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High Alloreactivity of Low-Dose Prophylactic Donor Lymphocyte Infusion in Patients with Acute Leukemia Undergoing Allogeneic Hematopoietic Cell Transplantation with an Alemtuzumab-Containing Conditioning Regimen

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The value of prophylactic donor lymphocyte infusion (pDLI) is unclear and differs among diseases and transplantation protocols. Experience with this approach in patients with acute leukemia undergoing hematopoietic cell transplantation (HCT) with an alemtuzumab-incorporating conditioning protocol is lacking. We conducted a single-center prospective study to investigate the applicability and efficacy of prophylactic donor lymphocyte infusion (pDLI) in patients with leukemia undergoing HCT with a low-dose alemtuzumab-containing conditioning regimen. Inclusion criteria were high-risk acute myelogenous leukemia, acute lymphoblastic leukemia, or increasing mixed chimerism. All patients included were tapered off of immunotherapy. Exclusion criteria were a history of ≥grade II or active graft-versus-host disease (GVHD). Of the 56 consecutive patients who underwent HCT with an alemtuzumab-containing regimen, 15 patients (8 with acute myelogenous leukemia and 7 with acute lymphoblastic leukemia) met the study inclusion criteria and received prophylactic DLI (total of 45 infusions) from 7 sibling donors and 8 unrelated donors. The first infusion was given at a median of 162 days posttransplantation. The median number of DLIs was 3, and the median cumulative $CD3^+$ cell dose was 2×10^6cells/kg. Six of the 8 patients (75%) who received pDLI while in mixed chimerism converted to stable, complete donor chimerism. Some 47% of DLI recipients developed GVHD (4 acute GVHD and 3 with chronic GVHD) after a median cumulative dose of 2×10^6 $CD3^+$ cells/kg. After a median follow-up of 575 days, 11 (73%) pDLI recipients were alive. All 4 deaths were due to GVHD-related causes. None of the patients who received pDLIs relapsed. Patients with leukemia who received low-dose pDLI after conditioning with alemtuzumab are at low risk for relapse; however, this approach is associated with a relatively high incidence of severe GVHD.

INTRODUCTION

Allogeneic hematopoietic cell transplantation (HCT) is an established treatment for high-risk (HR) hematologic malignancies; however, relapse remains a major cause of transplantation failure. Patients with acute myelogenous leukemia (AML) and acute lymphoblastic leukemia (ALL) who relapse after allogeneic HCT have a very poor prognosis [1]. Particularly for ALL, posttransplantation relapses cannot be rescued with currently available therapies [2]. Thus, strategies to prevent relapse are highly desirable, especially when some form of T cell depletion is applied. Based on the observation that administration of donor lymphocyte infusion (DLI) can induce a significant graft-versus-leukemia (GVL) effect, which is more effective when the tumor burden is low, investigators have used prophylactic DLI (pDLI) to minimize the risk of relapse [3]. pDLI is also given to promote complete chimera states. States of mixed chimerism (MC) can have varying significance, depending on the underlying disease for which HCT was performed, transplantation protocol, chimerism assessment method, level of mixed chimerism, and lineage of the cells assessed in the chimerism assay [4]. Stable MC is a common finding after alemtuzumab-based conditioning, and whether MC in these protocols can predict relapse in acute leukemia patients remains controversial [5-7]. Increasing MC in AML and ALL, evaluated in serial determinations, allows identification of patients at greater risk for subsequent relapse [8-12].

The value of pDLI is still unclear and may differ among diseases and transplantation protocols. The impact of pDLI after alemtuzumab-incorporating HCT conditioning protocols has been evaluated in patients with lymphomas treated with reduced-intensity conditioning regimens [13-15]; however, experience is lacking in patients with acute leukemias, especially in the context of myeloablative-conditioned HCT. In a previous study, we found that low-dose alemtuzumab effectively prevented severe acute and chronic GVHD after sibling or matched unrelated donor allogeneic HCT [16]. To enhance the GVL effect, we amended that low-dose alemtuzumab study to include pDLI in patients at high risk for leukemia relapse. Here we report the applicability and efficacy of this approach.

