Adoptive immunotherapy with donor lymphocyte infusions after allogeneic hematopoietic cell transplantation following nonmyeloablative conditioning

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This study retrospectively analyzed data from 446 patients given hematopoietic cell transplants from HLA-matched related or unrelated donors after conditioning with 2 Gy total body irradiation with or without fludarabine and postgrafting immunosuppression with mycophenolate mofetil and cyclosporine following grafting. Fifty-three of 446 patients received donor lymphocyte infusion (DLI) with a median CD3 dose of 1×10^7 cells/kg. Their diagnoses included myelodysplastic syndrome (n = 10), acute leukemia (n = 10), chronic leukemia (n = 11), multiple myeloma (n = 9), lymphoma (n = 9), and solid tumors (n = 4). Patients received DLI for persistent disease (n = 8), disease relapse (n = 17), progressive disease (n = 12), low donor chimerism with disease (n = 11), or low chimerism with disease remission (n = 5). Seventeen of the 53 patients (32%) are alive with a median follow-up of 30 months; 5 are in complete remission (CR), 2 are in partial remission (PR), and 10 have stable or progressive disease. Nine of 53 patients (17%) developed grades II to IV acute graft-versus-host disease. Of 48 patients receiving DLI for treatment of disease, 7 achieved CR and 5 PR, with an overall response rate of 25%. Six of 16 patients who received DLI for chimerism had increases in donor chimerism leading to sustained engraftment, whereas 10 eventually rejected their grafts. In conclusion, DLI is a potential treatment strategy, with acceptable toxicity, for patients with persistent, relapsed, or progressive disease after nonmyeloablative hematopoietic cell transplantation. (Blood. 2004;103:790-795)

Introduction

Based on studies in a canine model,[1] a nonmyeloablative conditioning regimen for allogeneic hematopoietic cell transplantation (HCT) was developed for patients with malignant diseases.[2-6] In contrast to traditional myeloablative conditioning regimens that use high doses of radiation or chemotherapy or both to suppress host immune responses and eradicate diseases, this approach relies almost exclusively on graft-versus-host effects for eradication of the underlying diseases. Since 1997, more than 600 patients who were not deemed candidates for conventional HCT because of age or medical infirmities were given hematopoietic grafts from HLA-matched related or unrelated donors using nonmyeloablative conditioning with 2 Gy total body irradiation (TBI) with or without fludarabine followed by posttransplantation immunosuppression with mycophenolate mofetil (MMF) and cyclosporine (CSP).

This regimen results in initial mixed chimerism in the majority of patients,[2-6] but complete donor chimerism is likely necessary to eliminate underlying malignant diseases. Donor lymphocyte infusions (DLIs) have been advocated to convert stable mixed chimerism into full chimerism[7-11] and have been used successfully in patients with persistent, relapsed, or progressive disease after conventional HCT to exert graft-versus-tumor (GVT) effects, most notably in patients with chronic myeloid leukemia (CML).[12-14] Although mixed donor/host chimerism, including the persistence of host antigen-presenting cells, may provide suitable conditions for immunotherapeutic strategies through DLI, there is little published information on administration, efficacy, and toxicity of such strategies in this setting. We therefore performed a retrospective analysis on the use of DLI after nonmyeloablative HCT.

Patients and methods

Patients

A total of 446 consecutive patients underwent nonmyeloablative allogeneic HCT between December 1997 and December 2002. Fifty-three of the 446 received DLIs at the Fred Hutchinson Cancer Research Center and the University of Washington Medical Center (n = 19) or the Veteran's Administration Medical Center (n = 3), all in Seattle; University of Leipzig, Germany (n = 14); Stanford University (n = 8); University of Torino, Italy (n = 3); City of Hope National Medical Center (n = 2); Baylor Medical Center, Dallas (n = 2); University of Colorado (n = 1); and University of Arizona (n = 1). Results were analyzed as of December 2002. Included in this study were data from patients with malignant diseases treatable by allogeneic HCT who were ineligible for conventional allogeneic HCT because of age (> 50 years), concomitant medical conditions, or

