24 M	Sib	PBSC	8.4	68	HCVAD, HDAC/MTX	N (Extramedullary)
3	56 M	URD	BM	6.4	61	Mylotarg	N (Extramedullary)
5	61 F	Sib	BM	121.3	40	IDR + AraC	Y
17	40 F	Sib	PBSC	16.8	53	IDR + AraC	N (BM FCM <5%)
7	38 M	Sib	BM	63.8	50	Mylotarg	N (Cytogenetic)
8	20 M	Sib	PBSC	5.8	75	HDAC + MIT	N (Extramedullary)
10	60 F	Sib	PBSC	25.9	68	IDR + AraC	Y
11	33 M	Sib	PBSC	6.0	50	IDR + AraC + ETP	Y
12	22 M	Sib	PBSC	7.5	54	MIT + ETP	Y
14	35 F	Sib	PBSC	17.9	77	MIT + ETP	Y
15	21 F	Sib	PBSC	3.2	36	FLAG + IDR	N (BM FCM 5%)
Acute lymphoblastic leukemia							
1*	24 F	Sib	BM	13.4	63	HDAC + ETP	N (BM FCM 0.1%)
4	34 M	URD	PBSC	8.7	62	HCVAD	Y
16	19 F	Sib	PBSC	18.5	83	VCR + DNR + DEX + PEG-ASP	Y
9*	21 F	URD	PBSC	11.9	91	VCR + DNR + PSL, FLAG	N (BM FCM 0.1%)
13	29 M	URD	PBSC	8.4	52	VCR + AraC + MTX	Y
6	50 F	Sib	PBSC	11.8	50	VCR + DNR + PSL + L-ASP	N (Cytogenetic)

HCT indicates hematopoietic cell transplantation; Sib, sibling donor; URD, unrelated donor; PBSC, peripheral blood stem cell; BM, bone marrow; HCVAD, fractionated cyclophosphamide + vincristine + dexamethasone + doxorubicin; HDAC, high-dose cytarabine; IDR, idarubicin; AraC, cytarabine; FCM, flow cytometry; MIT, mitoxantrone; ETP, etoposide; FLAG, fludarabine + cytarabine + granulocyte colony-stimulating factor; DNR, daunorubicin; PSL, prednisone; VCR, vincristine; L-ASP, L-asparaginase; DEX, dexamethasone; PEG-ASP, polyethylene glycosylated asparaginase.
 *Philadelphia chromosome-positive acute lymphoblastic leukemia.

8 patients (47%) had CR and 9 (53%) had hematologic, cytogenetic, flow cytometric, or molecular evidence of residual malignancy. Chimerism tests showed 95% to 100% donor-derived T cells and myeloid cells in blood samples from all patients before DLI.

Toxicity of IL-2

All 17 patients could be evaluated for toxicity. The toxicities of IL-2 therapy are summarized in Table 2. Nausea, vomiting, diarrhea, body weight gain, skin rash, and cardiovascular toxicities were frequent, but reversible. The incidence of diarrhea and rash increased in a dose-dependent fashion. Overall, grades 3-4 toxicities were observed in 1 (14%) patient at level I, 2 (22%) at level Ia, and 1 (100%) at level II. Administration of IL-2 was discontinued in 2 (22%) patients at level Ia because of the onset of grade III aGVHD (case #13) and liver toxicity (case #15), respectively, and in 1 (100%) patient at level II because of capillary leak syndrome (case #6). One patient died with capillary leak syndrome attributable to IL-2 (case #6). Pre-specified study toxicity directly or indirectly related to IL-2 was observed in 1 (14%) patient at level I (GVHD), 2 (22%) at level Ia (GVHD and IL-2 related), and 1(100%) at level II (IL-2 related) (Table 3).