MATERIALS AND METHODS

Patients and Study Design

Fifty-six consecutive patients with AML or Philadelphia chromosome (Ph)-negative ALL who underwent HCT with an alemtuzumab-based conditioning regimen were registered for the prospective, Institutional Review Board– and Ethics Committee–approved pDLI study. Patients were scheduled to receive pDLI if they had HR AML, ALL, or increasing MC. Increasing MC was defined either as newly developed MC or a >5% increase

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in host cells in subsequent chimerism analyses. HR AML was defined as AML with the following features: unfavorable karyotype according to the Southwest Oncology Group/Eastern Cooperative Oncology Group criteria [17], secondary or biphenotypic AML, induction failure AML, or greater than first complete remission (CR1) at the time of transplantation. Ph^+ patients were not offered pDLI, but received imatinib as posttransplantation therapy instead. Patients were eligible for pDLI if they had no evidence of relapse, no active infection or other transplantation-related complications requiring therapy, no history of acute GVHD grade II or greater or active GVHD at the time of planned DLI, off immunotherapy, and provided written informed consent. Sibling donors had to consent for leukapheresis without growth factor mobilization.

To ensure availability of DLIs from unrelated donors, we routinely isolated and froze small aliquots of DLIs from sufficiently large ($>5 \times 10^6$ cells/kg) peripheral blood (PB) stem cell (PBSC) grafts. Requests from unrelated donors for a second apheresis procedure for the purpose of a pDLI were not done. pDLI administration was planned to commence 2-4 weeks after cessation of the prophylactic cyclosporine or earlier in case of increasing MC. Patients were evaluated every 2-3 weeks after each pDLI and considered for subsequent pDLIs 4-6 weeks later, only if GVHD (any grade) or other toxicities (grade $\geq$2 according to the National Cancer Institute's Common Terminology Criteria for Adverse Events, version 3.0) had not presented. Patients were scheduled to receive up to 6 lymphocyte infusions. According to the study protocol, occurrence of GVHD, toxicity, and treatment-related mortality (TRM) were assessed in real time. The number of planned infusions was reduced if $\geq$3 patients experienced side effects. As a result of this design, the last 8 patients in our study cohort were scheduled to receive up to 3 pDLIs. The starting pDLI dose was 0.5×10^6 cells/kg in the unrelated donor setting and 0.75×10^6 cells/kg in the sibling setting, respectively, with an acceptable deviation of $\pm 0.25 \times 10^6$ cells/kg in the actual number of infused cells. pDLI dosage escalation was allowed up to 1×10^6 $CD3^+$ cells/kg for unrelated donors and 1.5×10^6 $CD3^+$ cells/kg for sibling donors (except in 1 sibling donor recipient, who received a dose of 3×10^6 $CD3^+$ cells/kg because of increasing MC under pDLI).

Conditioning Regimens, Donors, Grafts, and Chimerism

Patients with AML received standard chemotherapy-based myeloablative conditioning, and those with ALL received TBI-based myeloablative conditioning. Patients age >55 years and those with severe comorbidities received a reduced-toxicity myeloablative regimen consisting of fludarabine, carmustine, and melphalan or thiotepa [16]. The stem cell source was granulocyte colony-stimulating–mobilized PBSCs in all but 2 cases. Donors were HLA-identical siblings or volunteer unrelated donors (VUDs) with at least 7 of 8 (A, B, C, and DRB1) HLA allele matches. Patients received alemtuzumab (Mab-Campath; Genzyme, Leiden, the Netherlands) in a deescalating dosage manner. Criteria used for deescalation of alemtuzumab have been described in detail previously [16]. In brief, the first cohort of 10 patients received a total dose of 20 mg of alemtuzumab given from day -2 to day -1. After real-time evaluation (ie, of engraftment kinetics, occurrence of acute GVHD, and TRM), the total dose was deescalated to 15 mg in 6 patients and to 10 mg in 36 patients. Four patients who underwent HCT during the last time frame received 20 mg of alemtuzumab because of a low risk for relapse. Cyclosporine was given at a dose of 5 mg/kg starting at day -3. In patients without GVHD, cyclosporine was tapered starting at day +60 after sibling donor HCT or after HCT performed for advanced disease (>CR1) and starting at day +100 in cases of VUD HCT. Chimerism was assessed via PCR amplification of informative microsatellite markers and semiquantitative analysis after gel electrophoresis or, in some cases, after capillary electrophoresis, as described in detail previously [10]. Chimerism in PB cells was assayed weekly up to day +100, at 3-week intervals from day +100 to day +180 and at day +270 and day +365, or whenever the patient was seen in the outpatient clinic. Whenever we found MC in PB, we further evaluated chimerism in Ficoll gradient-separated mononuclear cells and/or $CD3^+$ subsets after their isolation using magnetic microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany). Bone marrow (BM) samples were routinely analyzed at days +30 and +100 and whenever indicated.