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preceding extensive therapies such as failed autologous or allogeneic HCT. Transplantations were performed either using HLA-identical related or unrelated donors with intermediate- to high-resolution molecular matching for HLA-A, HLA-B, and HLA-C, and high-resolution molecular matching for HLA-DRB1 and HLA-DQB1.[2,5] The study protocols were approved by the institutional review boards of the participating institutions, and written informed consents were obtained from all patients. Patient characteristics are summarized in Table 1. Underlying malignant diseases were multiple myeloma (MM; n = 106), non-Hodgkin lymphoma (NHL; n = 76), myelodysplastic syndrome (MDS; n = 69), acute myeloid leukemia (AML; n = 55), chronic lymphocytic leukemia (CLL; n = 37), chronic myeloid leukemia (CML; n = 37), Hodgkin disease (HD; n = 24), acute lymphocytic leukemia (ALL; n = 12), myeloproliferative disorders (n = 9), renal cancer (n = 13), cervical cancer (n = 2), breast cancer (n = 2), osteosarcoma (n = 1), and melanoma (n = 3). Median patient age was 54 years (range, 5-74 years). Fifty-five percent of patients (n = 246) had high-risk diseases, defined as refractory/advanced disease or relapse after preceding high-dose HCT.

Treatment and evaluations

Patients were conditioned for HCT by either 2 Gy TBI alone (n = 112) or combined with 3 doses of fludarabine, 30 mg/m^2/d, from day −4 to −2 (n = 334). TBI was given at 7 cGy/min from dual cobalt 60 sources or linear accelerators on day 0 followed by infusion of donor hematopoietic cells. In 426 patients, the source of donor cells was granulocyte colony-stimulating factor (G-CSF)–stimulated peripheral blood cells, whereas 20 patients received bone marrow grafts from unrelated donors. Postgrafting immunosuppression consisted of CSP and MMF. CSP was given orally at 6.25 mg/kg twice daily from day −3. In patients given grafts from related donors, CSP taper was started on day +35 or +56, whereas patients receiving transplants from unrelated donors received a longer course of immunosuppression with CSP taper beginning day +100. MMF was given orally twice daily at 15 mg/kg beginning on day 0 and discontinued on day +27 in patients given grafts from related donors or 2 to 3 times daily and tapered off starting on day +40 in patients given grafts from unrelated donors.

Percentages of donor chimerism among peripheral blood T cells, granulocytes, and unfractionated marrow were assessed at days 28, 56, 84, 180, and 360 after HCT using either polymerase chain reaction (PCR)–based analysis of polymorphic microsatellite markers in recipients of sex-matched transplants or fluorescence in situ hybridization to detect X and Y chromosomes[15] for recipients of sex-mismatched transplants. Full donor chimerism was defined as 95% or more donor CD3$^+$ T cells and graft rejection as less than 5% donor CD3$^+$ T cells.

Acute and chronic graft-versus-host disease (GVHD) were graded as previously described.[16] Disease responses were assessed as follows. A complete remission (CR) was defined as no detectable disease at the morphologic, biochemical, clinical, or radiologic level. In patients who achieved CR, PCR was used to detect bcr-abl transcripts (CML),[17] clonal immunoglobulin gene rearrangements[18] (lymphoid malignancies), or other chromosomal translocations. A partial remission (PR) described a more than 50% reduction in measurable tumor, a more than 50% reduction in serum paraprotein levels in multiple myeloma, or a response to less than 30% marrow blasts in patients with acute leukemia.

Toxicities were determined using the National Cancer Institute Common Toxicity Criteria (CTC), version 2.0 (National Cancer Institute, Bethesda, MD).

Statistical analyses

Five diagnostic groups were defined: acute leukemia, chronic leukemia, MDS, lymphoma/MM, and solid tumors. Survival was estimated by the Kaplan-Meier method. Statistical comparisons of response were based on the χ^2 test. Analysis of survival was based on the proportional hazards regression model. All *P* values are derived from likelihood ratio statistics and are 2-sided.