GVHD

All patients were evaluated for acute GVHD (Table 3). Six of 17 (35%) patients developed grades II-IV aGVHD after the IL-2 therapy, with a median onset at 55 days (range: 8-74) after DLI. One of the 6 patients (case #13) developed grade III aGVHD during IL-2 induction therapy beginning 8 days after

DLI. This patient did not receive IL-2 maintenance therapy. The 5 patients who developed grades III-IV aGVHD were all treated with high-dose prednisone with or without other immunosuppressive medications such as a calcineurin inhibitor and mycophenolate mofetil. In 2 of the 5 patients (cases #4 and 12), the dose of prednisone could not be decreased to <1 mg/kg/day within 1 month. The incidence rate of grades III-IV aGVHD was significantly higher in patients with unrelated donors than in those with related donors (3 of 4 patients versus 2 of 13 patients, *P* = .022). Absolute lymphocyte counts at and after DLI are shown in Figure 1. Nine of 17 patients had lymphopenia (absolute lymphocyte count <1000/mm³) at the time of DLI. Grades III-IV aGVHD after DLI were more prevalent in patients with lymphopenia than in those with higher lymphocyte counts (5 of 9 patients versus 0 of 8 patients, *P* = .012). Fourteen patients could be evaluated for cGVHD. Nine (64%) of the 14 developed cGVHD, and 8 (89%) of the 9 had extensive cGVHD. The incidence rate of cGVHD was not statistically different between patients with related donors and those with unrelated donors (5 of 10 patients versus 3 of 4 patients, *P* = .39). We found no statistically significant difference in the incidence of acute and cGVHD according to the dose level of IL-2.

Patient Outcome

All 8 patients in CR at DLI remained in CR after DLI plus IL-2 therapy. Two of 9 patients (cases #3 and 15, both with AML) not in CR at DLI had a CR after DLI plus IL-2 therapy. Overall, 10 of the 17

Table 2. IL-2 Infusion-Related Toxicity According to Dose Level

Toxicity	IL-2 Induction Dose Level					
	I (n = 7)		Ia (n = 9)		II (n = 1)	
	No.	%	No.	%	No.	%
Nausea/vomiting						
All grades	4	(57)	6	(67)	1	(100)
Grades 3-4	0	(0)	0	(0)	0	(0)
Diarrhea						
All grades	2	(29)	4	(44)	0	(0)
Grades 3-4	1	(14)	1	(11)	0	(0)
Body weight gain						
All grades	6	(86)	7	(78)	1	(100)
Grades 3-4	0	(0)	0	(0)	1	(100)
Kidney						
All grades	2	(29)	1	(11)	1	(100)
Grades 3-4	0	(0)	0	(0)	1	(100)
Skin						
All grades	2	(29)	6	(67)	1	(100)
Grades 3-4	0	(0)	1	(11)	0	(0)
Liver						
All grades	1	(14)	1	(11)	1	(100)
Grades 3-4	0	(0)	1	(11)	1	(100)
Thrombocytopenia						
All grades	1	(14)	1	(11)	1	(100)
Grades 3-4	0	(0)	0	(0)	1	(100)
Lung						
All grades	0	(0)	0	(0)	1	(100)
Grades 3-4	0	(0)	0	(0)	1	(100)
Cardiovascular (including hypotension)						
All grades	3	(43)	3	(33)	1	(100)
Grades 3-4	0	(0)	0	(0)	1	(100)
Neurological						
All grades	1	(14)	0	(0)	1	(100)
Grades 3-4	0	(0)	0	(0)	0	(0)
Overall grades 3-4 toxicities	1	(14)	2	(22)	1	(100)

patients had a CR after chemotherapy plus DLI plus IL-2 therapy. Remission duration exceeded 1 year in 3 of 11 patients with AML (range: 12-47 months), but all 10 patients with remission had subsequent relapse. The median DFS was 8 months (range: 3-47) after DLI or the day of CR after DLI. Four patients received a second allogeneic HCT thereafter. To date, 1 patient (case #14) is alive at 5.4 years after chemotherapy plus DLI plus IL-2 therapy after a second allogeneic HCT for recurrent malignancy at 11 months after DLI plus IL-2 therapy. The other 16 patients died at a median of 11 months (range: 0.8-74) after DLI plus IL-2 therapy. The cause of death was leukemia except in the 1 patient who died with capillary leak syndrome. As shown in Figure 2, DFS at 1 year was 40% (95% confidence interval [CI], 5.2%-75%) for patients with AML in remission at DLI (group 1) and 8% (95% CI 0.5%-31%) for those with AML not in CR or ALL in any stage at DLI (group 2).

