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# Impact of Prophylactic Donor Leukocyte Infusions on Mixed Chimerism, Graft-versus-Host Disease, and Antitumor Response in Patients with Advanced Hematologic Malignancies Treated with Nonmyeloablative Conditioning and Allogeneic Bone Marrow Transplantation

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## ABSTRACT

**In an attempt to capture graft-versus-tumor effects without graft-versus-host disease (GVHD), the authors initiated a trial of nonmyeloablative allogeneic bone marrow transplantation (BMT) in patients with advanced hematologic malignancies, with the majority of patients having chemotherapy-refractory disease. Forty-two patients received an HLA-matched related donor BMT after a cyclophosphamide and antithymocyte globulin–based conditioning that also included thymic irradiation for patients who had not received prior mediastinal radiotherapy. Prophylactic donor leukocyte infusion (pDLI) at a dose of $1 \times 10^7$ $CD3^+$ cells per kilogram were given beginning 5 weeks post-BMT to 16 patients with mixed chimerism (MC) but without GVHD, whereas 26 patients did not receive pDLI, either because of GVHD or early relapse. Twelve of 16 patients (75%) receiving pDLI had T cell chimerism at the time of pDLI ≥40%. These patients, by day 100 post-BMT, either converted to full donor chimerism (FDC) (n = 10) or had an increase in or stable donor chimerism (n = 2) after pDLI. Four of 4 patients whose T cell chimerism was ≤20% at the time of pDLI, lost the graft. In contrast, only 5 of 18 evaluable patients (28%) not receiving a pDLI converted to FDC by day 100 post-BMT, 7 maintained MC, and 10 of an evaluable 22 lost the graft. Patients who had undergone a previous autologous stem cell transplant had a higher rate of conversion to FDC (69% *v* 31%) and higher incidence of GVHD (69% *v* 34%) compared with those who did not have a previous autologous SCT. Eleven of 16 patients (69%) who received a pDLI achieved a remission with 50% 1-year progression-free survival rate and 44% 3-year overall survival rate. Nineteen of 42 patients (45%) had ≥grade II acute GVHD, including 12 after BMT and 7 after pDLI. Approximately one third of patients, after having initial MC, eventually lost their donor graft. The authors conclude that (1) pDLI has the potential to convert MC to FDC; (2) sustained remissions can be achieved in patients with chemorefractory hematologic malignancies who receive a pDLI, albeit with a significant risk of acute GVHD; and (3) the degree of donor T cell chimerism at the time of pDLI is predictive of the fate of MC, ie, donor T cell chimerism ≥40% or ≤20% at the time of pDLI correlates with conversion of MC or loss of the graft, respectively.**



## KEY WORDS

Mixed chimerism • Full donor chimerism • Donor leukocyte infusion • Graft versus host disease • Graft versus tumor effect

## INTRODUCTION

The prognosis of patients with advanced chemorefractory hematologic malignancies is universally dire. Whereas allogeneic SCT after myeloablative conditioning has the curative potential for a fraction of these patients, the treatment-related mortality rate associated with this treatment is exceedingly high. Alternative strategies, such as nonmyeloablative chemotherapy and allogeneic SCT followed by donor leukocyte infusion (DLI), currently are being evaluated in patients with chemo-refractory hematologic malignancies with the aim of achieving a durable antitumor response while reducing transplant-related mortality.

Both experimental and clinical data indicate that cell-mediated immunotherapy, known as graft-versus-tumor (GVT) effect, plays a major role in the eradication of disease in patients with hematologic malignancies treated with allogeneic stem cell transplantation after myeloablative conditioning [1-4]. The most striking and direct evidence supporting the concept of a GVT response was reported by Kolb et al. [5], who showed that complete remission of relapsed chronic myelogenous leukemia (CML) after allogeneic hematopoietic stem cell transplantation could be achieved by simply infusing donor leukocytes [6-8]. Subsequently, a GVT effect has been shown convincingly in patients with lymphoma [9-11], multiple myeloma [12-16], and chronic lymphocytic leukemia [17]. However, toxicities associated with myeloablative conditioning as well as the occurrence of significant acute or chronic graft-versus-host disease (GVHD) after therapeutic donor lymphocyte infusions (DLI) remain substantial.

It has been shown that mixed lymphohematopoietic chimerism can be achieved in mice across full major histocompatibility complex barriers after both myeloablative and nonmyeloablative preparative regimens. These mixed chimeric mice are specifically tolerant to donor and host antigens. When nontolerant donor leukocyte infusions are given to these mixed chimeras several weeks after bone marrow transplantation (BMT), they convert to full donor-type chimeras as a result of a potent lymphohematopoietic graft-versus-host reaction, but GVHD does not occur [18,19]. Furthermore, delayed infusions of donor leukocytes have been proven to mediate a powerful antitumor effect in murine fully allogeneic chimeras and in mixed chimeras [20] without causing GVHD. Thus, a delay in DLI in animals receiving a nonmyeloablative conditioning regimen can permit lymphohematopoietic GVH reactions, which are associated with GVT effects, to occur without causing GVHD in the nonlymphohematopoietic tissues. Based on the principle of reduced transplant-related mortality associated with nonmyeloablative conditioning therapy and the attempt to maximize the GVT effect by delayed DLI, we have performed HLA-matched donor transplants in patients with chemotherapy-refractory hematologic malignancies using a nonmyeloablative preparative regimen consisting of cyclophosphamide, peritransplant antithymocyte globulin, thymic irradiation, cyclosporine, and delayed prophylactic DLI (pDLI). This nonmyeloablative allografting strategy is novel in that it uses chemotherapy and vigorous host and donor T cell depletion for the intentional induction of mixed chimerism (MC) as an immunologic platform for DLI with the aim of separating GVHD from GVT effects while maximizing antitumor reactivity. Here we report the impact of pDLI on the status of MC and its therapeutic potential in achieving antitumor responses in patients with advanced lymphohematopoietic malignancies.