Definitions and Statistical Analysis

Data were evaluated as of November 2011. The current analysis includes some patients who were reported previously [16] and were analyzed here with respect to pDLI administration and with a longer follow-up. We classified post-DLI GVHD according to both clinical features and time of onset. GVHD with acute-like features was graded according to standard criteria [18], and GVHD with chronic-like features was graded according to severity using National Institutes of Health consensus criteria [19]. Patients who developed post-DLI GVHD (any grade) were treated with high-dose steroids (2 mg/kg daily for 3 days), with the dosage tapered according to response. Patients who did not respond within 5 days received cyclosporine, with the addition of mycophenolate mofetil for resistant cases. pDLI-related mortality (ie, TRM) was defined as death after pDLI therapy from causes other than relapse. GVHD, TRM, and relapse rates were estimated as cumulative incidence curves (NCSS, Kaysville, UT), with relapse as a competing risk for TRM, death in remission as a competing risk for relapse, and death without GVHD a competing risk for GVHD. Estimates of overall survival (OS) and relapse-free survival (RFS) were obtained by the method of Kaplan and Meier where patients were censored at last follow-up if still alive. Differences between subgroups were compared using the Fisher exact test for categorical data and the Mann-Whitney *U* test for continuous data. Statistical significance was based on $P < .05$. (Graph Pad Prism, CA).

RESULTS

Stratification and Patient Characteristics

The study design is summarized in Figure 1. Out of 56 patients who underwent allogeneic HCT with low-dose alemtuzumab conditioning, 42 HR patients (27 with AML and 15 with ALL) met the inclusion criteria and were screened for pDLI. Fifteen patients (36%) received at least 1 pDLI (Figure 1). Twenty patients (48%) were not eligible for pDLI owing to GVHD in 9 patients, early death in 4 patients, early relapse in 4 patients, and transplantation complications requiring treatment (3 patients; 2 with thyroiditis and 1 with Bell's palsy neuropathy). Another 7 patients (17%) did not receive pDLI even though they were considered potential pDLI recipients. In 4 of these cases, donor lymphocytes were not available (1 VUD BM transplant, 2 VUD PBSC transplants but no donor lymphocytes were frozen, 1 sibling donor refused to donate), and the remaining 3 patients did not consent to pDLI administration.

Details of the 15 pDLI recipients are given in Table 1. In total, 45 pDLI administrations were given to these 15 patients (median age, 29 years; range, 17-65 years). Seven patients had ALL (47%), 6 had HR AML (40%), and 2 had standard-risk AML (13%). At the time of pDLI, 8 patients (53%) demonstrated MC in PB. Eight patients (53%) received lymphocytes from a VUD, and 7 patients (47%) received lymphocytes from a sibling donor. The first pDLI was given at a median of 162 days (range, 78-426 days) after HCT. The median number of infusions was 3 (range, 1-6), and the median cumulative dose given was 2×10^6 $CD3^+$ cells/kg (range, 0.7-7 $\times 10^6$). Recipients of sibling pDLI received a median dose of 2×10^6 $CD3^+$ cells/kg (range, 1-7 $\times 10^6$), whereas recipients of VUD pDLI received a median dose of 1.6×10^6 $CD3^+$ cells/kg (range 0.7-5.4 $\times 10^6$) ($P = .27$).