Protocol Amendments and Termination of the Study

The protocol was amended twice during the study period. First, the protocol was amended to treat subse-

quent patients at an intermediate dose of IL-2 (dose level Ia), because of an unexpected death in the first patient treated at dose level II. Second, although the toxicities observed with level Ia did not meet the stopping rule according to the original protocol definition, 2 of the 7 patients treated in this group developed clinically significant GVHD. Based on careful review and reflection, the investigators decided to amend the protocol to treat 20 additional patients at dose level I. The next 2 patients (cases #14 and 15), however, received IL-2 at dose level Ia, in violation of the protocol. The study was closed 3 years later because of slow patient accrual.

DISCUSSION

Although combination of IL-2 therapy with DLI is a potentially effective approach for treatment of relapsed leukemia after allogeneic HCT, only 2 prior studies have reported the results of this type of treatment [7,19]. Both studies reported encouraging results after IL-2 administration in patients with recurrent malignancy that had not responded after DLI, but only 4 and 2 patients in the 2 studies, respectively, had acute leukemia. In addition, the safety and efficacy of this treatment could not be fully evaluated because of heterogeneity in DLI dose and schedule, the presence or absence of prior chemotherapy, dose, and schedule of IL-2, and the use of T cell-depleted grafts. To the best of our knowledge, the current study is the first prospective phase I/II clinical trial of IL-2 plus DLI designed to treat a population that included only patients with AML or ALL that had relapsed after T cell-replete HCT. All patients were treated with chemotherapy followed by a single standardized dose of DLI plus 15 days of IL-2.

In our study, IL-2 therapy was associated with dose-dependent toxicities, consistent with previous reports of studies using IL-2 in various settings [17,18,22]. Although most of the toxicities were reversible, intolerable toxicities included severe GVHD and severe capillary leak syndrome. Although stopping

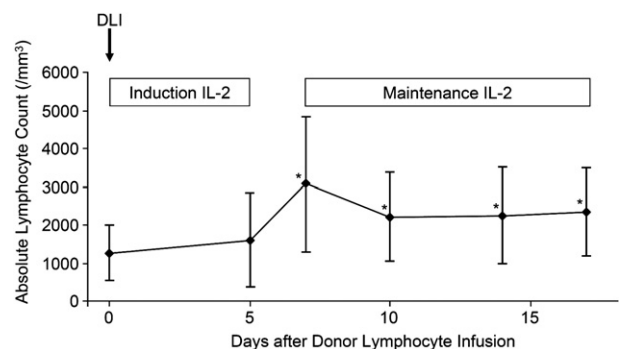


Figure 1. Absolute lymphocyte counts at and after DLI+IL-2 therapy. The * indicates statistically significant differences from values before DLI+IL-2, using a paired Mann-Whitney U-test.

Table 3. Outcomes after DLI plus IL-2 Therapy

Study Case #	IL-2 Dose Level	GVHD after DLI		Study Toxicity*	Remission after DLI	Remission Duration after DLI (Months)
		Acute	Chronic			
Acute myeloid leukemia						
2	I	0	NA	N	N	—
3	I	III	Extensive	N	Y	33
5	I	III	Extensive	N	Y†	35
17	I	0	Extensive	N	N	—
7	Ia	0	NA	N	N	—
8	Ia	0	0	N	N	—
10	Ia	II	Extensive	N	Y†	9
11	Ia	0	Extensive	N	Y†	5
12	Ia	IV	Extensive	Y (GVHD)	Y†	47
14	Ia	0	0	N	Y†	6
15	Ia	0	0	N	Y	3
Acute lymphoblastic leukemia						
1	I	0	0	N	N	—
4	I	III	Extensive	Y (GVHD)	Y†	8
16	I	0	0	N	Y†	5
9	Ia	0	Limited	N	N	—
13	Ia	III	Extensive	Y (IL2)	Y†	12
6	II	0	NA	Y (IL-2)	N	—

GVHD indicates graft-versus-host disease; DLI, donor lymphocyte infusion; N, no; Y, yes; NA, not assessable.