## PATIENTS AND METHODS

Forty-two patients with advanced hematologic malignancies, including 13 who had relapse after autologous stem cell transplantation (AuSCT), received an HLA-matched (at A, B, and DR loci) related donor BMT between 1997 and 2001 based on a nonmyeloablative MC protocol conducted at the Massachusetts General Hospital in Boston. Patients showing no response to chemotherapy after diagnosis or at the time of relapse were considered to have *treatment-refractory* disease (n = 37; 16 in the pDLI group and 21 in the non-pDLI group); those showing evidence of response were considered to have *chemosensitive* disease (n = 2). Three patients underwent BMT without being treated at the time of relapse and were said to have *untreated relapse* at the time of the transplantation. Written informed consent was obtained from each patient before enrollment on this Institutional Review Board–approved protocol.

### Patient Characteristics

The diagnosis, age, and disease characteristics for the 42 recipients are shown in Table 1. The median age of these 42 patients was 42.5 (range, 22 to 62) years, and the median time from diagnosis to allogeneic BMT was 14 (range, 5 to 116) months. Eligibility criteria included a diagnosis of chemotherapy-refractory hematologic malignancy, defined as achievement of less than a partial response or disease progression during salvage chemotherapy or relapse after autologous stem cell transplants; Eastern Cooperative Oncology Group performance status of 0, 1, or 2; age $\leq$65 years; and adequate organ function. Patients and their donors were typed for HLA-A and HLA-B using standard serologic techniques and for HLA-DR and -DQ using sequence specific primer, or sequence specific oligonucleotide probe analyses. Based on their disease status and previous therapy (eg, an autologous

**Table 1.** *Patient Characteristics*

| | |
|---|---|
| **Patient No.** | **42** |
| **Sex (M/F)** | **25/17** |
| **Median age (yr)** | **43** |
| **(range)** | **(22-62)** |
| **Disease (%)** | |
| **NHL** | **24 (57)** |
| **Intermediate grade** | **18 (75)** |
| **Low grade** | **6 (25)** |
| **HD** | **7 (17)** |
| **AML** | **4 (10)** |
| **ALL** | **1 (2)** |
| **CLL** | **3 (7)** |
| **MDS** | **1 (2)** |
| **MM** | **2 (5)** |
| **Related donor source (%)** | |
| **HLA-genotypically identical** | **41 (98)** |
| **HLA-phenotypically identical** | **1 (2)** |
| **Sibling donors** | **41 (98)** |
| **Sex matched donors** | **33 (67)** |
| **No. of prior therapies median (range)** | **3 (1-7)** |
| **Time from diagnosis to BMT (d)** | **668** |
| **median (range)** | **(150-3474)** |
| **Previous auto transplant (%)** | **13 (30)** |

NHL indicates non-Hodgkin's lymphoma; HD, Hodgkin's disease; AML, acute myloid leukemia; ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; MM, multiple myeloma; MDS, myelodisplastic syndrome; BMT, bone marrow transplant.

stem cell transplant), patients were believed to have a prohibitively high risk of mortality after conventional allogeneic stem cell transplantation or a very high risk of posttransplantation relapse.

## Treatment Protocol

Nonmyeloablative conditioning therapy consisted of cyclophosphamide, 50 mg/kg/d, administered intravenously on 3 or 4 consecutive days on days −6 or −5 through −3; thymic irradiation (700 cGy) given on day −1 in patients who had not received previous mediastinal radiotherapy; antithymocyte globulin (ATG), 15 to 30 mg/kg on days −2, −1, and +1 or −1, +1, +3 and +5; and cyclosporine beginning day −1 (5 mg/kg daily intravenously, reduced to 3 mg/kg on day +4, switched to oral administration when tolerated at a dose of 6 mg/kg every 12 hours), then tapered to discontinuation in the absence of GVHD by ≥35 days post-SCT. The initial cohort of patients (n = 4) received a total of 200 mg/kg cyclophosphamide. For subsequent patients, the dose was reduced to 150 mg/kg because of cardiac toxicity experienced in 2 patients. The dose and schedule of ATG were changed (currently 20 mg/kg on days −1, +1, +3 and +5) to reduce toxicities and achieve better in vivo donor T cell depletion. Because of previous mediastinal radiotherapy, 10 patients did not receive thymic irradiation.

*Bone Marrow Transplantation.* A target number of $3 \times 10^8$ nucleated cells per kilogram of recipient body weight was collected by marrow aspiration in the operating room from donors under spinal, epidural, or general anesthesia. In the case of minor ABO incompatibility, plasma was removed from donor marrow before infusion, and in the case of major ABO incompatibility, red blood cells were depleted from the marrow using a CS-3000 cell separator (Baxter-Fenwal, Round Lake, IL). The median number of nucleated cells infused was $2.74 \times 10^8$ cells per kilogram of recipient body weight (range, $0.6 \times 10^8$ to $12 \times 10^8$/kg).