Toxicity after pDLI

Overall, 7 of the 15 pDLI recipients (47%; 3 sibling donor, 4 VUD) developed GVHD after a median cumulative $CD3^+$ cell dose of 2×10^6 cells/kg (range, 0.7-7 $\times 10^6$) and at a median of 75 days (range, 33-343 days) after the first pDLI and 36 days (range, 11-126 days) after the last pDLI, respectively (Figure 2). GVHD developed after a median cumulative $CD3^+$ dose of 2.5×10^6 cells/kg (range, 2-7 $\times 10^6$) in sibling pDLI recipients and 1.5×10^6 cells/kg (range, 0.7-4.5 $\times 10^6$) in unrelated pDLI recipients ($P = .28$). Four of 8 (50%) MC pDLI recipients developed GVHD, whereas 3 of 7 (43%) CC recipients experienced GVHD. In univariate analysis, we could not find a statistically significant difference between the 7 subjects who experienced post-DLI GVHD and the non-GVHD pDLI recipients (Table 2). The number of pDLIs and the total cell dose given were dependent on GVHD occurrence and thus could not be included as variables in the analysis.

Of the 7 patients with GVHD, only 1 case was mild and easily treatable. Four patients (27%) developed acute-like GVHD at a median time of 51 days (range, 33-75 days) after

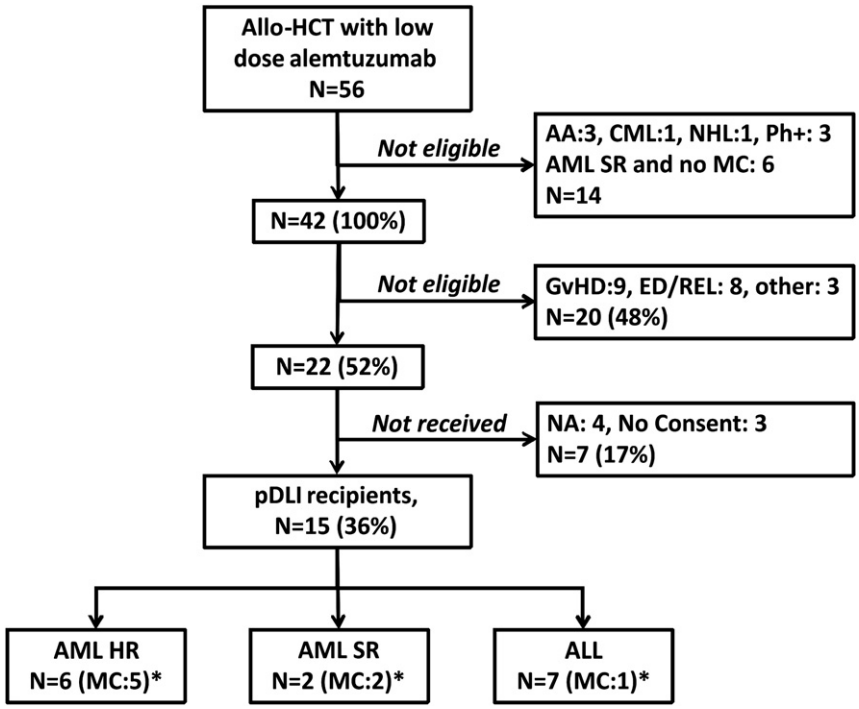

Figure 1. Outline of the study design and patient flow. AA, aplastic anemia; CML, chronic myelogenous leukemia; ED/REL, early death or early relapse; NA, not available; NHL, non-Hodgkin lymphoma; SR, standard risk. *: Numbers in parentheses indicate the number of patients with MC.

the first pDLI. One sibling donor recipient presented with grade I skin GVHD that resolved with steroids, and 3 recipients (20%; 2 VUD, 1 sibling donor) experienced fatal, steroid-refractory grade III liver and/or gut acute-like GVHD, even though the total $CD3^+$ cell dose was $<1 \times 10^6$cells/kg in 2 of them. Chronic-like GVHD was seen in 3 subjects (20%) at 147-343 days after the first pDLI; 2 VUD recipients developed moderate skin chronic-like GVHD, which resolved only after prolonged (>3 months) immunotherapy in both cases, and patient 7 experienced fatal bronchiolitis obliterans at 183 days after the first pDLI. Regarding other toxicities, patient 6 presented with isolated reversible thrombocytopenia after his fourth pDLI administration, and patient 14 developed increased creatine-phosphokinase levels after 2 subsequent pDLI administrations, which ultimately resolved spontaneously.