*Death related to acute or chronic GVHD, development of acute or chronic GVHD with inability to decrease the dose of prednisone to less than 1 mg/kg/day within 1 month after initiating treatment for GVHD, or life-threatening or fatal organ toxicity attributable to IL-2.

†Also remission before DLI following chemotherapy.

rules for excessive toxicity defined in the original protocol were not met before the study was closed, we observed study toxicities in 2 of 9 patients treated at dose level Ia (2.0×10^6 IU/m²/day) and a death associated with IL-2 administration at dose level II (3.0×10^6 IU/m²/day). From these results, we conclude that the MTD of IL-2 induction therapy by continuous infusion after DLI appears to be dose level I (1.0×10^6 IU/m²/day). This MTD dose is much lower than IL-2 dose of $6-18 \times 10^6$ IU/day for 3 days by subcutaneous injection combined with DLI reported by others [7,19], but comparison is difficult because half-life of IL-2 in plasma is as short as 2.4 hours after subcutaneous administration [23].

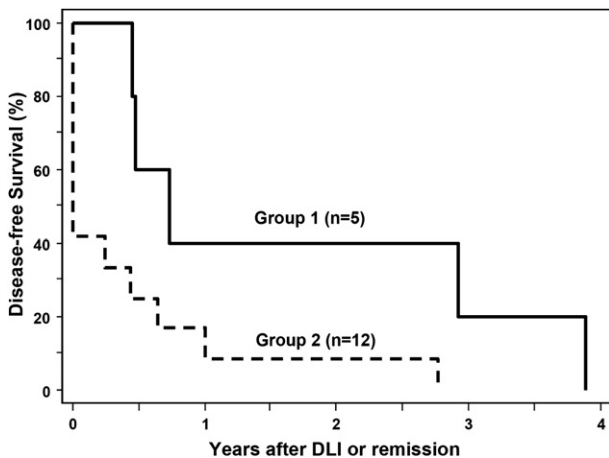


Figure 2. Disease-free survival for patients with AML in complete remission at DLI (group 1) and for those with AML not in remission or ALL in any stage at DLI (group 2).

The effects of IL-2 depend on the dose, the route of administration, and the clinical setting. For example, subcutaneous administration of low-dose IL-2 ($0.6-1.0 \times 10^6$ IU/m²/day) has been found to increase the number of CD4⁺CD25⁺FOXP3⁺ T regulatory cells [24] and may ameliorate GVHD in the setting of steroid-refractory cGVHD [25]. In contrast, higher IL-2 dose regimens have been found to increase alloreactivity, promote GVL responses [7,19], and may also increase the severity of GVHD when given after DLI by continuous infusion at doses between $1.0-3.0 \times 10^6$ IU/m²/day, as observed in our study.

Our results can be interpreted in comparison with previous studies of chemotherapy followed by DLI without IL-2 (Table 4) [8-10]. First, the incidence rates of grades II-IV and III-IV aGVHD in our study are similar to those reported after DLI without IL-2. Second, development of early grade III aGVHD (day 8 after DLI) was observed in 1 patient (case #13) in our study, but no such cases were observed in previous DLI studies without IL-2. Third, the 50% incidence of extensive cGVHD in our study was somewhat higher than the 14% to 32% incidence reported in previous studies. These findings suggest that our IL-2 regimen might induce early aGVHD and might increase the incidence of cGVHD when combined with DLI after T cell-replete HCT.

