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REVIEW

Donor lymphocyte infusions: the long and winding road: how should it be traveled?

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Donor lymphocyte infusions (DLI) often are used after allo-SCT to augment the graft-versus-tumor effect. Timing of infusion varies according to indication, for example to treat tumor recurrence, as a planned strategy to prevent disease relapse in the setting of T-cell-depleted grafts or non-myeloablative conditioning regimens, or as a method to convert mixed to full donor chimerism. The optimal strategy of timing, use of cytotoxic conditioning, cell dose and cell product composition, and so on, for DLI administration remains unclear. Despite varied techniques, DLI may lead to 3-year disease-free survivals (DFS) in excess of 60% for all CML patients and approach 90% in patients with only molecular or cytogenetic relapse. Other hematologic malignancies appear much less responsive, as less than 50% of patients respond and provide, at best, 3-year DFS rates of 20–50%. Multiple myeloma patients have overall response rates of 40–45% after DLI, suggesting benefit in relapsed disease, but limited experiences for diseases such as Hodgkin's lymphoma, myelodysplasia and ALL preclude recommendations for use of DLI at this time. Regardless of the indication, treatment-related mortality after DLI is 5–20% and more than one-third of patients will develop acute and/or chronic GVHD after DLI. The risks of these complications appear related, in part, to donor source, cell dose and therapy prior to DLI. Although there are no definitive answers, the information gleaned from published literature suggests that DLI should be administered early after relapse or as a prophylactic strategy in patients receiving T-cell-depleted grafts, and patients with bulky or aggressive disease may benefit from disease reduction prior to DLI.
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Introduction

Relapse, occurring in nearly 40% of all hematologic malignancy patients, remains the most common cause of death after allogeneic hematopoietic cell transplantation (HCT).[1] GVHD contributes greatly to the morbidity of HCT and results in about 15% of deaths. Methods to decrease GVHD, such as *in vitro* and *in vivo* T-cell depletion (TCD), result in increased risks of relapse, as both GVHD and the graft-versus-tumor (GVT) effects are mediated by alloreactive T cells. Management of relapse after allogeneic HCT relies on augmentation of the GVT effect either by reduction or by elimination of immune suppression or administration of donor lymphocyte infusions (DLI).[2]

DLI is used in nearly all malignant diseases for which allogeneic HCT is performed. The published literature, however, is heterogeneous with respect to reporting methods of cell collection (steady state vs mobilization), timing (that is, after chemotherapy, in early relapse, and so on), cell dose infused, and even cell sub-type used. Furthermore, many published studies include multiple diseases and provide little information regarding disease-specific outcomes. Herein, we present a comprehensive review of DLI literature published from 1995 to 2008 and offer guidelines for use of this modality. This review is organized to assess use as therapeutic DLI (tDLI), pre-emptive DLI (pDLI) and DLI in specific diseases. Only studies that were limited to specific disease types were included in the disease-specific sections. Publications that included treatment of varied diseases are discussed in the tDLI and pDLI sections. Newer areas of investigation to augment GVT are discussed separately.

Definitions

Because the literature addressing DLI is heterogeneous, we attempted to standardize definitions across studies. Not all clinical end points were reported in every study. Overall survival (OS) is recorded from the time of DLI. PFS, leukemia-free survival (LFS) and disease-free survival (DFS) are defined as alive without disease or disease progression after DLI. Treatment-related mortality (TRM) is defined as death due to any cause except the underlying

malignancy. Reporting of acute GVHD was limited to grades II–IV and chronic GVHD included both limited and extensive diseases. As cell type and cell dose varies considerably, all values are reported as $\times 10^6$ cells/kg to allow direct comparisons. The majority of studies have reported the T-cell dose defined as $CD3^+$ cells but specific cell types are noted where appropriate. The majority of lymphocytes were collected as steady-state lympho-apheresis, although it is noted when G-CSF-mobilized cells were utilized. Pre-DLI therapies are noted when incorporated as part of therapy.