*Donor Leukocyte Collection and Infusion.* In the absence of clinically evident GVHD, patients were eligible for pDLI, beginning on day +35 posttransplantation in an effort to convert their mixed chimeric state to a state of full-donor hematopoiesis. Unmobilized donor leukocytes were obtained by leukapheresis using a COBE Spectra cell separator (Cobe Laboratories, Lakewood, CO) according to the manufacturer's instructions. Pheresis durations were 120 to 240 minutes and yielded a median of $1.4 \times 10^8$/kg recipient body weight (range, 0.7 to 2.0) mononuclear cells and $0.8 \times 10^8$/kg recipient body weight (range, 0.3 to 1.1) $CD3^+$ T cells. The target number of $CD3^+$ T cells per infusion given to 16 patients for conversion of MC to a state of full donor hematopoiesis was $1 \times 10^7$/kg. In 4 instances, as part of the original protocol, 1 to $5 \times 10^7$ $CD3^+$ T cells per kilogram were given in a second pDLI on or about day +56 posttransplant. Higher numbers of $CD3^+$ T-cells (range, $1 \times 10^7$ to $2.98 \times 10^8$/kg) as therapeutic DLI (tDLI) were given to 11 patients for treatment of progressive or relapsed disease.

## Supportive Care

Patients were cared for either in HEPA-filtered or laminar air flow rooms. All patients had triple lumen Silastic (Dow Corning, Midland, MI) central venous catheters. Anti-infective measures included co-trimoxazole for *Pneumocystis carinii* prophylaxis from admission until day −1, then 3 times weekly after resolution of neutropenia; levofloxacin, 500 mg daily, until resolution of neutropenia, fluconazole beginning on day −1 at a dose of 400 mg, which was reduced to 200 mg daily on day 0; and intravenous acyclovir at a dose of 250 mg/m$^2$ every 8 hours beginning on day −1, which was changed to a dose of 400 mg twice daily after resolution of neutropenia. Febrile neutropenia was treated with broad spectrum antibacterial agents usually consisting of vancomycin and ceftazidime. All blood products were irradiated with 2500 cGy using a cesium irradiator and were transfused through a third-generation leukocyte depletion filter. Initial treatment of acute GVHD consisted of corticosteroids (methylprednisolone), initially at a dose of 1 to 2mg/kg, then tapered as tolerated.

### Analyses of Chimerism

Analyses of microsatellite variable number of tandem repeat (VNTR) or short tandem repeat (STR) markers were performed on peripheral blood leukocytes from the patients and their donors before BMT. Each assay included mixtures of pretransplant donor and recipient DNA at different ratios, which were used to estimate the percentage of donor or recipient cells present in the posttransplant samples. For early patients, CD3-enriched and CD3-depleted populations were isolated from the peripheral blood using MACS beads (Miltenyi Biotec, Sunnyvale, CA). Recently, granulocytes (myeloid cells) were isolated by centrifugation over a density gradient (Isolymph, Gallard Schlesinger, Carle Place, NY), after which CD3-enriched cells (lymphoid cells) were isolated from the mononuclear cell population using MACS beads. Weekly microsatellite analyses were performed on peripheral blood leukocytes beginning on day +7 through day +100 posttransplant and on days +28 and +100 on bone marrow aspirate samples.

### Status of Remission

At approximately day +100 post-BMT, all evaluable patients underwent restaging evaluations including computed tomography (CT) scans of chest, abdomen, and pelvis; a gallium scan when applicable; and bone marrow aspirate and biopsy. Patients with no evidence of disease based on these studies were determined to be in complete remission, and those with more than 50% reduction in measurable disease were considered to have achieved a partial remission. A few patients with residual radiographic abnormalities on CT scans were required to have a negative Gallium scan as a criterion for complete remission (CR). Evaluable patients underwent follow-up studies (CT scans, Gallium scan, bone marrow examination as indicated) every 6 months for at least 3 years after BMT.

### Statistical Analysis

Descriptive statistics are summarized as a percentage or as the median and range where appropriate. Comparison between groups of the analyzed parameters were performed using Fisher's exact test. A $P$ value of less than .05 was considered significant. Overall survival and progression-free survival rates were calculated using the Kaplan-Meier method.

## RESULTS

### Prophylactic versus Therapeutic Donor Lymphocyte Infusions

In an attempt to convert a mixed chimeric state to full donor chimerism (FDC) and achieve a putative GVT effect, hopefully without clinical GVHD, 16 of 42 patients (38%) received pDLI at $1 \times 10^7$ $CD3^+$ cells per kilogram starting as early as 5 weeks posttransplant (Table 2), including 4 patients (UPN 181,