Chimerism and Outcome

Of the 8 patients who received pDLI while in MC (median 7.5% recipient cells in PB; range, 5%-15%), 6 (75%) converted to complete chimerism (CC) after a median of 2.5 pDLI administrations (range, 1-3) and 1.7×10^6 cumulative $CD3^+$ cells/kg (range, 0.7-7 $\times 10^6$). All remained in stable CC after a median follow-up of 717 days (range, 232-1445 days) after the first pDLI. The 2 patients who did not convert to CC had a drop in the number of recipient cells to <5% and remained in CR nearly 1 year after their first pDLI.

At a median follow-up of 575 days (range, 310-1786 days) after HCT and 367 days (range, 212-1445 days) after the first pDLI, 11 of 15 (73%) pDLI recipients were alive (median Karnofsky score, 100%; range, 90%-100%), and 4 recipients died. Death was attributed to post-DLI GVHD causes in all cases. Thus, DLI-related mortality was 27%, and mortality in patients who developed post-DLI GVHD was 57%. The estimated OS and RFS for pDLI recipients was 87% +/− 9% at 1-year and 72% +/− 12% at 2 years. None of the patients who received pDLIs relapsed. Of note, of the 7 patients who were considered eligible for pDLI but did not receive lymphocytes owing to logistical hurdles (5 with AML and 2 with ALL), 3 (43%) relapsed after a median follow-up of 619 days (range, 143-965 days). Outcomes of eligible pDLI candidates who received immunotherapy and those who did not receive immunotherapy are shown in Figure 3. The 2 groups are too small to allow for statistical comparison.

DISCUSSION

Given that immunotherapy seems to be more effective in stages of impending, rather than overt, relapse, perhaps the most appropriate platform for DLI is in a prophylactic setting [3]. The first report of prophylactic immunotherapy dates back to 1995 [20]. Evaluation of pDLI in patients with leukemia in several transplantation settings have demonstrated the challenges of this approach [21-27]. However, with the absence of randomized studies, the value of pDLIs in leukemia remains unclear. Recently, a prospective collaborative mutlicenter study of childhood AML has suggested that pDLI can be administrated safely on the basis of MC and may result in improved survival [12]. The benefit of pDLI may differ based on disease and transplantation-related factors. To our knowledge, this is the first study that prospectively evaluates the applicability and efficacy of pDLIs in patients with acute leukemias receiving with a conditioning regimen incorporating alemtuzumab. Efforts were made to predetermine variables to reduce bias. First, patients included were clearly defined either at transplantation (HR AML, Ph^- ALL) or by using objective markers (ie, MC) after transplantation. Second, to avoid logistical issues in unrelated HCT, we routinely cryopreserved donor lymphocytes from the original graft. Third, starting doses and subsequent doses were given within a narrowly defined range, and the total

Table 1
Characteristics of the pDLI Recipients

Patient	Age	Disease	Stage at HCT	Conditioning	Donor	Chimerism at First pDLI	First pDLI, Day	Number of pDLIs	Cumulative $CD3^+$ Cell Dose ($\times 10^6$/kg)	Post-pDLI GVHD, Grade/Organ(s)	Day of GVHD after First/Last pDLI	Days of Follow-up	Current Status
1	58	SR AML	CR1	RTC	VUD	MC	426	6	5.4	No	—	1786	Alive, CR, CC
2	29	SR AML	CR1	HD	Sibling	MC	158	3	7	Acute, I/skin	59/28	1603	Alive, CR, CC
3	58	HR AML	Relapse	RTC	Sibling	MC	78	3	2.5	Acute, III/liver	43/11	310	Dead (GVHD)
4	43	HR AML	CR1	HD	VUD	CC	161	5	4.5	Chronic, moderate/ skin	343/126	1491	Alive, CR, CC
5	20	B-ALL	CR1	HD	VUD	CC	197	2	0.9	Acute, III/liver, gut	33/18	414	Dead (infection)
6	17	B-ALL	CR1	HD	Sibling	CC	162	4	5.5	No	—	1219	Alive, CR, CC
7	20	B-ALL	CR2	RTC	Sibling	CC	119	3	2	Chronic, severe/ lung	183/36	486	Dead (GVHD)
8	30	B-ALL	CR1	HD	Sibling	CC	148	2	2	No	—	1051	Alive, CR, CC
9	34	HR AML	CR1	HD	VUD	MC	167	1	0.7	No	—	1042	Alive, CR, CC
10	28	T-ALL	CR1	HD	VUD	MC	85	1	0.7	Acute, III/gut	75/75	382	Dead (infection)
11	65	HR AML	CR1	RTC	VUD	MC	182	3	2	Chronic, moderate/ skin	147/84	742	Alive, CR, CC
12	49	HR AML	CR1	HD	VUD	MC	218	4	3.2	No	—	575	Alive, CR, MC
13	26	HR AML	CR1	HD	VUD	MC	223	3	1.3	No	—	531	Alive, CR, MC
14	24	B-ALL	CR1	HD	Sibling	CC	155	3	1.5	No	—	435	Alive, CR, CC
15	26	B-ALL	CR1	HD	Sibling	CC	174	2	1	No	—	387	Alive, CR, CC