Miller et al. [26] found that lymphodepletion enhanced immune responses, causing severe GVHD after DLI, possibly by increasing the concentrations of cytokines such as IL-15 and IL-7 [27]. In our study, the risk of grades III-IV aGVHD was increased in patients who had lymphopenia at the time of DLI plus

Table 4. Summary of Previous Reports of DLI without IL-2 Followed by Chemotherapy for Relapsed Acute Leukemia and Current Study

Parameters	Study			
	Collins et al. [8]	Schmid et al. [10]	Levine et al. [9]	Current
Design	Retrospective	Retrospective	Prospective	Prospective
Disease, no. of patients				
Acute myeloid leukemia	7*	124*	50*	11
Acute lymphoblastic leukemia	3*	0	0	6
DLI dose, $\times 10^6/\text{kg}$ CD3	2.2 (mean)†	0.1 (median)‡	1	1
Grades II-IV acute GVHD	46%†	34%‡	45%§	35%
Grades III-IV acute GVHD	22%†	NA	28%§	29%
Chronic GVHD	61%†	46%‡	36%§	64%
Chronic extensive GVHD	32%†	26%‡	14%§	50%
1 year DFS after DLI	54% in AML 33% in ALL	NA	34% among patients in CR at DLI§	40% in AML CR at DLI, 8.3% in others

DLI indicates donor lymphocyte infusion; GVHD, graft-versus-host disease; NA, not assessable; DFS, disease-free survival; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; CR, complete remission.

*Data abstracted from the entire cohort.

†Data represent the entire study cohort of 140 patients treated for various diagnoses, with or without chemotherapy before DLI.

‡Data represent the entire study cohort of 171 AML patients. Of the 171 patients, 124 received chemotherapy before DLI, and information was missing in 7 patients.

§Data represent the entire study cohort of 65 patients, including 50 with AML and 15 with other diagnoses.

IL-2. Recent studies have shown that high-dose melphalan, fludarabine, and cyclophosphamide all cause lymphdepletion with increased concentrations of IL-15 and IL-7 [28-30]. Although these specific drugs were infrequently used in our patients, many types of chemotherapy before DLI might have similar effects.

Durable CD8⁺ T cell memory responses have a strict requirement for IL-2, but recent studies highlight a biological role of IL-2 in facilitating immune tolerance through generation of CD4⁺CD25⁺FOXP3⁺ T regulatory cells [31]. In fact, several studies have shown that IL-2 administration increases the number of T regulatory cells in cancer patients [32,33] and after CD4⁺ DLI [24]. Therefore, IL-2 given after DLI for treatment of recurrent acute leukemia after HCT may not represent the best approach for augmenting GVL effects associated with DLI in the future. Other γ_c -signaling cytokines such as IL-7, IL-9, IL-15, and IL-21 may offer alternative choices for augmenting GVL, because most of these cytokines have positive effects on the maintenance of CD8⁺ T cell survival and function in vivo but do not affect growth of T regulatory cells [34-36].

This study was closed because of slow patient accrual, and the small sample size limits the ability to draw definitive conclusions regarding the efficacy of DLI plus IL-2. Nonetheless, the 1-year DFS rates of 40% among patients with AML in remission at DLI were not inferior to the 34% to 54% DFS rates after chemotherapy followed by DLI without IL-2 in previous studies (Table 4) [8]. The remission duration exceeding 1 year in 3 patients treated for recurrent AML was notable. These results support the previous finding by Nadal et al. [19] that DLI plus IL-2 treatment was more effective for patients with AML than for those with other diseases. Previous studies using continuous intravenous

IL-2 at doses as low as 0.2-0.6 IU/m²/day after allogeneic HCT showed that IL-2 promoted expansion of NK cells but did not increase the numbers of CD3⁺ T cells [17,37]. Recent studies have shown that mismatches of killer immunoglobulin-like receptors (KIR) were associated with reduced rates of leukemia relapse through alloreactive NK cells, particularly for patients with AML [15,38]. By augmenting NK alloreactivity, IL-2 may augment GVL effects, particularly in patients with AML. In conclusion, MTD of continuous intravenous IL-2 induction therapy combined with DLI appears to be 1.0×10^6 IU/m²/day. IL-2 after DLI might increase the incidence of cGVHD, although the antileukemia efficacy of this treatment remains to be determined.

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