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Leukemia Lineage-Specific Chimerism Analysis and Molecular Monitoring Improve Outcome of Donor Lymphocyte Infusions

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A retrospective analysis was performed of 118 patients with hematologic malignancies who received donor lymphocyte infusions (DLIs) after allogeneic stem cell transplantation (ASCT). Treatment was either given because of hematologic relapse (n = 44), molecular/cytogenetic relapse (n = 52), or other causes (n = 22). Molecular relapse was in most cases based on leukemia lineage-specific chimerism analysis. Patients with acute leukemia and myelodysplastic syndrome showed a 3-year survival of 42% if DLI treatment was given because of molecular relapse, compared to 16% in hematologic relapse ($P < .006$). In multivariate analysis, there was a correlation between response to DLI and nonhematologic relapse (risk ratio [RR] 3.36, $P = .001$), chronic graft-versus-host disease (cGVHD) (RR = 1.51, $P = .005$), and late relapse (RR = 2.06, $P = .017$). The overall incidences of acute GVHD (aGVHD) grades I-II and grades III-IV aGVHD were 33% and 8.5%, respectively. Probability of cGVHD was 33%. The development of aGVHD or cGVHD did not significantly influence the response of DLI in patients with molecular/cytogenetic relapse. However, the development of cGVHD was significantly associated with a better response in patients with hematologic relapse because only 4 of 29 patients without cGVHD responded compared to 7 of 12 with cGVHD ($P = .007$). The development of cGVHD increased significantly if DLI was given >12 months after ASCT (46% versus 27%, $P = .04$). In contrast, time between ASCT and start of DLI treatment had no significant influence on the risk of developing aGVHD. To conclude, monitoring of leukemia lineage-specific chimerism is of utmost importance for DLI response after ASCT.

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KEY WORDS: DLI, GVHD, GVL, Chimerism, MRD, Relapse

INTRODUCTION

Allogeneic stem cell transplantation (ACST) can be curative and give long-term survival in a high proportion of patients with hematologic malignancies and nonmalignant disorders of the immunohematopoietic system [1,2]. The graft-versus-leukemia (GVL) effect is of major importance for the success of ASCT in the treatment of malignant disease [3]. There is a strong correlation between graft-versus-host disease (GVHD) and GVL as previously described [4,5]. Clinically, a significant GVL effect is induced by chronic rather than acute GVHD (cGVHD, aGVHD) [6]. Despite this, relapse of the underlying malignant disease remains the major obstacle after ASCT in patients with leukemia.

Augmentation of the GVL effect by either withdrawal of immunosuppression and/or donor lymphocyte infusion (DLI) is one major treatment strategy for relapse after ASCT [7]. T cells play a major role in inducing GVL effects after ASCT, and patients who receive T cell-depleted grafts have an increased risk of relapse after ASCT [4,8].

The effect of DLI for treating relapse of the underlying disease has been substantiated in several studies [9,10]. However, T cells are also the major cause of GVHD, which can be detrimental to the patient [11].The likelihood of developing GVHD after DLI depends on several factors such as the cell dose and the time interval between ASCT and DLI [12,13].

DLIs are used in nearly all malignant diseases for which ASCT is performed, but with highly variable efficacy. The best results have been obtained in patients

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with chronic myelogenous leukemia (CML), where there has been a high response rate in comparison with acute myelogenous leukemia (AML) and acute lymphoblastic leukemia (ALL) [9,10]. Leukemia cell lineage-specific chimerism analysis may allow identification of patients with threatening relapse of ALL and AML after ASCT several months earlier than conventional methods [14,15]. This may improve the results of DLI in patients with acute leukemia because treatment is started at a lower tumor burden [16]. In addition, CML patients treated with DLI because of molecular relapse do better than those with a hematologic relapse [17].

In the present study, we performed a retrospective analysis of 118 patients with hematologic malignancies who received DLI mainly because of relapse. Because we have been treating patients based on molecular relapse from the beginning of the last decade if at all possible, we wanted to evaluate the clinical impact of this approach.

MATERIAL AND METHODS

Patients and Donors

In total, 118 consecutive patients who received DLI at the Karolinska University Hospital, Huddinge, between April 1991 and September 2005, were included in the study. The main indication for DLI treatment was relapse of the underlying hematologic malignancy after ASCT. Ninety-six patients were treated for either morphologic relapse (n = 44) or for cytogenetic/molecular relapse (n = 52). Twenty-two patients were treated for other reasons: 4 because of Epstein-Barr virus (EBV) lymphoma, 7 with threatening rejection, 4 with extramedullary relapse, and 7 received DLI as prophylactic treatment because of high-risk leukemia. Median interval between ASCT and first dose of DLI was 212 (24-4800) days. All patients and donors were typed using PCR-SSP high-resolution typing for both HLA class I and II alleles [18]. Apart from patients with HLA-identical related donors (n = 58) and unrelated donors matched for HLA-A, -B, and -DRβ1 (n = 44), 11 patients with allele-mismatched donors were included. Five patients received an HLA-A, -B, and -DRβ1 major antigen mismatched graft. Patient and donor characteristics are summarized in Table 1. The study was approved by the ethics committee at Karolinska Institutet, Huddinge University Hospital in Stockholm (DNR 425/97).