Table 1. Patient and clinical characteristics

Characteristic	All patients	DLI
Total no. of patients studied	446	53
Median age, y (range)	54 (5-74)	56 (24-72)
Sex, no. M/F	285/161	42/11
Sibling donor/unrelated donor, no. patients	306/140	52/1
Graft source, PBSC/BM, no. patients	426/20	53/0
Conditioning, no. patients		
TBI only	112	32
Fludarabine/TBI	334	21
Disease, no. patients		
MM	106	9
NHL	76	4
Follicular	10	0
High grade	28	2
Mantle cell	21	0
Other	17	2
MDS	69	10
AML	55	9
CLL	37	6
CML	37	5
HD	24	5
ALL	12	1
Myeloproliferative disorders	9	0
Renal carcinoma	13	2
Cervical carcinoma	2	0
Breast carcinoma	2	1
Osteosarcoma	1	0
Melanoma	3	1
Median time to DLI from transplantation, d (range)	—	99 (48-1228)
Median follow-up from DLI for patients alive, mo (range)	—	30 (4-59)

PBSC indicates peripheral blood stem cells; BM, bone marrow; and —, not applicable.

Results

DLI and patients

Fifty-three (12%) of the 446 patients received DLIs, 52 after HCT from related donors (MRDs) and one after HCT from an unrelated donor (URD; Table 1). No scheduled immunosuppressive therapy was used after DLI. Patients received DLI for relapsed disease (n = 17), progressive disease (n = 12), persistent disease (n = 8), chimerism with persistent/relapsed or progressive disease (n = 11), and low or falling chimerism without evidence of disease (n = 5; Table 2). Patients were eligible for DLI once all immunosuppression had been discontinued without any flare of GVHD for at least 3 weeks. All but the one patient, who received a transplant from an unrelated donor and therefore DLI from a G-CSF–stimulated frozen product, received fresh nonmobilized donor lymphocytes. Thirty-one of the 53 patients had been conditioned with 2 Gy TBI alone and 22 had received the combination of fludarabine and 2 Gy TBI. All patients had received G-CSF–stimulated peripheral blood cell grafts. The median age of the patients receiving DLI was 56 years (range, 24-72 years). The underlying diagnoses were AML (n = 9), ALL (n = 1), CML (n = 5), CLL (n = 6), MM (n = 9), MDS (n = 10), NHL (n = 3), HD (n = 5), Waldenström macroglobulinemia (n = 1), melanoma (n = 1), breast cancer (n = 1), and renal cancer (n = 2). Seven patients received chemotherapy before DLI consisting of idarubicin/fludarabine/cytarabine and G-CSF in 2 patients with AML and one with ALL, paclitaxel/adriamycin in one patient with breast carcinoma, cyclophosphamide in one patient with MM, ara-C in one patient with MDS, and cisplatin/ara-C and dexamethasone in one patient with HD. The 46

Table 2. DLI characteristics and outcome

Characteristic	No.
DLI no. 1	53
Median cell dose	1×10^7 $CD3^+$ cells/kg
Median time from HCT, d (range)	99 (48-1228)
DLI no. 2	17
Median cell dose	3.2×10^7 $CD3^+$ cells/kg
Time from HCT, d (range)	158 (64-692)
DLI no. 3	3
Median cell dose	3×10^8 $CD3^+$ cells/kg
Time from HCT, d (range)	237 (174-393)
Indication for DLI, no. patients	
Relapse	17
Progressive disease	12
Persistent disease	8
Low or falling chimerism/disease*	11
Low or falling chimerism	5
Response if DLI for disease*, no. patients (%)	12/48 (25)
CR	7
PR	5
Chimerism response if DLI for disease; n = 28†	
Complete chimerism (≥ 95% donor cells)	19
Stable mixed chimerism (5%-94% donor cells)	6
Graft rejection/loss (≤ 5% donor cells)	3
Chimerism response if DLI for low or falling chimerism; n = 16	
Complete chimerism (≥ 95% donor cells)	3
Stable mixed chimerism (5%-94% donor cells)	4
Graft rejection/loss (≤ 5% donor cells)	9
No. patients alive/dead	17/36
Causes of death, no. patients	
Disease progression/relapse	32
GVHD related	3
Infection	1

*Disease is persistent/progressive or relapsed disease.
†Of 37 patients receiving DLI for disease, 28 were evaluable for chimerism.

remaining patients did not receive any form of antitumor therapy before DLI.