**Table 2.** *Outcomes of Patients Receiving Prophylactic-DLI*

| UPN | Diagnosis (Age, yr)* | AuSCT | % of Donor T cell Chimerism | | pDLI | | Time to Chimerism Conversion (Days post-BMT) | Acute GvHD (Grade) | Response | PFS (mo) | OS (mo) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Pre-pDLI | Post-pDLI | Day 1 | Day 2 | | | | | |
| 21 | NHL (35) | Yes | 60 | ≥99 | 35 | | 84 | No | CR | >52 | Alive >52 |
| 181 | NHL (41) | | 80 | ≥99 | 35 | 56 | 73 | IV | CR | 6 | 6 |
| 234 | NHL (22) | | 50 | ≥99 | 34 | 62 | 91 | No | CR | >52 | Alive >52 |
| 254 | HD (27) | | 40 | ≥99 | 36 | 57 | 82 | IV | PR | 4 | 5 |
| 285 | CLL (50) | | 95 | ≥99 | 37 | | 48 | I | CR | 19 | 19† |
| 317 | NHL (39) | | 60 | ≥99 | 35 | | 55 | III | PD | 2 | 3 |
| 322 | NHL (50) | | 20 | <1 | 36 | 64 | | No | PR | 14 | Alive >38 |
| 328 | NHL (61) | | 50 | 50 | 40 | | 91 | No | PR | 12 | 18 |
| 329 | HD (55) | Yes | 40 | 99 | 41 | | 91 | I | CR | 11 | 11‡ |
| 334 | HD (24) | Yes | 40 | 95 | 44 | | 70 | IV | PD | 1 | 3 |
| 349 | CLL (45) | | 5 | <1 | 42 | | | No | PD | 2 | Alive >34 |
| 360 | HD (42) | | 5 | <1 | 37 | | | No | CR | >32 | Alive >32 |
| 395 | MM (51) | Yes | 95 | 99 | 35 | | 42 | II | CR | >25 | Alive >25 |
| 402 | NHL (43) | | 5 | <1 | 36 | | | No | PD | 2 | 13 |
| 403 | NHL (57) | Yes | 80 | 99 | 36 | | 68 | IV | CR | >23 | Alive >23 |
| 409 | NHL (42) | Yes | 40 | 99 | 44 | | 70 | II | PD | 2 | 4 |

PD indicates progressive disease; NHL, non-Hodgkins lymphoma; HD, Hodgkin's disease; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; MM, multiple myeloma; MDS, myelodisplastic syndrome; AuSCT, prior autologous stem cell transplant; PFS, progression-free survival; OS, overall survival.

*Age at the time of BMT.

†UPN 285 died of infection.

‡UPN 329 died of acute myocardial infarction; both were in CR at the time of death.

**Table 3.** *Outcomes of Patients Not Receiving Prophylactic DLI*

| UPN | Diagnosis (Age, yr)* | AuSCT | GVHD | tDLI (d) | % of Donor T cell Chimerism | | Graft Loss (d) | FDC (d) | Response | PFS (mo) | OS (mo) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Day 28 | Day 100 | | | | | |
| 259 | ALL (40) | | No | No | 50 | | NE | | PD | <1 | 1 |
| 275 | NHL (43) | | No | No | 10 | | 43 | | CR | 7 | 16 |
| 282 | NHL (57) | | No | Yes (35) | 5 | | 35 | | PD | <1 | 2 |
| 283 | AML (44) | | No | Yes (38) | 10 | | 40 | | PD | 1 | 13 |
| 344 | NHL (35) | | No | Yes (35) | 50 | | NE | | PD | <1 | 1 |
| 216 | MDS (59) | Yes | No | Yes (41) | 20 | | 61 | | PD | 1 | 8 |
| 384 | NHL (59) | | No | Yes (35) | 20 | | 85 | | PD | 1 | 12 |
| 410 | HD (34) | | No | Yes (44) | 10 | | 40 | | PD | <1 | 4 |
| 160 | MM (56) | Yes | II | No | ≥99 | | | 35 | CR | 12 | Alive >20 |
| 194 | NHL (44) | | I | Yes (120) | 1 | | 28 | | PR | 1 | 6 |
| 218 | AML (44) | | I | No | 50 | | 63 | | PD | 1 | 2 |
| 351 | HD (43) | Yes | I | No | 70 | 95 | | | CR | >34 | Alive >34 |
| 358 | NHL (57) | | I | Yes (42) | 80 | | 50 | | PD | 1 | 2 |
| 376 | AML (58) | | I | No | NA | | NE | | PD | <1 | 1 |
| 377 | NHL (41) | | I | No | 40 | | 34 | | PD | 1 | 1 |
| 201 | NHL (45) | | II | No | 60 | | | 93 | PR | 7 | 17 |
| 238 | HD (27) | Yes | II | Yes (371) | 60 | 80 | | | CR | 9 | 34 |
| 268 | CLL (55) | | II | No | 60 | 95 | | | CR | >46 | Alive >46 |
| 279 | NHL (62) | | II | No | 95 | | NE | | PD | 1 | 1 |
| 368 | NHL (36) | Yes | II | No | 90 | | | 92 | PD | 3 | 7 |
| 386 | AML (57) | | II | Yes (54) | 40 | | | 89 | PD | 1 | 23† |
| 396 | NHL (36) | | II | Yes (292) | 70 | 95 | | | PD | 2 | 11 |
| 398 | NHL (52) | | II | No | 80 | 80 | | | CR | >24 | Alive >24 |
| 346 | NHL (46) | Yes | III | No | 40 | 40 | | | PD | 2 | 3 |
| 352 | NHL (38) | Yes | III | No | 80 | | | 45 | PD | 1 | 6 |
| 247 | AML (27) | | IV | No | 60 | 80 | | | CR | 3 | 3 |

PD indicates progressive disease; tDLI, therapeutic donor leukocyte infusion; FDC, full donor chimerism; GL, graft loss; NHL, non-Hodgkins lymphoma; HD, Hodgkin's disease; AML, acute myloid leukemia; ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; MM, multiple myeloma; MDS, myelodisplastic syndrome; AuSCT, prior autologous stem cell transplant; PFS, progression free survival; OS, overall survival; NE, not evaluable.