tDLI vs pDLI

Therapeutic DLI is administered to patients in whom the desired outcome of transplant has not been achieved, that is the treatment of relapse (most situations), and more recently, the correction of incomplete donor chimerism after non-myeloablative or reduced intensity conditioning (RIC). pDLI involves the planned administration of T cells at some interval after allogeneic HCT, often in the setting of donor graft TCD. Infusion of pDLI generally is withheld in the setting of active GVHD.

tDLI

Management of relapse remains the most common indication for DLI. Several reports have been published and summaries of the largest of these are provided in Table 1.[3–19] Almost 40% of the patients reported in the table had CML. Even within studies, there was no standardization of cell dose or pre-DLI treatment. Pre-DLI chemotherapy (including single-agent hydroxyurea or IFNα) was administered to 20,[7]13,[8] 14,[10] 11,[3] 7[19] and 25 patients.[4] Not all patients, however, were assessed for response to chemotherapy prior to DLI. The reported end points also varied, although responses obtained and the development of acute and chronic GVHD were universally reported.

These studies demonstrate improved response for patients with CML compared with other hematologic malignancies. As illustrated in Table 1, a total of 197 patients with CML received DLI and 139 responded (71%) compared with responses in only 73 (24%) of 298 patients with other hematologic malignancies. In the studies reporting OS, patients with CML fared better than those with other malignancies (Table 1). In addition, the development of GVHD, particularly in diseases other than CML, improved response rates and survival.[3,5,7]

GVHD was a frequent complication of DLI with 35% of patients developing acute GVHD and 33% developing chronic GVHD and rates increased with higher T-cell doses infused.[5,8] Raiola *et al.*[5] noted that 23% of patients receiving more than 100×10^6 CD3/kg in a single infusion developed acute GVHD, when compared with only 6% of patients receiving 1 log lower. Similarly, Verdonck *et al.*[8] found that only 1 of 10 patients receiving 10×10^6 CD3/kg developed acute GVHD, when compared with 22 of 28 patients receiving at least 25×10^6 CD3/kg. Although the interval between transplant and DLI had no impact on the development of GVHD, the longer the time from transplant to relapse, the more likely the patient was to respond to DLI.[5]

The percentage of donor chimerism at the time of DLI also impacted upon outcomes. Patients who had more than 50% donor chimerism, including full donor chimeras, demonstrated not only a 4.5-fold improved likelihood of CR but also a 3.4-fold increased risk of GVHD.[4] However, patients who were mixed chimeras at the time of DLI had an increased risk of post-DLI BM aplasia.[3,8]

Few studies examine the role of DLI after RIC transplants.[9,11,19] Marks *et al.*[9] reported on 81 patients (MRD = 71 and URD = 10) transplanted for malignancy (non-Hodgkin's lymphoma, NHL = 29; CML = 12; multiple myeloma, MM = 11; AML = 10; CLL = 9 and other diseases = 10). Patients received DLI for stable or progressive disease ($n = 41$), mixed chimerism ($n = 18$), as part of a preemptive strategy ($n = 10$), or for other indications ($n = 2$). Nine patients received pre-DLI cytoreductive treatment. Patients were eligible for multiple DLI infusions with an escalating dose regimen. Consecutive infusions occurred at a median of 42 and 45 days after the first and second DLI, respectively. The cell dose infused was 5×10^6 CD3/kg for the first infusion, 10×10^6 CD3/kg for the second ($n = 34$) and 50×10^6 CD3/kg for the third infusion ($n = 15$). Similar to the previous studies, a higher median total cell dose infused ($> 10 \times 10^6$ CD3/kg) and development of GVHD improved response. The 2-year OS for the entire cohort was 55% (95% CI: 42–56). A retrospective analysis by Shaw *et al.*[11] reported on the outcomes of DLI in patients who received TCD with Campath as part of RIC conditioning. Patients were not eligible for DLI if they had evidence of GVHD or were still receiving immune suppression. Patients received DLI with a median dose of 5×10^6 CD3/kg for relapse ($n = 20$), residual disease ($n = 15$) and mixed chimerism ($n = 9$). For patients with disease at the time of DLI, 22 responded including 15 patients who obtained a CR. Only five of nine patients with no active malignancy and mixed chimerism became full donor chimeras. An increased risk of GVHD and improved response were seen in patients with active disease who had mixed chimerism and became full donor chimeras after DLI.