Median time to the first DLI from HCT was 99 days (range, 48-1228 days; Table 2). A median of 1×10^7 (range, 1×10^7 to 1.5×10^8) $CD3^+$ cells/kg was given with the first administration of DLI. In 17 patients, a second infusion of DLI with a median dose of 3.2×10^7 (range, 3.2×10^7 to 3.2×10^8) $CD3^+$ cells/kg was given at a median of 39 days (range, 6-324 days) following the first DLI. Three patients received a third dose of DLI consisting of 1×10^8 $CD3^+$ cells/kg.

Toxicity

The overall incidence of acute GVHD grades I to IV after DLI was 19% (n = 10; Table 3). Specifically, grade II GVHD was seen in 6 patients, grade III in 2 patients, and grade IV in one patient.

Table 3. GVHD after DLI

	No. of patients; n = 53	% patients
Acute GVHD, grade	10	19
I	1	2
II	6	11
III	2	4
IV	1	2
Chronic GVHD	18	34
Limited	8	15
Extensive	10	19

Chronic GVHD occurred in 18 patients (34%) and was extensive in 10 patients (19%). One patient died from complications related to GVHD. Among the 17 patients receiving 2 doses of DLI, acute GVHD grade II occurred in 2 and chronic GVHD in 6 patients (limited in 5; extensive in 1). Among the 3 patients given 3 doses of DLI, none developed acute GVHD and one developed chronic GVHD. Among the 11 patients with a history of acute GVHD (grades I-III) before the first DLI, none had developed acute GVHD but 9 developed chronic GVHD (limited in 5; extensive in 4). There was a significant positive correlation between the level of chimerism and GVHD. Acute GVHD was seen in 7 (31%) of 22 patients with full donor chimerism, 2 (22%) of 9 with mixed chimerism, and in none of the patients who rejected their grafts (n = 12; $P = .03$).

Ten (25%) of 40 evaluable patients experienced transient myelosuppression with granulocyte counts less than 0.5×10^9/L or platelet counts less than 20×10^9/L. This complication occurred a median of 7 days (range, 4-26 days) after DLI and lasted for a median of 18 days (range, 4-50 days). All patients eventually experienced full recoveries of their granulocyte and platelet counts.

No statistically significant influences of sex, age, DLI dose, or time point of DLI were found in respect to GVHD.

Survival and disease response

Thirty-six of the 53 patients receiving DLI have died, primarily because of relapse/progression of their underlying disease (n = 32) but also from GVHD-related causes (n = 3) or infection (n = 1; Table 2). Seventeen patients (32%) are alive with a median follow-up after DLI of 30 months (range, 4-59 months). Figure 1 shows the Kaplan-Meier plots of survival for all 53 patients. Estimated survivals were 51% at 6 months and 43% at 1 year. Five patients (1 with AML, 1 with MDS/AML, 2 with MM, and 1 with CML) are in CR, and 2 (1 with Waldenström macroglobulinemia and 1 with MDS) are in PR. Two patients have stable disease, and 8 are alive with relapse/progression.

Forty-eight patients received DLI for the treatment of relapsed, progressive, or persistent disease. In contrast, 131 of the 446 patients studied relapsed or progressed with their underlying disease but did not receive DLI. Among the 131, there were 23 patients with acute leukemia, 20 with chronic leukemia, 24 with MDS, 53 with MM/lymphoma, and 11 with solid tumors. The 6-month and 12-month survival estimates for these patients were 22% and 11% for acute leukemia, 69% and 57% for chronic leukemia, 17% and 8% for MDS, 59% and 43% for MM/lymphoma, and 36% and 9% for solid tumors, respectively.

Among the subgroup of 48 patients who received DLI for the treatment of relapsed, progressive, or persistent disease, 33 (69%)

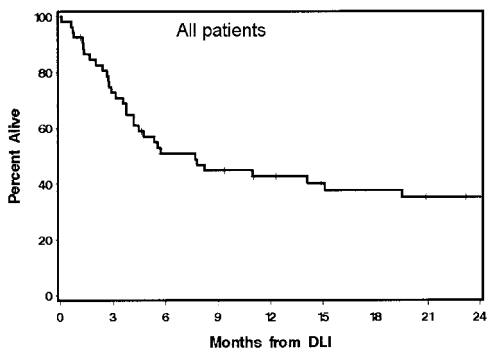

Figure 1. Survival after DLI. Kaplan-Meier plot of survival after DLI for 53 patients. The survival estimates were 51% at 6 months and 43% at 1 year.