B-ALL indicates B cell acute lymphoblastic leukemia; HD, high dose; RTC, reduced-toxicity conditioning containing fludarabine, thiotepa, and carmustine [16]; SR, standard risk.

number of administrations was predefined and then redefined after interim evaluation.

DLI confers a risk of GVHD, especially at higher dosages. Despite the relatively low cumulative doses of lymphocytes administered in our cohort (median, 2×10^6 $CD3^+$ cells/kg), 7 of the 15 pDLI recipients experienced GVHD. Importantly, in all but 1 case, GVHD occurring after pDLIs had significant ramifications, with 3 patients requiring prolonged immune suppression and 4 patients ultimately dying of GVHD-related complications. Two patients developed lethal GVHD after very low $CD3^+$ cell doses ($<1 \times 10^6$ cells/kg), which impelled us to reduce the total number of infusions from the initially planned 6 total infusions to 3 total infusions in the last 8 patients enrolled in the study. The small number of patients and the fact that the majority (73%) of pDLI recipients received a relative narrow dosage range ($0.7\text{-}2.5 \times 10^6$ $CD3^+$ cells/kg) precludes any statement regarding the exact dose that can be given safely. Lutz et al. [27] also reported a high incidence of GVHD (67%) after pDLI in patients with ALL, which prompted them to reduce the doses in subsequent patients by approximately 10-fold (from 10^7 to 10^6 cells per kg). Thus, although DLI doses up to 10×10^6 $CD3^+$ cells/kg appear to be safe when given in the relapsed setting [28], we suggest that much smaller doses should be given in the prophylactic setting. Lutz et al. [27] reported that ALL patients who received pDLI while in MC had a higher rate of GVHD than those who received lymphocytes while in CC. In our series, by administrating one log lower doses, including both AML and ALL patients and incorporating alemtuzumab-based T cell depletion in the conditioning, we could not verify this observation. Furthermore, we could not identify any other clinical factors predictive of post-DLI GVHD. In our study, administration of mobilized pDLI resulted in a similar incidence of GVHD as steady-state pDLI, despite the fact that the former were derived from unrelated donors and the latter were derived from related donors. Retrospective analyses in relapsed patients have suggested a comparable incidence of GVHD in granulocyte colony-stimulating factor–primed or unprimed DLIs and graft-derived DLIs [29-32]. Identification of biological markers that could predict the outcome of pDLI therapy is warranted [33].

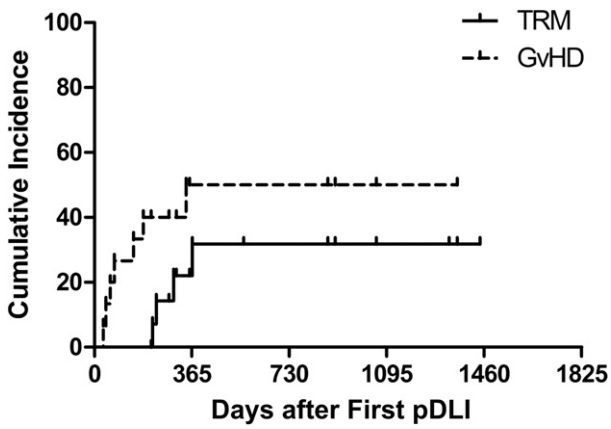

Figure 2. Toxicity in pDLI recipients. Cumulative incidence rates of pDLI-related GVHD and TRM measured from the time of first pDLI.