On the basis of available data, tDLI, in all its varied forms, clearly provides benefit to patients with CML. These patients have high response rates and more than 70% of patients are alive at 5 years after DLI. This result far outweighs the risks of acute and chronic GVHD. In addition, TRM accounts for approximately 15% of all patient deaths following tDLI. Given the risks of TRM and the morbidity of GVHD, which occurs in approximately one-third of patients after DLI, it is unclear if DLI is warranted in other hematologic malignancies.

pDLI

Owing to increased relapse rates after TCD allogeneic HCT, several studies have examined the utility of pDLI to minimize tumor recurrence.[20–28] The outcomes of the largest of these studies are reported in Table 2.

In the series by Montero *et al.*,[22] the allograft contained a fixed dose of T cells ($0.02–0.1 \times 10^6$ CD3/kg) with planned

Table 1 Studies of therapeutic DLI that included multiple diseases

Study/follow-up after DLI	*n*	*Diseases*	*Donor source (N)*	*Cell dose ($\times 10^6$) cells are CD3+ T cells unless noted (N)*	*aGVHD grade II–IV*	*cGVHD*	*Response*	*OS*	*PFS/LFS*	*TRM*
Collins[7] 194 days	140	CML = 56; AML = 46; ALL = 15; MDS = 6; NHL = 6; MM = 5; Other = 6	MRD (121) MmRD (7) URD (12)	220 ± 120/kg (26) 70 ± 297 MNC/kg (78) 480 ± 344 TNC/kg (29) Unknown (7)	58/125	51/84	CR: 45/140 (CML = 33; AML = 6; ALL = 2; MDS = 2; MM = 2)	NR	NR	At 1 year 14% (8–20)
Raiola[5] 15 months	100	CML = 51; AML = 19; ALL = 10; LPD = 10; MPD = 3; MDS = 7	MRD (87) MmRD (7) URD (6)	BDR: > 50/kg ($n = 14$) ESD: median number of DLI = 4; median total dose = 76/kg ($n = 86$)	21/100	6/100	CR: 47/100 (CML = 35/51; other = 12/49)	At 10 years 36%	At 5 years (for those obtaining CR) 85%	9/100
Huff[4] CML: 857 days Other: 383 days	83	CML = 22; MM = 20; AML = 13; MDS = 12; ALL = 3; HL = 8; NHL = 5	MRD	CML: 10/kg MM, MDS = 50/kg AML/ALL, HL, NHL = 100/kg dose escalation permitted	29/83	27/83	ORR: 37/83 (CML = 14; MM = 8; AML = 6; MDS = 2; ALL = 1; HL = 3; NHL = 3	CML CP/cyto rel at 2 years: 16/17 Others at 1 year: 9/66	NR	22/83
Chiorean[3] 59 months	42	CML = 24; AML = 10; MDS = 6; ALL = 1; JMML = 1	MRD (34) MmRD (1) URD (7)	CML: 100/kg Other diseases: total of x3 lympho-aphereses	20/42	14/42	CR: 25/42 (CML = 18; AML = 6; MDS = 1)	CML at 5 years 75% (58–92) Other at 2 years 17% (0–34)	NR	NR
Micheallet[10] 58 months	30	CML = 14; AML = 9; ALL = 4; NHL = 1; MDS = 1; MM = 1	MRD (27) URD (3)	BDR: 10–264/kg ($n = 10$) ESD: 1–300/kg ($n = 20$)	4/30	3/30	ORR: 18/30 (CML = 14; AML = 2; ALL = 1; NHL = 1)	At 3 years CML: 80% Other: 48%	NR	NR
Schattenberg[16] 47 months	26	CML = 15; AML = 6; ALL = 4; MDS = 1	MRD	70/kg multiple DLI (9) median total dose: 110/kg	8/26	10/19	CR: 12/26 (CML = 11; AML = 1)	NR	NR	NR
Peggs[19] NR	46	MM = 19; HL = 13; NHL = 10; CLL/PLL = 3; CML = 1	MRD (32) URD (14)	ESD: 1/kg (33); 3/kg (23); 10/kg (26); 30/kg (16); 100/kg (10) Given every 3 months	12/45	10/41	ORR: 21/34 CR: 9/34 (MM = 1; HL = 5; NHL = 1; CLL = 1; CML = 1)	NR	NR	5/46
Verdonck[8] NR	28	CML CP = 9; CML AP/BC = 5; MM = 5; AL = 9	MRD (28) URD (2)	1–330/kg multiple DLI (8)	18/28	16/21	ORR: 19/28 (CML = 13; AL = 1; MM = 5)	NR	NR	6/28