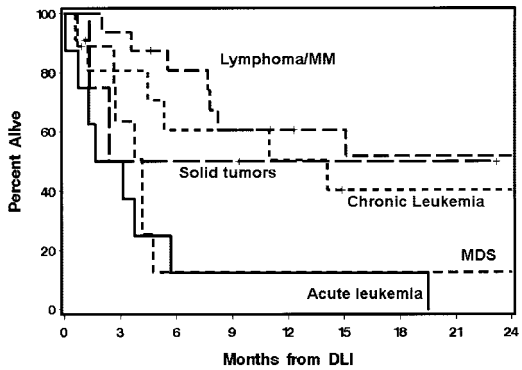

Figure 2. Diagnosis and survival after DLI for disease. Kaplan-Meier plots of survival after DLI depending on diagnoses. Survival estimates at 6 and 12 months were 81% and 61% for B-cell malignancies, 61% and 51% for chronic leukemia, 50% and 50% for solid tumors, 13% and 13% for acute leukemia, and again 13% and 13% for MDS, respectively.

died, mainly due to disease (n = 29) but also due to infection (n = 2) or GVHD (n = 2). Fifteen (31%) of the 48 are alive. The 6-month and 1-year estimates of survival were 50% and 41%, respectively. Seven patients achieved CR and 5 PR, resulting in an overall response rate of 25%. CR was achieved in 3 patients with AML, 3 with MM, and one with CML. Four of these patients are alive; the remainder died either from subsequent relapse (n = 2) or from a GVHD-related cause (n = 1). PR was achieved in one each with MM, NHL, Waldenström macroglobulinemia, MDS, and renal cancer. Two of these patients died, one due to relapse and one due to infection. Of the subset of 7 patients receiving DLI in combination with preceding chemotherapy, 3 patients achieved CR (1 each with AML, MM, and ALL), one patient achieved PR (with HD), and no response was seen in 3 patients (one each with AML, breast carcinoma, and MDS). Among the 48 patients, a statistically significant influence of GVHD on disease response was found as 5 (63%) of 8 patients with GVHD had a best response of CR or PR, whereas only 7 (18%) of 40 patients without GVHD achieved such responses ($P = .01$). There was also a statistically significant difference in overall survival depending on diagnostic group ($P = .02$) with the best survivals at 6 months and 1 year for lymphoma (81% at 6 months and 61% at 1 year) and chronic leukemia (61% at 6 months and 51% at 1 year) and the worst survival for acute leukemia and MDS (both 13% at both time points; Figure 2). Patients with solid tumors had estimated survivals of 50% at 6 months and 1 year.

Chimerism

Of 37 patients who received DLI for the treatment of disease, 28 were evaluable for changes of T-cell chimerism after DLI. In the other 9 patients, either no evaluation of chimerism was available or the patients died before any subsequent evaluation of chimerism after DLI (Table 2). Nineteen of the 28 patients had complete chimerism (95%-100% donor chimerism) after DLI. Nine of the 19 were already complete chimeras before DLI. Ten had increases from stable mixed chimerism (> 5%-95%) to complete chimerism. Six patients remained stable mixed chimera with 5% to 93% donor chimerism before and after DLI. Three patients lost their grafts (< 5% donor chimerism) after administration of DLI. The patients who subsequently rejected their grafts had donor CD3 chimerisms of 55%, 50%, and 35%, respectively, before DLI.

To assess the influence of chimerism on disease response, we included the response of the 11 patients receiving DLI for chimerism with evidence of disease in the evaluation (total n = 39). There was a significant correlation between the level of chimerism after DLI and disease response; no responses were seen among the patients losing their grafts (n = 9), whereas 3 of 10 patients with mixed chimerism and 9 of 20 patients with full chimerism showed disease response ($P = .02$). All patients who responded with either CR or PR had chimerism levels of more than 90% donor cells after DLI.