*Age at the time of BMT.

†This patient with secondary AML had evidence of progressive disease within a month after BMT, converted to full donor chimerism after tDLI with the achievement of sustained remission, but died of marantic endocarditis (in association with recurrent AML) 2 years after BMT.

234, 254, 322) who received a second pDLI ($1 \times 10^7$ $CD3^+$ cells per kilogram, n = 3; and $5 \times 10^7$ $CD3^+$ cells per kilogram, n = 1) between days 56 and 64 post-BMT. CSA was discontinued ≥24 hours before the administration of pDLI. Eighteen patients (43%) did not receive the pDLI because of the emergence of GVHD or initial suspicion of GVHD (Table 3). Eleven of 42 patients (26%) were given tDLI(s) for posttransplant disease progression (early progression, n = 8; late relapse, n = 3).

### Status of Chimerism

All patients had initial MC, defined as more than 1% peripheral blood donor myeloid or T cells. Patients who had less than 1% donor chimerism in both lymphoid ($CD3^+$) and myeloid ($CD3-$) lineages were declared to have lost their donor graft. Twelve of 16 patients (75%) receiving pDLI either converted to FDC (n = 10; 63%) with a median conversion time of 70 (range, 35 to 91) days post-BMT (or, median 32 days post-pDLI), or had increased or stable donor chimerism (n = 2) after pDLI by day 100 post-BMT, whereas 4 of 16 (25%) patients lost the donor chimerism despite pDLI (Table 2). In contrast, only 5 of 18 evaluable patients (28%; 8 patients who died within 2 months of BMT were removed from the analysis) who did not receive a pDLI converted to FDC, with 4 converting spontaneously by day 100 post-BMT and one converting only after tDLI; 7 patients maintained stable MC, and 10 of 22 evaluable patients ultimately lost their grafts in spite of early and late tDLIs. Among 10 patients (out of 11 evaluable patients) who received tDLI, 7 lost the donor graft, 2 maintained MC, and one achieved FDC. Of 13 patients who previously underwent an autologous stem cell transplant 9 achieved DFC (69%), 3 had sustained MC, and 1 lost the graft. In contrast, only 9 of 29 patients (31%; $P = .02$) who had not had a previous autologous stem cell transplant achieved FDC (Table 4).

**Table 4.** *Influence of Au-SCT on Mixed Chimerism and GVHD*

| | Prior Autologous Transplant (n = 13) | No Prior Autologous Transplant (n = 29) | *P* Value |
|---|---|---|---|
| **FDC** | 9 (69%) | 9 (31%) | .02 |
| **GL** | 1 (7%) | 11 (37%) | .04 |
| **aGVHD** | 9 (69%) | 10 (34%) | .03 |

FDC indicates full donor chimerism; GL, graft loss; aGVHD, acute graft-versus-host disease.

### Level of Donor T Cell Chimerism at the Time of pDLI as a Predictor of the Fate of Mixed Chimerism Post-pDLI

As shown in Figure 1, all patients who underwent conversion to FDC or had an increase in donor chimerism after pDLI had T-cell chimerism of ≥40% (range, 40% to 95%) at the time of pDLI. All patients whose T cell chimerism was ≤20% at the time of pDLI lost their grafts post-pDLI. Five patients in the non-pDLI group who underwent conversion to FDC as well as 7 patients in this group who maintained stable MC had ≥40% donor T cell chimerism during the time frame when pDLIs were given to other patients (Table 3). However, by day 100 post-BMT the conversion to FDC after pDLI in patients with ≥40% donor T cell chimerism at the time of the DLI was higher (10 of 12; 83%) compared with non-pDLI group, in which only 5 of 18 patients (28%) who had ≥40% donor T cell chimerism at approximately 35 days post-BMT achieved FDC. These results indicate that the degree of donor T cell chimerism at the time of pDLI was predictive of the fate of MC post-pDLI, ie, donor T cell chimerism ≥40% or ≤20% at the time of pDLI correlated with conversion of MC or loss of graft, respectively.

### Graft-versus-Host Disease

Twelve of 42 patients (29%) had grade II-IV acute GVHD after the BMT (grade II, n = 9; grade III, n = 2; grade IV, n = 1), and 8 of these patients achieved remissions (Table 3). As shown in Table 2, 7 of 16 patients (44%) had grade II or above acute GVHD after pDLI (grade II, n = 2; grade III, n = 1; grade IV, n = 4), with 4 achieving remissions (CR, n = 3; PR, n = 1). Two of 4 patients (UPN 181, 254) who received a second pDLI had grade IV GVHD with conversion to FDC. Seven patients in the pDLI group and 2 in the non-pDLI group achieved remissions without GVHD or with grade I only GVHD (Table 4). Nine of 13 patients (69%) who had undergone a previous autologous SCT had grade II or above acute GVHD either after the BMT (n = 5) or after pDLI (n = 4) in comparison with the entire series, in which only 10 of 29 patients (34%) had GVHD ($P = 0.03$), suggesting that extensive prior therapy is a strong predictor for the development of GVHD (Table 4). Only 2 patients (UPN 181, 334) died of GVHD (grade IV). Acute GVHD was staged and graded using the Seattle criteria, as described [21].