Table 1. Patient and Donor Characteristics

N	118
Diagnosis	
AML	29
ALL	24
CML	39
MDS	14
Other hematological malignancy	12
Reason for DLI	
Hematologic relapse	44
Cytogenetic/molecular relapse	52
Other causes	22
Sex (M/F)	68/50
Age	37 (3-63)
Donors:	
HLA-identical siblings	58
Major HLA-Ag MM, unrelated	5
Matched, unrelated	44
Allele MM, unrelated	11
Stem cell source	
BM/PBSC	64/54
Conditioning	
TBI+Cy	46
fTBI+Cy	23
Bu+Cy	13
RIC	36
GVHD prophylaxis	
CsA + MTX	109
CsA + MMF	3
TCD	6
Number of DLIs per patient	
One	44
Two or more	74
Bulk DLI	34
Escalating DLI	84

AML indicates acute myelogenous leukemia; ALL, acute lymphocytic leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome; DLI, donor lymphocyte infusion; M, male; F, female; MM, mismatch; BM, bone marrow; PBSC, peripheral blood stem cells; FTBI, fractionated total body irradiation; Cy, cyclophosphamide; Bu, busulfan; RIC, reduced-intensity conditioning; GVHD, graft-versus-host disease; CsA, cyclosporine; MTX, methotrexate; MMF, mycophenolate mofetil; TCD, T cell depletion.

Conditioning and GVHD Prophylaxis

The myeloablative conditioning regimens consisted of intravenous (i.v.) cyclophosphamide (60 mg/kg daily) for 2 consecutive days in combination with either 3 Gy of fractionated total body irradiation (fTBI) for 4 consecutive days, or busulfan (Bu) at 4 mg/kg/day for 4 days (Table 1) [19]. Cyclophosphamide (60 mg/kg on 2 consecutive days) combined with 10 Gy of TBI (with the lungs shielded to receive no more than 9 Gy), was given to 46 patients. Reduced-intensity conditioning (RIC) was given to 36 patients and in the majority of patients it consisted of fludarabine (30 mg/m^2 for 6 days) in combination with oral Bu (total dose 8 mg/kg) as previously described [20]. All patients with unrelated donors received antithymocyte-globulin (ATG) (Thymoglobulin; Genzyme, Cambridge, MN, USA) for 3-5 days before transplant, with a total dose of 6-8 mg/kg [21]. The majority of patients (n = 109) received cyclosporine A (CsA) combined with 4 doses of methotrexate (MTX) as prophylaxis against GVHD [22]. During the first month, blood CsA levels were kept at 100-150 ng/mL in patients with sibling donors and 200-300 ng/mL in patients with a matched unrelated donor (MUD). In the absence of GVHD, CsA was discontinued after 3-4 months when HLA-identical sibling donors were used and after 6 months when

unrelated donors were used. In most cases where DLI was used as treatment for relapse, immunosuppressive therapy was discontinued prior to the first infusion. However, in 13 patients who received DLI for various reasons, immunosuppressive therapy was continued.

Analysis of Chimerism and BCR-ABL

DNA from donor and recipient pretransplantation samples was extracted using standard protocols (MagNA Pure, Roche, Switzerland). To evaluate lineage-specific chimerism, CD3, CD19, and CD33-positive cells were selected from peripheral blood using antibody-labeled magnetic beads (Dynal, Oslo, Norway). The methodology and sensitivity of chimerism analysis in the various cell lineages has been described elsewhere [14,23,24]. We have previously reported a sensitivity of 2×10^{-4} when CD19-positive cells were collected in a leukemia-affected cell-lineage specific manner and 4×10^{-4} for CD33-positive cells [14,15]. In all patients transplanted after 2001, chimerism analysis was routinely performed. As part of the posttransplantation routine, recipient samples were collected for monitoring of chimerism status on days +14, +21, +28, and every other week up to 3 months, and then monthly thereafter. The method of detection of BCR-ABL has been described elsewhere [25,26]. Briefly, quantification was done by competitive PCR using plasmid constructs containing a modified *BCR-ABL* fusion gene. A sensitivity of 10^{-6} was obtained with this method after 40 cycles of PCR amplification. Patients were monitored on a monthly basis during the first 6 months after ASCT and then in general after every third month. Detection with BCR-ABL was performed in all patients with CML and in patients with ALL expressing BCR-ABL.

DLIs

Donor cells were collected by leukapheresis on 1 or more occasions, depending on the cell yield. Thirty-four patients received a single bulk dose of fresh donor lymphocytes (all lymphocytes that could be collected were given at a single time point), median 2×10^8 $CD3^+$ cells/kg (range: 1×10^6- 3.6×10^9 $CD3^+$ cells/kg).