The reported incidence of TRM after prophylactic DLI ranges between 6% and 52% [21-27]. In the present study, the cumulative incidence of TRM was 27%. Barrett et al. [21] reported a significant increase in TRM when pDLI was given early after BM transplantation. Although in our cohort, the timing of the first pDLI varied according to clinical and chimeric status, it is noteworthy that both patients who received their first pDLI before day +100 died due to GVHD. Therefore, we agree with other groups that have suggested withholding pDLI beyond day +100 [27]. All cases of TRM were attributed to GVHD. In all but 1 case (which was lost to follow-up), our patients were instructed to immediately report any symptoms and were routinely seen in the outpatient clinic every 2-3 weeks after each pDLI. Thus, we do not believe that the high mortality rate of GVHD is related to a delay in steroid initiation. Given that GVHD occurred within 11-126 days after the last lymphocyte infusion, our data underscore the need for close and prolonged monitoring after each pDLI. However, because most patients begin to return to their pretransplantation lifestyle rhythms at the time of planned pDLI, both practical and psychosocial considerations might discourage them from prophylactic

Table 2
GVHD after pDLI

	pDLI Recipients with GVHD	pDLI Recipients without GVHD	*P* value
Patients, n (%)	7 (47)	8 (53)	
Age, years, median (range)	29 (20-65)	28 (17-58)	.73
Disease, n (%)			
AML	4 (57)	4 (50)	1.00
ALL	3 (43)	4 (50)	
Conditioning, n (%)			
High dose	4 (57)	7 (87)	.28
Reduced toxicity	3 (43)	1 (13)	
Alemtuzumab dose, n (%)			
20 mg	4 (57)	2 (25)	.31
10 mg	3 (43)	6 (75)	
Donor, n (%)			
Sibling	3 (43)	4 (50)	1.00
Unrelated	4 (57)	4 (50)	
Sex, n (%)			
Male	3 (43)	5 (62)	.62
Female	4 (57)	3 (38)	
Sex-mismatched HCT, n (%)			
Yes	3 (43)	1 (13)	.28
No	4 (57)	7 (87)	
Day of first pDLI, median (range)	158 (78-197)	170 (148-426)	.12
MC at first pDLI, n (%)			
No	3 (43)	4 (50)	1.00
Yes	4 (57)	4 (50)	
% MC at first pDLI, median (range)			
PB MC	5 (0-15)	3 (0-15)	.85
T cell MC	5 (0-30)	10 (0-30)	.67