### Disease Response and Survival Outcomes

As is summarized in Table 5, 11of 16 patients (69%) who received pDLI(s) achieved a remission (CR, n = 8; PR, n = 3); 7 of these patients with a CR underwent conversion to FDC, whereas one with a CR eventually (by day +60) lost the graft. Nine of 26 patients (35%) in the non-pDLI group achieved a remission (CR, n = 7; PR, n = 2); one with a CR underwent conversion to FDC, 5 with a CR had stable MC, and one with a CR lost the graft (Table 5). The median overall survival rate of patients receiving pDLI was 18 (range, 3 to 52) months with a 50% one-year

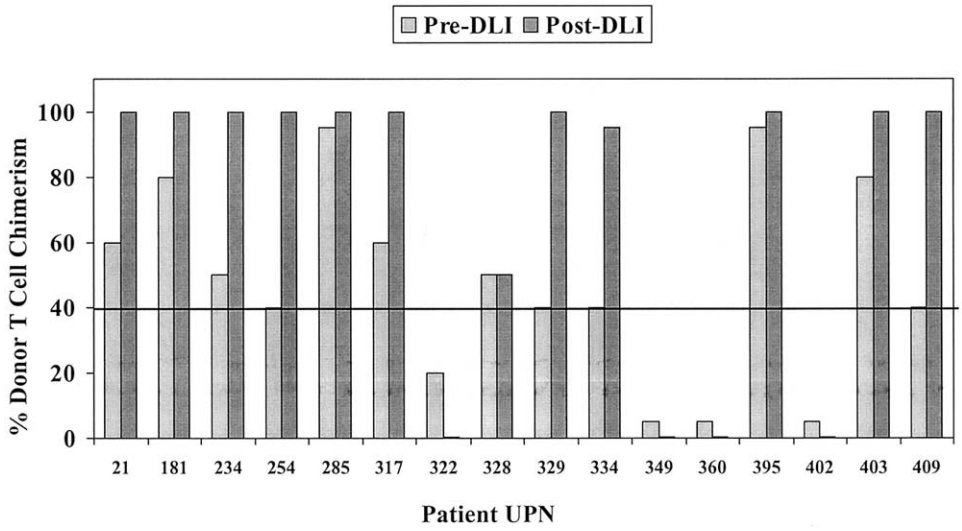


**Figure 1.** The pattern of donor T cell chimerism before and after pDLI, as determined by microsatellite analysis of peripheral blood performed once weekly until day +100 post-BMT.

**Table 5.** *Chimerism and Disease Response*

| | FDC | SMC | GL |
|---|---|---|---|
| **pDLI (n = 16)** | | | |
| **CR** | 7 | | 1 |
| **PR** | 1 | 1 | 1 |
| **PD** | 2 | 1 | 2 |
| **Total** | 10 | 2 | 4 |
| **No pDLI (n = 26)** | | | |
| **CR** | 1 | 5 | 1 |
| **PR** | 1 | 0 | 1 |
| **PD** | 3 | 2 | 8/12† |
| **Total** | 5* | 7 | 10 |

FDC indicates full donor chimerism; SMC, stable mixed chimerism; GL, graft loss.

*Four of 5 patients had spontaneous FDC and one had FDC after tDLI.

†Eight of 12 patients had evidence of loss of chimerism; 4 patients were not evaluable.

progression-free survival rate (Figure 2B) and 44% 3-year overall survival rate (Figure 2A). Seven of 16 pDLI recipients, including 6 of 11 who achieved remission, remained alive at a median of 2.5 (range, 1.5 to 4) years (Table 2; Figure 2A). Two patients died of nonrelapse causes (myocardial infarction and infection; both were in CR at the time of death); 5 patients died of relapsed disease and 2 of GVHD. One patient (UPN 402) with non-Hodgkin's lymphoma (NHL) lost evidence of donor chimerism after nonmyeloablative BMT, had recurrent disease, and underwent myeloablative allografting. He then had bronchiolitis obliterans with organizing pneumonia (BOOP) and died of respiratory failure; autopsy showed no evidence of lymphoma. One patient (UPN 349) with chronic lymphocytic leukemia who lost the graft had Richter's transformation and underwent myeloablative allogeneic SCT followed by DLI for recurrent disease; she remained in CR 14 months after the second allogeneic transplant. In contrast, the median overall survival rate was 6.5 (range, 1 to 46) months, and 1-year progression-free survival rate was 15% in patients who did not receive pDLI because of GVHD or suspicion of GVHD or early relapse, regardless of whether early or late tDLI(s) were given for disease progression. Four of 26 patients (15%) in the non-pDLI group remained alive at a median of 2 (1.4 to 3.5) years; 20 died of progressive disease, one died of infection and one of GVHD and posttransplantation lymphoproliferative disorder (PTLD). The 1-year progression-free survival in the whole cohort of patients was 29% with an overall survival rate of 30% at 2 years (Figure 2A and B). Twenty-two of the total 42 patients did not achieve an antitumor response.