The remaining patients were treated according to a cell dose escalating protocol. Donor cells obtained from the leukapheresis were cryopreserved with autologous plasma and DMSO. Based on an estimated cell loss of 30% to 50% in the preservation procedure itself, the cell doses were doubled before freezing. The escalations in dose were done in steps of 0.5 to 1 log starting between 1×10^5 to 1×10^6 $CD3^+$ cells per kg, depending on the type of donor, the degree of HLA match, and the history of GVHD in the recipient. No patients with current aGVHD were treated. The time intervals between doses were generally 3 to 5 weeks. A median dose of 1×10^7 $CD3^+$ cells/kg (range: 1×10^5-2×10^8 $CD3^+$ cells/kg) was given.

Ten patients received chemotherapy prior to DLI, all because of hematologic relapse of ALL (n = 4), or AML (n = 5), or CML with blast crisis in 1 case.

Different immunosuppressive and immunostimulatory regimens, such as interleukin (IL)-2, were used in conjunction with DLI as previously described, especially in the early years [27]. Briefly, 6 patients received IL-2 alone and 8 patients were given MTX after DLI in combination with various IL-2 regimens.

Diagnosis and Treatment of GVHD

aGVHD and cGVHD was diagnosed on the basis of clinical symptoms and/or biopsies (skin, liver, gastrointestinal tract, or oral mucosa) according to standard criteria [28,29]. aGVHD was not restricted to any time point after transplantation, but could appear after day 100 and at any time after DLI. The patients were treated for grade I aGVHD using prednisolone, starting at 2 mg/kg/day, which was tapered after the initial response. In severe cases, ATG, methylprednisolone, MTX, psoralene and ultraviolet light (PUVA), or treatment with mesenchymal stem cells was used [30,31].

cGVHD was initially treated with CsA and steroids.

Definition of Relapse, Pancytopenia, and Rejection

Response to DLI was defined as follows: complete response (CR) in bone marrow (BM) after hematologic relapse, conversion to BCR-ABL-negative PCR, conversion to full donor chimerism in relevant cell lineage, absence of leukemic clone according to flow cytometry, or disease remission based on cytogenetic analysis—that is, absence of metaphases with the Philadelphia chromosome in BM.

In this study, molecular relapse was defined as either increasing levels of BCR-ABL, or BCR-ABL ratio exceeding 0.02% on 3 occasions, or the ratio reaching 0.05% on 2 occasions. Molecular relapse concerning chimerism analysis was defined as recurrence of recipient cells in the leukemia-affected cell lineage as described previously [14,15]. If mixed chimerism was detected in the leukemia-affected cell lineage in peripheral blood in 2 consecutive samples, immunosuppression was discontinued if possible and DLI started. The sensitivity of chimerism analysis in BM is increased but specificity is lower [14]. Thus, if a patient showed mixed chimerism in the relevant cell lineage in BM, immunosuppression was tapered or discontinued and a new BM sample was generally analysed after 1 month. If mixed chimerism was decreasing, no DLI was given; if it continued to increase, DLI treatment was started.

Patients were, in general, analyzed with morphology and flow cytometry of BM aspirates at 3, 6, and 12 months, and then on an annual basis after ASCT. All results were obtained from the Department of Pathology, Karolinska University Hospital. A patient with acute leukemia with regenerating peripheral blood values was considered in clinical remission when <5% blast cells among 200 nucleated cells were found in a BM sample, on morphologic examination. Clinical leukemic relapse was defined as at least 20% blast cells in BM aspirates or the presence of extramedullary leukemic cells, that is, extramedullary relapse. Patients with myelodysplastic syndrome (MDS) were classified according to both the FAB Cooperative Group and the WHO classification.

Pancytopenia was defined as leukocyte counts of $<1.0 \times 10^9$/L, and/or platelet counts of $<20 \times 10^9$/L [32].

Statistical Methods

The probabilities of overall survival (OS) were estimated using the Kaplan-Meyer method and compared with the log-rank test [33]. The incidence rates of GVHD were estimated using a nonparametric estimator of cumulative incidence curves [34]. The competing event was death without GVHD within 100 days. Predictive analyses for GVHD were based on the proportional hazards model for subdistribution of competing risks. In predictive analysis for response, the Cox regression method was used. Factors analyzed were patient and donor age and sex, diagnosis, disease stage, aGVHD and cGVHD, time to relapse, type of relapse, nucleated cell dose, and conditioning. cGVHD was analyzed as a time-dependent variable. Analyses were performed using the cmprsk package (developed by Gray, June 2001), Splus 6.2 software, and Statistica software. The Mann-Whitney *U*-test was used to compare continuous variables and the Fisher exact test was used to compare the distribution of categoric variables.

RESULTS

DLI as Treatment for Hematologic Relapse

Forty-four patients were treated because of hematologic relapse. Patients who received DLI treatment because of early hematologic relapse showed a significantly inferior survival rate compared to those with relapse beyond 1 year after ASCT (Figure 1a). Only 3 of 24 patients (12.5%) with early hematologic relapse responded to DLI compared to 8 of 20 patients (40%) with relapse beyond 1 year ($P = .036$).