Sixteen patients received DLI for low or falling chimerism (Table 4). All but one of these patients had received 2 Gy TBI alone. Six patients with donor CD3 chimerisms between 30% and 79% had increases in chimerisms leading to sustained donor engraftment; 3 are alive, 2 with CR and one with PR. Nine patients with donor CD3 chimerism between 0% and 74% eventually rejected their grafts. Of these, 3 are alive, one in CR and one each with stable or progressive disease, whereas 6 patients died of relapse/progression. One of the 16 patients remained a stable mixed chimera but died of disease.

Discussion

Nonmyeloablative allogeneic HCT with a conditioning regimen consisting of 2 Gy TBI with or without fludarabine and posttransplantation immunosuppression with CSP/MMF results in initial mixed donor/host chimerism in the majority of patients. DLI has been proposed both to convert mixed donor chimerism into full chimerism and to exert strong GVT effects in patients with persistent, relapsed, or progressive disease. After conventional HCT, DLI has proved to be a powerful treatment option for some patients with recurrent malignant disease, but there are only limited published data on the use, efficacy, and toxicity of DLI after nonmyeloablative HCT.[19,20] We therefore retrospectively analyzed the experiences gained in patients treated with DLI within the nonmyeloablative HCT trials conducted at 8 academic centers.

Fifty-three patients were given DLI after nonmyeloablative HCT. Indications for DLI included persistent disease, disease relapse, progressive disease, and low or decreasing donor chimerism. Among the patients given DLI for the treatment of disease an overall response rate of 25% was observed, although many patients with rapidly progressing malignancies such as AML, ALL, MDS, high-grade NHL, and HD, were included in the study population.

Table 4. DLI for chimerism

Patient no.	Diagnosis	% Donor CD3 chimerism before DLI	% Donor CD3 chimerism after DLI	Disease response	GVHD after DLI	Status
1	MM	0	0	PD	No	Dead
2	AML	8	0	PD	No	Dead
3	CML	8	2	PD	No	Dead
4	CML	20	15	PD	No	Dead
5	CLL	29	0	Stable	No	Alive
6	CML	30	0	PD	No	Dead
7	AML	30	77	PD	No	Dead
8	MDS	35	0	PD	No	Dead
9	MDS/AML	43	93	CR	II	Dead
10	NHL	45	98	CR	III	Dead
11	AML	50	0	CR	No	Alive
12	CML	50	0	PD	No	Dead
13	MDS	62	91	PR	II	Alive
14	MM	74	0	PD	No	Alive
15	MM	75	100	CR	No	Alive
16	MDS/AML	79	95	CR	III	Alive

The 1-year estimate of survival was 36%. There was a clear difference in survival depending on diagnostic group with a better survival for chronic leukemia and lymphoma and the worst survival for acute leukemia and MDS. GVHD had a significant positive influence on disease response. In addition, we found a positive relationship between the level of chimerism and response because all patients responding to DLI had chimerism levels after DLI of above 90%. Only 16 patients received DLI for low or falling donor chimerism, and of these only 5 patients with donor chimerism levels of more than 40% before DLI eventually converted to full chimerism. The most serious toxicity of DLI was GVHD, with an overall incidence of grades II to IV acute GVHD of 17% and of extensive chronic GVHD of 19%. Irreversible aplasia after DLI was not seen, but transient myelosuppression occurred in 25% of evaluable patients. Treatment-related mortality was low, with only 4 deaths related to infection or GVHD (7%).