## DISCUSSION

Because of multiple variables, including the design and mission of the treatment strategy and the various factors that determined whether patients received a pDLI, the aim of this uncontrolled study was not to compare the outcomes between the pDLI and non-pDLI groups. Rather, we have focused on the patterns of chimerism in patients who received this cyclophosphamide-based nonmyeloablative transplant strategy with delayed DLI (pDLI) and the complications of the transplants and DLI. This transplant strategy was a direct translation of preclinical murine BMT studies [19] in which the achievement of stable MC assures a state of bidirectional, (ie, donor- and host-specific) tolerance [18, 22-26] and thus can serve as an immunologic platform for pDLI, which can convert the mixed chimeric state to a state of full donor hematopoiesis via a GVH [18-20, 27]. In animal models, this powerful GVH alloreaction against lymphohematopoietic cells has been shown to be capable of eradicating leukemia without causing clinically significant GVHD, even though donor T cells were given in numbers that would cause rapidly lethal GVHD in recipients who had just undergone radiation [20, 28].

The goals of our strategy were to deliberately induce initial MC followed by the administration of

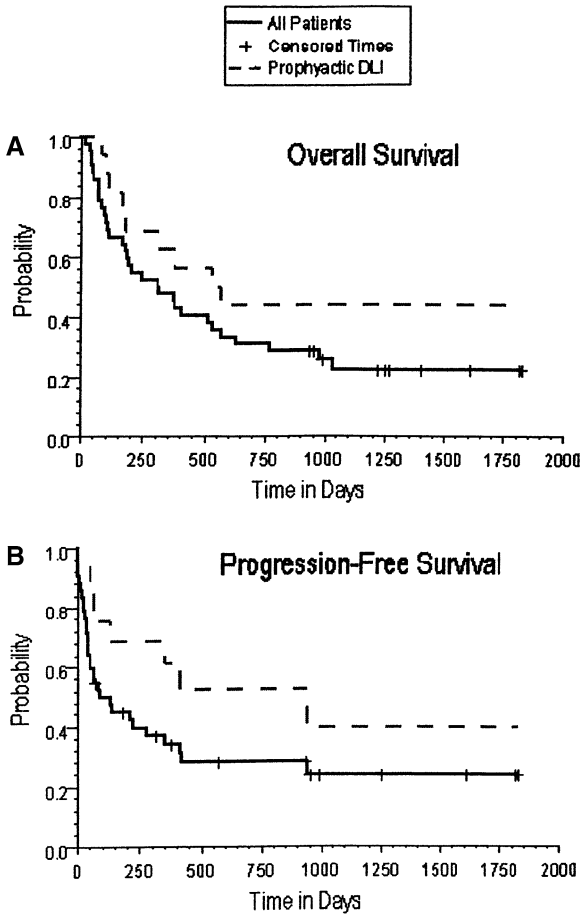


**Figure 2.** Kaplan-Meier (A) overall survival probability and (B) progression-free survival probability and for the 16 patients who received pDLI.

pDLI as early as possible after BMT to capture the maximal GVT effect, hopefully without the occurrence of GVHD [29,30]. Sixteen of 42 patients (38%) who did not develop clinical GVHD after BMT had their cyclosporine (the only posttransplant pharmacologic immunosuppressive drug given) rapidly tapered and received pDLI around 5 to 6 weeks after transplant. A response was achieved in 11 of these 16 patients. This clearly indicates a powerful immune-mediated antitumor effect, because most of these patients had previously shown resistance to cyclophosphamide, the only cytoreductive agent used in our conditioning regimen. Twelve of 16 patients either underwent conversion to FDC or had an increase in or stable donor chimerism after pDLI, whereas 4 of 16 lost the graft post-pDLI. With rare exception, the gradual achievement of complete remission after pDLI was associated with progressive conversion to FDC (Table 2), suggesting that a lymphohematopoietic graft-versus-host response mediated the GVT effect, as has been observed in the mouse model [20,28]. The persistence of antitumor responses in these patients correlated with the presence of sustained engraftment, highlighting the importance of the presence of donor T cells for inducing and maintaining the remission. One possible explanation for the potency of this GVT mechanism may be the presence of host-derived antigen-presenting cells (APC) in the mixed chimeric state at the time of pDLI. Host APC have been shown to be instrumental in eliciting GVH responses in a murine model of minor antigen-mismatched BMT [31]. Recently, Mapara et al.[20] showed in a murine leukemia model that GVT activity mediated by DLI is markedly superior in mixed chimeras compared with full chimeras, and that host-type APCs are important for the induction of maximal GVT effects. It is clear that the mixed chimeric state does not always reflect a state of tolerance, at least in humans, because many patients (n = 7) in the non-pDLI group who had sustained MC had GVHD with the achievement of CR (n = 5). The remissions in 2 patients who rejected the graft even after pDLI (Table 2) is perplexing and raises the question of whether a heightened immune-mediated host-derived antidonor response played a role in this antitumor effect.