Seven of 30 patients (23%) who did not develop aGVHD responded after DLI treatment, compared to 4 of 11 (36%) with aGVHD of grades I-II and none of the 3 patients with GVHD grades III-IV (ns). However, there was a significant correlation between development of cGVHD and a better response because only 4 of 31 patients without cGVHD responded, compared to 7 of 13 with cGVHD ($P = .007$). Six of these 7 patients had cGVHD before response.

Ten patients (9 with acute leukemia and 1 with CML in blast crisis) with hematologic relapse received chemotherapy before DLI. Only 3 patients showed a response. Median survival was 112 days (range: 21-1022). All 10 patients died: 5 because of relapse, 3 because of GVHD, and 2 because of infection.

Furthermore, the various MTX/IL-2 regimens had no clinical impact regarding response after DLI. In the majority of patients with hematologic relapse chimerism analysis was not performed because the chimerism method was not in routine use (n = 25). Twelve patients had evidence of decreasing donor chimerism before relapse but no treatment was started because of ongoing chimerism studies. Seven patients showed only donor chimerism before relapse but with several months interval between last sample and relapse.

DLI Because of Mixed Chimerism in Leukemia-Affected Cell Lineage

A total of 26 patients received DLI because of molecular relapse, defined as detection of mixed chimerism in the leukemia-affected cell lineage as previously described [14,15,35]. Three patients with CML responded to DLI, all of whom had myelogenous mixed chimerism and all whom were BCR-ABL positive. Two patients with B cell chronic lymphocytic leukemia with mixed chimerism in the B cell lineage responded. All DLIs given and the reason behind it, diagnoses, and molecular method used are summarized in Table 2.

Seven patients with ALL received DLI either because of mixed chimerism in the B cell lineage (n = 5) or T cell lineage (CD7 expressing T cell ALL) (n = 2) (Figure 2). Three patients responded, all of whom had molecular relapse at 6 months or later. Two of these patients are still alive. One patient died because of cGVHD more than 2 years after immunotherapy. Of the remaining patients, UPN 779 showed decreasing levels of remaining recipient B cells but the patient died of GVHD. Patient UPN 981 responded initially to chemotherapy and DLI but relapsed 10 months later.

Of the 7 patients with AML and mixed chimerism in the myeloid cell lineage, 6 responded (Figure 3). One patient (UPN 920) with an early molecular relapse and delayed DLI treatment did not show any response. In 2 patients with initial response (UPN 976 and 902), recurrence of the underlying AML was detected again 8 and 16 months after immunotherapy. Both died of relapse. Of the remaining 4 patients, 3 are