Abbreviations: aGVHD = acute GVHD; AL = acute leukemia; AP = accelerated phase; BC = blast crisis; BDR = bulk dosing regimen; cGVHD = chronic GVHD; CP = chronic phase; ESD = escalated dosing regimen; HES = hypereosinophilic syndrome; HL = Hodgkin's lymphoma; JMML = juvenile myelomonocytic leukemia; LPD = lymphoproliferative disorder; MDS = myelodysplasia; MF = myelofibrosis; MM = multiple myeloma; MmRD = mismatched related donor; MNC = mononuclear cells; MPD = myeloproliferative disorder; MRD = matched related donor; NHL = non-Hodgkin's lymphoma; NR = not reported; OS = overall survival; PLL = prolymphocytic leukemia; RIC = reduced intensity conditioning; TNC = total nucleated cell dose; TRM = treatment-related mortality; URD = unrelated donor.

Table 2 Studies of pre-emptive DLI which included multiple diseases

Study/follow-up	*n*	*Diseases*	*Donor source/ conditioning*	*Cell dose (× 10⁶) cells are CD3+ T cells unless noted*	*aGVHD grade II–IV*	*cGVHD*	*Relapse*	*OS*	*PFS/LFS*	*TRM*
Barrett[20] 20.5 months	38	Std risk: CML CP = 13; CML AP = 3; AML = 2 High risk: CML BC = 3; tMDS = 5; AML = 4; MM = 8	Related marrow TCD MA	Group 1($n = 26$): day + 30 = 2/kg; day + 45 = 5/kg Group 2 ($n = 12$): day + 30 = 10/kg	Group 1: 31.5% Group 2: 100%	10/24 (46%)	Std risk: 29 ± 13% High risk: 69 ± 23%	45.5 ± 8.5%	Std risk: 72 ± 9% High risk: 12 ± 10%	16/38 (42%)
Schaap[21] 3 years	82 (35 DLI)	AML = 28; ALL = 10; CML = 27; MDS = 7; MM = 10	Related marrow TCD MA	High dose ($n = 6$): 70/kg Low dose ($n = 25$): 10/kg	High dose: 5/6 patients Low dose: 8/25 patients	NR	DLI: 18% (5–31) No DLI: 44% (28–60)	DLI: 79% (63–95) No DLI: 63% (47–79)	DLI: 77% (63–91) No DLI: 51% (36–66)	2/35 (6%)
Montero[22] 47 months	138 (112 DLI)	Std risk: AML CR1 = 17; ALL CR1 = 5; CML CP1 = 42; MDS RA = 10; other = 3 High risk: AML > CR1 = 21; ALL > CR1 = 16; CML AP/BC = 12; MDS > RA = 8; other = 4	Related marrow TCD MA	10/kg Given between day +45 and day + 100 Single DLI ($n = 70$) Two DLI ($n = 42$)	53/138 (38%)	71/116 (61%)	20 ± 5%	58 ± 5 %	46 ± 5%	20 ± 4%
Ferra[23] 585 days	22 (12 DLI)	CML = 10; AML = 6; ALL = 3, MDS = 1, CLL = 1; HD = 1	Related PBSC CD34 selected MA	Day + 28 = 0.2/kg ($n = 12$) day + 60 = 0.2/kg ($n = 6$) day + 90 = 2/kg ($n = 3$)	28% (7–49)	38% (10–63)	54% (29–79)	65% (43–86)	33% (11–55)	3/22 (14%)
Nakamura[24] 510 days	51 (44 DLI)	CML CP = 13; CML AP = 4; AML = 14; MDS = 11; ALL = 4; MM = 2; CLL = 2; NHL = 1	Related PBSC TCD MA	Day + 45 = 10/kg ($n = 44$) day + 100 = 50/kg ($n = 31$)	33 ± 7%	21/46 (46%)	39 ± 10%	51 ± 10%	47 ± 9%	6 ± 3% (day 100)
Lee[28] 3 years	52 (38 DLI)	DLCL = 15; FL = 22; MCL = 4; Mediastinal NHL = 2; lymphoblastic = 4; Burkitt = 2; HL = 3	Related (33) URD (19) TCD MA	Varied based on patients GVHD risk category[a] 1 infusion$n = 38$ 2 infusions = 17 3 infusions = 5	1 DLI: 14/38 2 DLI: 9/17 3 DLI: 2/5	20/32 (63%)	23 ± 8%	34 ± 7%	34 ± 7%	27/52 (52%)