Clearly, the degree of donor chimerism, that is, the presence of ≥40% donor T cells at the time of pDLI, correlated with the conversion to increased or complete donor chimerism. Conversely, 4 of 4 patients whose donor T cell chimerism was ≤20% at the time pDLI lost their grafts after pDLI. This is consistent with the findings of Schattenberg et al. [32], who recently showed that the percentage of donor T lymphocytes correlated well with the outcome of tDLI in relapsed patients after T cell–depleted BMT. It is also interesting to note that 5 patients in the non-pDLI group who underwent conversion to FDC and 7 patients who had stable MC or increased DC had ≥40% donor T cell chimerism at the time when pDLI were given to other patients. These results suggest that there may exist a threshold for donor T cell chimerism post-BMT that dictates the fate of MC, ie, donor T cell chimerism ≥40% or ≤20% at the time (5 to 8 weeks posttransplant) of pDLI correlates with conversion of MC or loss of graft, respectively. Approximately one third of our patients, after having initial MC, eventually lost their donor graft, including 11 patients after prophylactic or tDLI. We recently have obtained evidence that loss of chimerism in these patients is caused by immune-mediated rejection. As in most such patients, this rejection appears to be mediated by an increased recovery of donor-reactive recipient $CD8^+$ cells as well as interleukin-2 (IL-2)-producing recipient T helper cells (A. Kraus and M. Sykes, unpublished data).

The major cause of treatment failure in patients with advanced lymphohematopoietic malignancies after nonmyeloablative allogeneic BMT has been early progression of their underlying disease, presumably caused by an insufficient GVT effect from the BMT alone in this group of patients with aggressive, often bulky disease. Immunotherapy in our protocol is intended to arise from the pDLIs, which were administered as early as 5 weeks after BMT. In their nonmyeloablative strategy, McSweeney et al. [33] used 2 drugs for GVHD prophylaxis after BMT, and occasionally they administered DLI only beyond 2 months post-BMT. Although we have shown that early discontinuation of CSA with the administration of pDLI is feasible in many cases, the incidence of grade II or above GVHD after BMT and pDLI (45%) remains substantial. This incidence of acute GVHD, however, is similar to the experience described in other studies [16,34]. At the Dana Farber Cancer Institute, pDLIs were administered 6 to 9 months after T cell–depleted myeloablative BMT in patients with multiple myeloma [16], whereas the Seattle group delivered the first DLI 56 to 326 days after nonmyeloablative BMT in patients with hematologic malignancies [33]. Possible reasons for the presence of GVHD in several of our patients include (1) the incomplete T cell depletion of the donor marrow (by ATG) induced either clinical GVHD after BMT or a subclinical GVHD, which was accentuated when nontolerant pDLIs were given; and/or (2) the proinflammatory conditions (upregulation of cell adhesion molecules and various cytokines and chemokines) that play a role in the pathogenesis of GVHD are different in heavily pretreated patients and not as short lived in duration as in animal models. In patients who had had a previous autologous stem cell transplantation, both conversion to FDC and acute GVHD were significantly increased in comparison with those who did not have prior AuSCT (Table 4), indicating that AuSCT is a predictor for the

development of GVHD, probably because of profound host immunosuppression caused by earlier therapies [30]. Although the incidence of acute GVHD was significant, only 2 patients in the pDLI group died of GVHD, including one who received 2 pDLIs according to our original protocol. Five patients achieved remissions with conversion of their MC with no (n = 3) or minimal grade I GVHD (n = 2) providing proof of the principle that lymphohematopoietic GVH reactions, with GVT effects, are separable from GVHD in humans, as in the mouse [20,28].

Remissions were seen in 20 of 42 patients (48%) with advanced malignancies, including 9 of 26 in the non-pDLI and 11 of 16 in the pDLI groups. Although the treatment outcomes in these patients with advanced lymphohematopoietic malignancies are highly encouraging, a comparison of survivals between pDLI and non-pDLI groups is not possible because the non-pDLI group may have been already preselected for adverse outcome on the basis of early relapse. Sustained antitumor responses were seen in both indolent and aggressive histologies of NHL, Hodgkin's disease, chronic lymphocytic leukemia, and multiple myeloma. In all patients with advanced acute myeloid or acute lymphoid leukemia and in many with aggressive NHL, early disease progression occurred. This likely reflects the inadequate cytoreduction conferred by cyclophosphamide and the inability to deliver pDLI before the tumor had advanced to a level that could not be controlled by DLI. It is likely that for aggressive malignancies, additional drug(s) with both immunosuppressive and cytoreductive properties to facilitate engraftment and control tumor growth might be needed in the conditioning regimen. However, enhanced transplant-related morbidity or more GVHD could occur with such intensification of conditioning therapy. Using a reduced intensity regimen of busulfan, fludarabine, and ATG, Slavin et al. [34] showed a 14-month survival rate of 77.4% in patients with mainly standard-risk hematologic malignancies. Among 26 patients, however, 15% succumbed to fatal acute GVHD and hepatic veno-occlusive disease. Other groups have used various combinations of fludarabine with cyclophosphamide [35-37], including many requiring dose escalation of conditioning chemotherapy [38] to reduce graft rejection and to have better impact on the disease remission.

Our cyclophosphamide-based nonmyeloablative conditioning regimen for HLA-matched BMT resulted in the reliable induction of initial MC. Although it is difficult to interpret the true effects of pDLI without an adequate control group, pDLI given after early cessation of GVHD prophylaxis has the potential to convert MC to FDC and induce sustained remission in patients with chemotherapy-refractory hematologic malignancies, albeit with a significant risk of acute GVHD. It may, however, be important to tailor the regimen to the underlying disease (indolent versus aggressive) and to revise the schedule of pDLI according to the patterns of chimerism (increasing or decreasing at the time of pDLI). Our current strategies are focusing on the optimization of in vivo T cell depletion with MEDI-507 (a humanized CD2 monoclonal antibody) and possibly the addition of ex vivo T cell depletion to achieve a GVHD-free environment after the BMT before pDLI.

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