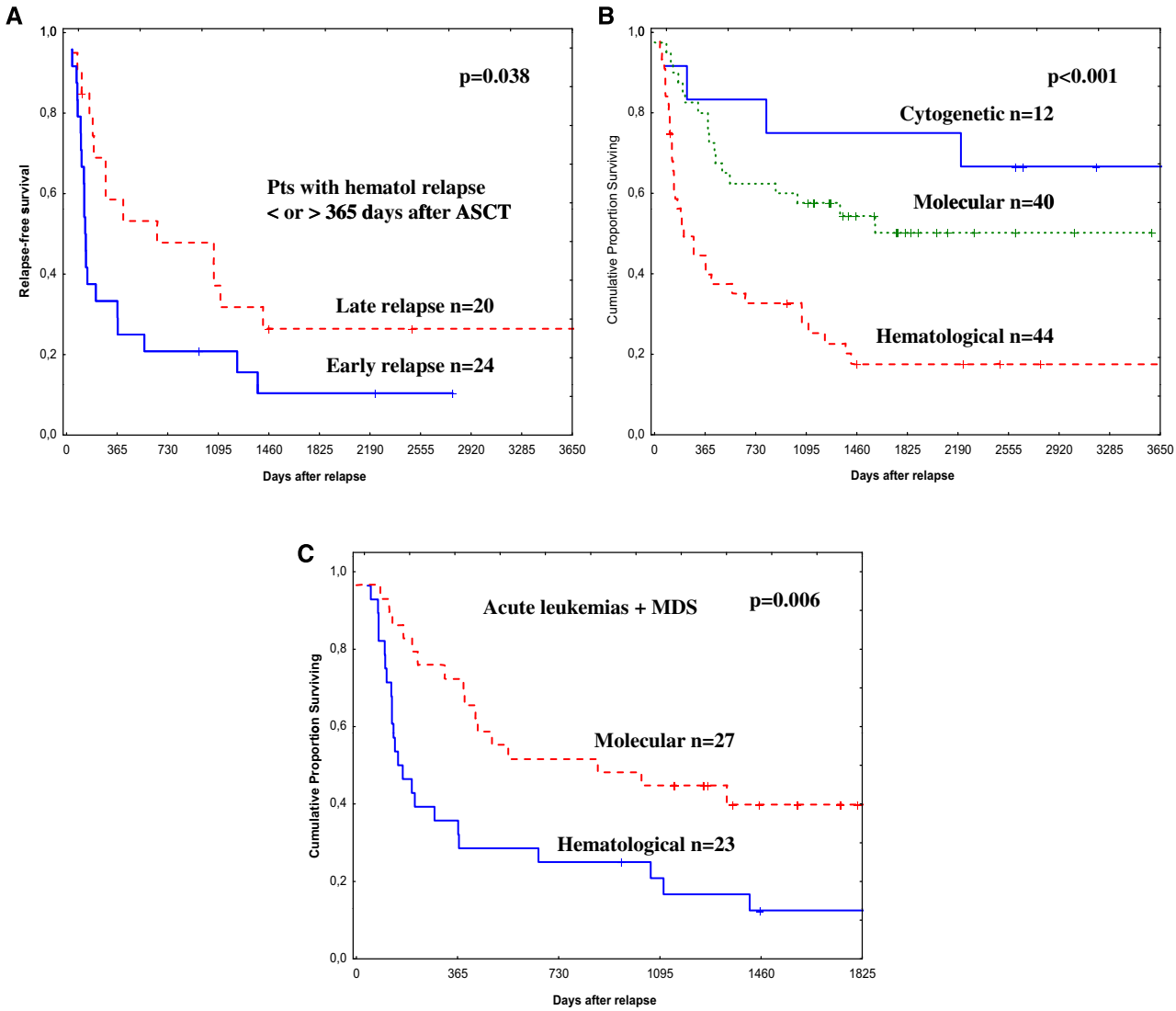

Figure 1. (A) Probability of survival in patients (pts) with early or late hematologic relapse after ASCT (at <365 days or >365 days, respectively). (B) Cumulative survival if DLI was given because of hematologic, molecular, or cytogenetic (only patients with chronic myeloid leukemia) relapse after ASCT. (C) Probability of survival in patients with acute leukemia and myelodysplastic syndrome (MDS) if DLI was given after hematologic or molecular relapse, the latter being ascertained mainly from chimerism analysis.

still alive. One patient later developed T cell ALL, and after receiving DLI the patient died of GVHD.

The results of DLI treatment in patients with MDS are shown in Figure 4. All patients were diagnosed with mixed chimerism in the myeloid cell lineage between 2.5 and 5 months after ASCT. Of the 5 patients, 4 responded. Three of them are still alive.

DLI as Treatment for BCR-ABL and Cytogenetic Relapse

Patients with cytogenetic response (CML patients only) showed the best survival (Figure 1b). Nine patients with CML were treated because of high or increasing levels of BCR-ABL and 1 patient with ALL. Three patients with CML developed mixed chimerism at the same time as they became BCR-ABL positive. Eight of these patients responded to DLI and became BCR-ABL negative. In 2 of these patients, DLI had to be combined with imatinib to induce long-lasting BCR-ABL negativity. Three patients died. One of them died of hematologic relapse (the patient who did not respond) and 2 responders died several years later because of septic shock.

Outcome after DLI for Molecular and Cytogenetic Relapse

The majority of patients with molecular relapse (n = 40) had acute leukemia or MDS (n = 27). The majority of these molecular relapses were based on detection of mixed chimerism in the leukemia-affected cell lineage as stated previously. Significantly better survival was seen if DLI treatment was given for molecular relapse as opposed to hematological relapse (Figure 1c).

In patients with late molecular/cytogenetic relapse 18 of 20 (90%) responded to DLI compared to 20 of 32

Table 2. Diagnoses and Reasons for Treatment with Donor Lymphocyte Infusions

	Morphologic Relapse (n = 44)	Cytogenetic Relapse (n = 12)	BCRABL (n = 10)	Mixed Chimerism (Relapse) (n = 26)	Mixed Chimerism (Rejection) (n = 7)	FACS (n = 7)	Others (n = 15)
CML (n = 39)	15	12	9	3*	2	0	1
AML (n = 29)	10	0	0	7	2	4	6
MDS (n = 14)	7	0	0	5	2	0	0
ALL (n = 24)	6	0	1	7	0	3	7
Other hematological malignancies (n = 12)	6	0	0	4	1	0	1

CML indicates chronic myelogenous leukemia; AMC, acute myelogenous leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphblastic leukemia; DLI, donor lymphocyte infusion.; EBV, Epstein-Barr virus.
Others: 4 patients with EBV lymphoma, 4 with extramedullary relapse and 7 patients receiving prophylactic DLI.
*Three patients with mixed chimerism and BCR-ABL positive at the same time point.

(63%) patients with relapse during the first year after ASCT ($P = .06$).

Development of aGVHD or cGVHD had no significant influence on the response to DLI in patients with molecular/cytogenetic relapse. In patients without aGVHD 16 of 24 (67%) responded to DLI treatment, compared to 19 of 23 patients (83%) with aGVHD grades I-II, and 3 of 5 patients with aGVHD grades III-IV (ns). Eighteen of 22 patients (82%) with cGVHD responded, compared to 20 of 30 (63%) without cGVHD ($P = .11$).