Most patients received one dose of DLI at a dose level of 1×10^7 $CD3^+$ T cells/kg. Dose escalation consisted of a second dose of DLI with 3.2×10^7 T cells/kg and a third dose of DLI with 1×10^8 T cells/kg. The starting dose of DLI used in our study corresponded to the dose of 1×10^7 $CD3^+$ T cells/kg, used by Mackinnon et al in a dose-escalation trial of DLI after myeloablative HCT, and found to be sufficient to induce potent GVT effects with a relatively low incidence of GVHD (12.5%).[21] DLI has been shown to be particularly effective for the treatment of relapsed CML after myeloablative allogeneic HCT with response rates of approximately 70%.[13,22,23] Although there was evidence for GVT effects with DLI in patients with other diseases, including CLL, MM, and lymphoma, reported response rates were lower than in patients with CML.[24-26] A definitive assessment of disease responses in individual diseases in our study was difficult due to the heterogeneous patient population and limited patient numbers. In addition, DLI was not uniformly used in all patients with disease relapse after nonmyeloablative HCT as 131 patients of the 446 patients studied who relapsed or progressed did not get DLI. This might lead to a selection bias toward patients with better overall condition and less aggressive disease. An analysis of the overall survival of these 131 patients seems not very different from the data seen among the patients treated with DLI, but a formal comparison is difficult due to the heterogeneity of the 2 groups. It was, however, promising that 12 patients achieved either PR or CR after DLI including patients with diseases known not to be particularly responsive to DLI. There was also evidence for some response to DLI in patients with renal cell carcinoma because one of 2 patients in the current study achieved a PR. These promising results were consistent with the notion that the state of mixed chimerism induced by nonmyeloablative HCT allows host dendritic cells in mixed chimeras to present polymorphic minor histocompatibility antigens to donor T cells thereby initiating GVT effects and increasing the efficacy of DLI.[27] The significantly lower survival rate of patients with acute leukemia and advanced MDS might be due to the fact that the tempo of tumor growth outpaces the GVT effects of the donor cytotoxic lymphocytes.[19,28] Results could be possibly improved by using salvage chemotherapy immediately before DLI in these patients.[29] This additional step was taken in only 7 patients in the current study, resulting in 3 CRs and one PR after DLI. However, these limited numbers do not allow for a formal evaluation of the role of chemotherapy preceding DLI in the nonmyeloablative setting and this strategy is therefore evaluated in a prospective trial.

Similar to observations made with a reduced intensity regimen, using cyclophosphamide, thymic irradiation, and antithymocyte globulin, DLI given to patients with mixed donor/host chimerism induced disease responses.[8,27] As observed previously by others[8,19] all responding patients in the current study had chimerism levels of above 90% after DLI, supporting the concept that full chimerism is necessary to eradicate the malignant clone and exert sufficient GVT effects. Our study found a significant positive influence of GVHD on disease response similar to reports on DLI after myeloablative HCT[13,24,30] but in contrast to studies in a murine model where GVT effects were achieved without GVHD if given in the setting of mixed chimerism.[31,32] The 17% incidence of acute grade II to IV GVHD was somewhat lower than the 25% overall and 15% grades III to IV incidences reported in another study on DLI after nonmyeloablative HCT,[19] but close to those in reports using a similar dose of 1×10^7 $CD3^+$ cells/kg DLI after conventional transplantation.[21,33] Severe, but in general reversible, pancytopenia has been reported to occur in 20% to 50% of patients[24,33,34] after DLI, which is similar to the 25% we observed in our patients.

The attempt to induce a state of full chimerism with DLI in patients with low or falling donor chimerism in our study was only successful in patients with chimerism levels more than 40%. This is consistent with observations made by Dey et al who only observed increases in donor chimerisms or conversions to full chimerism when DLI was given to patients with 40% or higher donor chimerism after nonmyeloablative HCT.[20] In subsequent patients not receiving DLI, we observed spontaneous increases of chimerism or conversion to full chimerism after comparable initial chimerism levels, so that it is unclear whether the conversions observed here can be attributed to DLI. Similar chimerism kinetics was observed in those patients who showed disease responses after DLI. The ineffectiveness of DLI to induce full donor chimerism in patients with low levels of mixed chimerism in the current HCT protocols warrants the evaluation of new strategies such as the administration of additional immunosuppressive agents or chemotherapy before DLI to overcome graft rejection.

In conclusion, this study shows that DLI has limited but definite effectiveness in the treatment of patients with relapse or progression of disease after nonmyeloablative HCT and that toxicities are acceptable. The data presented here will be the basis for further prospective studies in larger patient cohorts and individual diseases to assess disease responses and study new treatment strategies such as the combined use of chemotherapy and DLI.

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