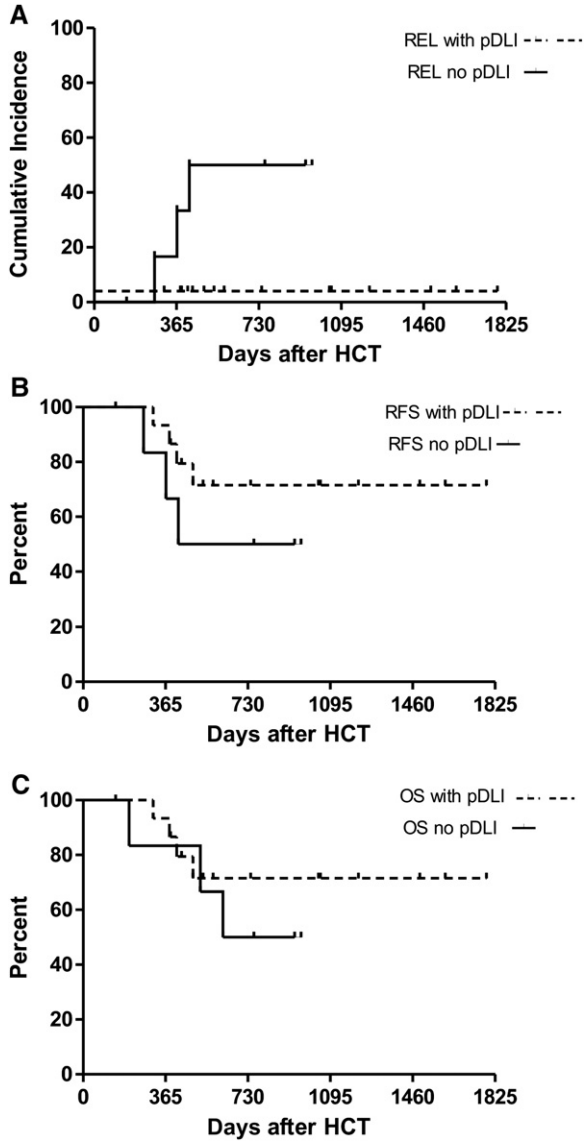

Figure 3. Outcomes of eligible pDLI candidates with pDLI and without pDLI. (A) Cumulative incidence rates of relapse (REL) (A), relapse-free survival (RFS) (B), and overall survival (OS) (C).

immunotherapy, especially when its value is still unproven. Indeed, in the present study, the 3 pDLI candidates who did not consent to participating in the study reported such concerns.

Because pDLIs were given while our patients were free of leukemia and in a nonrandomized manner, drawing conclusions regarding efficacy is difficult. The fact that all patients with MC improved chimeric status in close relation to pDLI administration suggests a graft-versus-lymphohematopoietic system effect of low-dose pDLI. However, the MC in our patients was relatively low (5%-15% recipient cells), and spontaneous conversions of low-percentage chimerism have been reported [34,35]. Thus, it remains unclear whether the conversions observed here can actually be attributed to pDLIs. Furthermore, conversion to complete chimerism is not clear evidence of GVL activity. None of the pDLI recipients relapsed, resulting in a 2-year posttransplantation probability for RFS and OS of 72%. A comparative statistical analysis between patients with and without pDLI treated with the uniform low-dose alemtuzumab protocol (a total of 56 patients), although appealing, is not valid, given that selection of the patients for pDLI is obvious (patients in CR with no GVHD, no early death, or relapse).

The present study has some significant limitations. It was a single-center study with a relatively small number of patients. Even though it focused on patients with acute leukemias receiving a uniform alemtuzumab-based GVHD prophylaxis protocol, the study population was heterogeneous. Despite the study's prospective nature, it did not include a control arm. The time of the first pDLI administration was not fixed, but varied according to patients' clinical and chimeric status, and donor lymphocytes were collected using different methods in sibling and unrelated recipients. Therefore, no concrete conclusions can be made regarding optimal cell dose, timing, and intervals of administration, or use of mobilized graft-derived versus steady-state DLIs [32].

Despite these limitations, however, the data reported herein are informative. Our data show that low doses of donor lymphocytes after alemtuzumab-incorporating conditioning are feasible and likely maintain significant antileukemic activity, as suggested by the absence of relapse in the pDLI recipients. Moreover, our data indicate that even low $CD3^+$ cell doses may induce severe and fatal GVHD when given in a prophylactic setting, emphasizing the need for more careful selection of patients scheduled to receive pDLIs. Novel, sensitive methodologies of monitoring minimal residual leukemia may improve the selection of patients who are in real need of prophylactic immunotherapy (eg, *WT1* gene expression, disease-specific mutation monitoring, multiparameter flow cytometry [36]). Finally, our data indicate that pDLI probably should not be given very early and not before day +100 after transplantation. We suggest starting pDLI at doses $\leq 5 \times 10^5$ $CD3^+$ cells/kg and to escalate, if at all,

within a very narrow range. In addition, pDLI recipients should be monitored very closely after administration, and prompt initiation of full-dose therapeutic immunosuppression should be started if signs or symptoms of GVHD develop.

In conclusion, our data illustrate that patients with leukemias who receive low-dose pDLI after conditioning with alemtuzumab are at low risk for relapse; however, this approach is associated with a relatively high incidence of severe GVHD. Further systematic studies are needed to determine whether the alemtuzumab-based T cell depletion had an impact on the high alloreactivity of low-dose pDLIs identified in the present study. Collaborative multicenter randomized trials in individual diseases and transplantation protocols are needed to clarify the value of pDLI. Data such as ours presented here may aid in the design of such studies. The use of engineered lymphocytes through insertion of suicide genes is a highly promising strategy that may increase the safety of pDLIs [37,38].

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