Multivariate Analysis of Response

In multivariate analysis, the response to DLI was significantly associated with nonhematologic relapse (n = 52, $P < .001$), cGVHD (n = 29, $P = .005$), and late relapse ($n = 38$, $P = .017$) (Table 3). Other factors analyzed such as patient and donor age and sex, diagnosis, disease stage, aGVHD, time to relapse, type of relapse, nucleated cell dose, and conditioning did not show ant significant association with response to DLI.

Complications, Timing, and Causes of Death after DLI

The overall incidences of mild-to-moderate aGVHD (grades I-II) and severe aGVHD (grades III-IV) in the present study were 34% and 8.5%, respectively. Univariate analysis for factors affecting incidence of aGVHD of grades II-IV showed a slight positive effect of unrelated donors ($P = .07$) and a tendency of a lower incidence in patients with hematologic relapse ($P = .06$). None of these factors showed any significance in multivariate analysis, however. Seven of 44 (16%) patients with hematologic relapse developed aGVHD grades II-IV compared to 14 of 40 (35%) patients with molecular relapse ($P = .08$).

cGVHD was diagnosed in 39 of the 118 patients (33%). Nineteen patients had mild cGVHD, 17 moderate, and 3 developed severe cGVHD. The incidence of cGVHD was more common if DLI was given >12 months after ASCT. Twenty-one of 79 patients (27%) who were treated with DLI <12 months after ASCT were diagnosed as having cGVHD, compared to 18

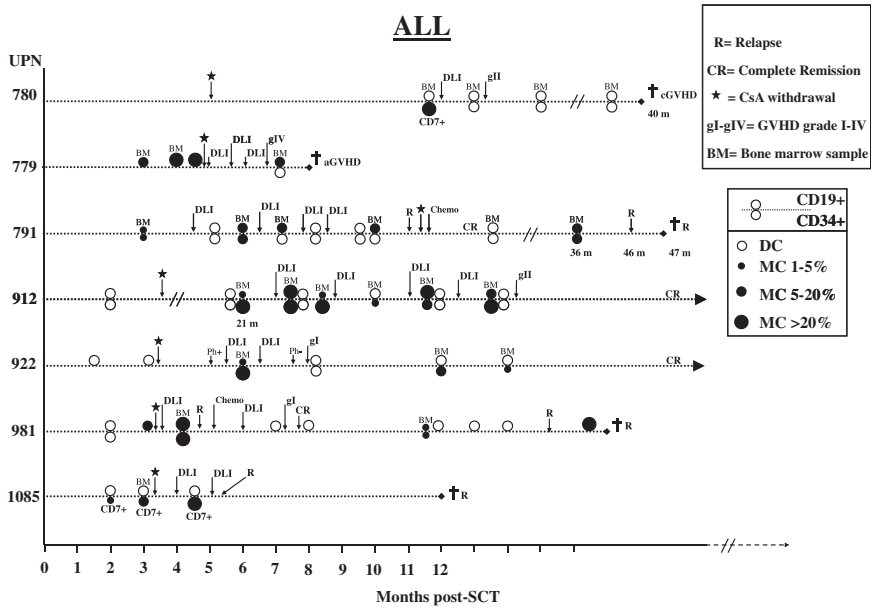

Figure 2. Chimerism analysis of leukemia-affected cell lineage in patients with acute lymphoblastic leukemia (ALL) who received DLI after ASCT.

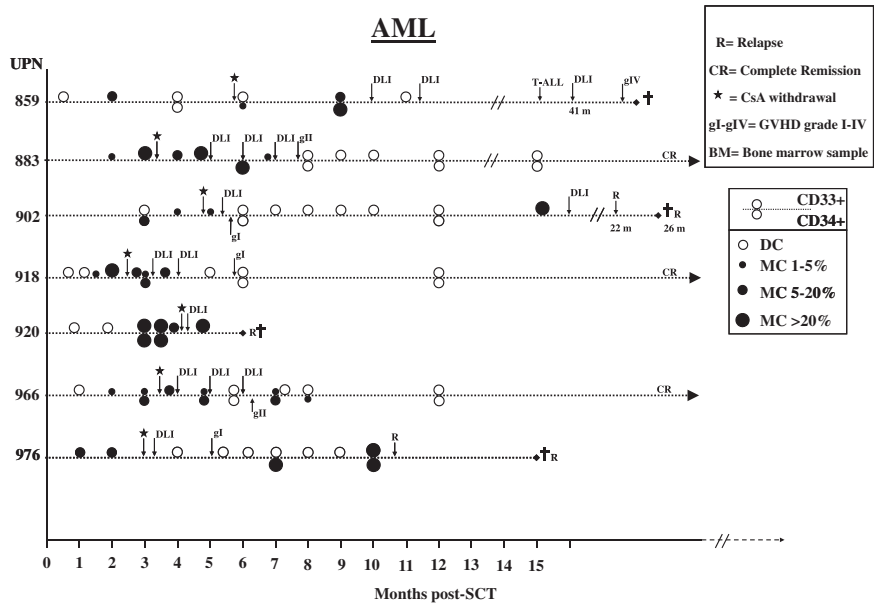

Figure 3. Chimerism analysis of leukemia-affected cell lineage in patients with acute myelogenous leukemia (AML) who received DLI after ASCT.

of 39 patients (46%) who were treated with DLI beyond 1 year ($P = .04$).

In this study, DLI administered as a bulk-dose regimen (BDR) was not associated with a higher incidence of aGVHD grades III-IV compared to escalating-dose regimens (EDR): 8.8% and 8.3%, respectively.

Three patients in this study developed pancytopenia after DLI. Two of them later rejected their grafts, and the third patient died of relapse. All 3 patients showed mixed chimerism of 20% to 30% recipient cells in the myeloid and $CD34^+$ cell lineage before DLI. Ten other patients developed isolated thrombocytopenia, defined as $<10 \times 10^9$/L, 8 of whom had a fast, spontaneous recovery. Because few patients developed cytopenia after DLI, no difference between hematologic relapse and cytogenetic/molecular relapse could be detected.

Time between ASCT and the start of DLI treatment did not significantly influence the risk of developing aGVHD. Of the 28 patients who received DLI before day 100, only 4 (14%) developed aGVHD grades III-IV and 13 (46%) aGVHD grades I-II. In 10 of these 28 patients, immunosuppressive treatment was still given because of fear of aGVHD. For patients with DLI treatment between day 100 and day 365 (n = 51), 15 (29%) developed aGVHD grades I-II and only

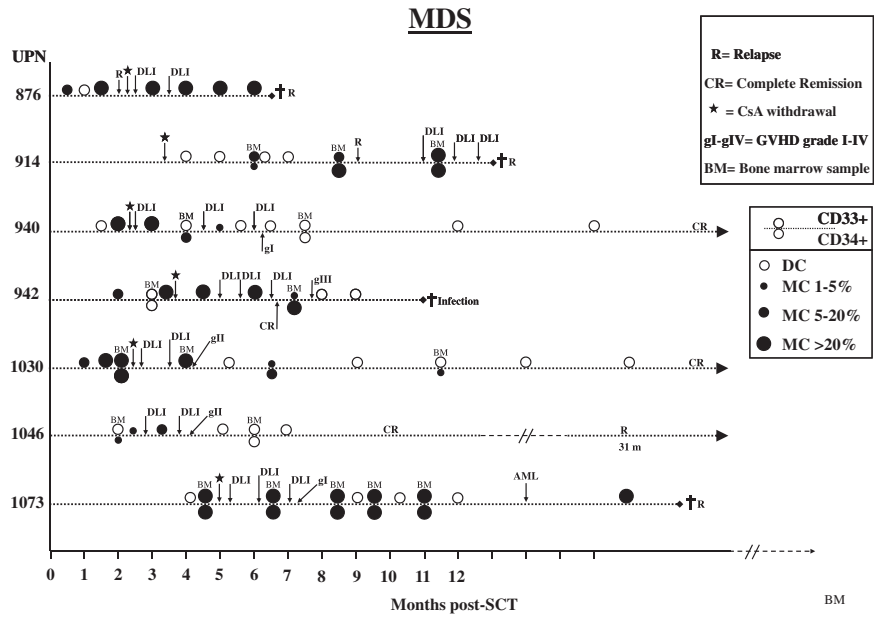

Figure 4. Chimerism analysis of leukemia-affected cell lineage in patients with myelodysplastic syndrome (MDS) who received DLI after ASCT. In UPN 876 and 914, DLI was given after hematologic relapse.

4 (8%) developed severe GVHD. Beyond 1 year (n = 39), 2 (5%) developed severe aGVHD and 12 (31%) developed aGVHD grades I-II.

Of the 118 patients included in this study, 66 died after DLI treatment. The commonest cause of death was relapse of the underlying malignancy (n = 42). Other causes of death were: GVHD (n = 11), septic shock (n = 3), pneumonia (n = 3), other infections (n = 3), and other causes (n = 4).

DISCUSSION

The GVL effect after ASCT is of utmost importance in the treatment of patients with leukemia. Weiden et al. [5] described GVL as early as in 1979, where patients who developed GVHD had relapse rate that was 2.5 times less than for those without it. With the use of DLI, the GVL effect may be increased after ASCT. Pioneering work using DLI after ASCT was done almost 2 decades ago [36]. Increased GVL effect in patients with leukemia may also be accomplished by abrupt withdrawal of immunosuppression after ASCT [37].

It has previously been shown that response to DLI is predicted by tumor burden [27,32,38]. This is in agreement with the findings in the present study, where patients with hematologic relapse did significantly worse than patients with cytogenetic or molecular relapse. According to the multivariate analysis, hematologic relapse was the strongest predictor of poor outcome. Because of the lower efficacy of DLI in patients with hematologic relapse, several studies have combined DLI with chemotherapy [39,40]. In the latter 2 studies, complete remission was achieved in 47% and 63% patients, respectively. OS was 19% and 31% at 2 years. In the present study, however, the combination of chemotherapy and DLI was not successful because none of the 10 patients survived this therapy even though 3 patients responded.

It was previously found that DLI induces long-lasting remission in most patients with CML, but only in a minority of patients with acute leukemia [9]. However, as indicated in the present study, the response may be improved if DLI is started based on molecular monitoring of the underlying disease. Interestingly, patients with acute leukemia and MDS showed a significantly better response and OS if DLI was started because of molecular relapse. This was, in most cases, based on detection of recurrence of recipient cells in the leukemia-affected cell lineage. We have previously shown that this finding is an early sign of relapse in patients with ALL and AML [14,15]. This is further substantiated by the fact that patients with ALL or AML who did not respond to DLI later on developed hematologic relapse. However, it should be recognized that the persistence of recipient cells, especially CD33-positive cells, might be normal myelogenous cells and not necessarily part of the leukemic clone. It should also be acknowledged that patients diagnosed directly in hematologic relapse might have had more aggressive diseases than those diagnosed in molecular relapse. There are several other limitations with this study that may influence the results such as the long time frame (1991-2005), different cells doses, and additional use of IL-2 and MTX. However, of the 26 patients who were treated because of detection of recipient cells in the leukemia-specific cell lineage, 20 patients responded. Interestingly, 3 of 7 with ALL and 6 of 7 with AML responded. This is in agreement with the findings of Bader et al. [16], that early therapy, including rapid withdrawal of immunosuppressive therapy and DLI, increases the response and survival in patients with acute leukemia. The best result in the present study was with DLI given to patients with CML with molecular or cytogenetic relapse, which is in accordance with the results of previous studies [17]. Leukemia lineage-specific chimerism analysis may reach a reasonable sensitivity to detect a threatening relapse and enable DLI at an earlier time point [14,15]. However, PCR methods such as BCR-ABL are preferentially used because a higher sensitivity is achieved and the method is also disease-specific [26]. How leukemia lineage-specific chimerism analysis compare with other methods such as flow cytometry is currently being evaluated at our center.

Another interesting finding is that cGVHD is of significant importance for response in patients with hematologic relapse, whereas it does not seem to be a necessity for patients with molecular relapse. A stronger immunologic response is probably needed to achieve remission in patients with a larger tumor burden. As indicated in previous studies, the time from ASCT to relapse also plays a role in efficacy of DLI because patients with late relapse respond significantly better [38]. However, the response to DLI does not appear to be influenced by whether there is myeloablative or reduced conditioning or whether the donor is related or unrelated according to multivariate analysis. This is also in agreement with a recent study by the EBMT Acute Leukemia Working Party involving 399 patients with AML [38].

According to the findings of this study, it is safe to give DLI early after ASCT, which contrast with the results of previous studies [13]. Few clinical studies have been reported concerning DLI early after ASCT, but several animal studies have shown that DLI is much more likely to cause GVHD if given soon after an intensive preparatory therapy [41]. Twenty-eight patients received DLI before day 100 in this study and only 4 developed aGVHD grades III-IV. Almost half of the patients developed aGVHD grades I-II, but this is desirable among these patients [4]. However, 10 of these patients were still on immunosuppressive

Table 3. Multivariate Analysis Concerning Factors Related to Response of Donor Lymphocyte Infusion (DLI)

Factor	No (n)	Yes (n)	RR	95% CI	*P*-value
Late relapse	58	38	2.06	1.14-3.72	.017
Nonhematologic relapse	44	52	3.36	1.69-6.68	<.001
Chronic GVHD	67	29	1.51	1.13-2.01	.005

GVHD indicates graft-versus-host disease; CI, confidence interval; RR, relative risk.
Patients who received DLI because of rejection or as prophylaxis were excluded from analysis. Concerning diagnosis, acute leukemia and MDS were compared against all other diagnoses. Chronic GVHD is analyzed as a time-dependent variable.

therapy at the time of DLI. The majority of patients responded to DLI despite ongoing immunosuppressive therapy, but with poor response in patients with threatening relapse. Thus, with patients in the latter category, immunosuppressive therapy should be discontinued before DLI is initiated.

In contrast to previous studies [32,38,42], few patients developed severe GVHD and pancytopenia after DLI in the present study. One explanation for this may be that all our patients who received unrelated donor transplants were treated with ATG before ASCT, which is correlated to a reduced risk of developing GVHD [21]. Pancytopenia only occurred in 3 patients and another 10 patients developed thrombocytopenia, which recovered spontaneously in 8 of them. One reason for this is that the majority of patients received DLI treatment based on molecular methods and chimerism analysis. If treatment with DLI is started at hematologic relapse with a high proportion of recipient cells, the risk of pancytopenia may be increased [9].

We can conclude that, in contrast to what has been reported previously, it is safe to administer DLI early after ASCT with regard to induction of GVHD and pancytopenia. It also appears to be beneficial when DLI is given because of molecular relapse of acute leukemias, with increased response rate and survival. These data highlight the necessity that DLI treatment should be based on minimal residual disease and monitoring of chimerism as opposed to morphologic relapse.

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