Abbreviations: aGVHD = GVHD; AP = accelerated phase; cGVHD = chronic GVHD; CP = chronic phase; DLCL = diffuse large-cell lymphoma; FL = follicular lymphoma; HD = Hodgkin's disease; MA = myeloablative conditioning; MCL = mantle cell lymphoma; MM = multiple myeloma; NHL = non-Hodgkin's lymphoma; NR = not reported; OS = overall survival; RA = refractory anemia; Std = standard; TCD = T-cell depleted; tMDS = therapy-related myelodysplastic syndrome; TRM = treatment-related mortality.

[a]GVHD Risk category: low risk = matched-related donor, $\leqslant$35 years; intermediate risk = matched-related donor, 36–50 years; matched URD $\leqslant$50 years; High risk = all other patients.

DLI of 10×10^6 CD3/kg administered between day +45 to day +100 provided patients did not have grade 2 or greater acute GVHD. Seven patients died early, whereas others did not receive DLI because of the development of GVHD ($n=15$), and CML in molecular CR (mCR; $n=4$). The patients reported by Schaap *et al*.[21] also received a fixed T-cell dose at HCT (median 0.7×10^6 CD3/kg; range 0.6–1.5). Patients received planned DLI at a median of 22 weeks (range: 12–40) post-HCT, provided the post-HCT immunosuppression was discontinued for at least 2 months without evidence of active chronic GVHD and no history of acute GVHD above grade 1. Similarly, Nakamura reported on patients receiving a smaller fixed T-cell dose (median 0.083×10^6 CD3/kg), followed by planned non-mobilized cryopreserved DLI of 10×10^6 CD3/kg at day +45 and a second infusion of 50×10^6 CD3/kg at day +100. DLI was withheld if patients were receiving corticosteroids for active acute GVHD. Alternatively, Lee *et al*.[23] and Ferra *et al*.[24] selected DLI dosing based on the risk of GVHD and/or relapse risk. Disease status and outcomes are reported in Table 2. In summary, the risk of relapse ranged from 18 to 69% with TRM occurring in 6–52%. This translated into a DFS that exceeded 40% at 2 years.[21–25]

Sohn *et al*.[26] reported outcomes of 17 patients with refractory or high relapse risk hematologic malignancies (primary refractory AML = 7; refractory ALL = 3; CML blast crisis = 1; refractory NHL = 2; refractory MM = 1; RAEBT = 1 and acute leukemia in CR 4 = 4) undergoing a myeloablative allogeneic HCT who received a planned second infusion of G-CSF-mobilized PBSC, not DLI, from a matched sibling donor with a median of 50×10^6 $CD3^+$ cells/kg between day +40 and day +120 (median 66 days). Ten patients did not receive the subsequent PBSC infusion due to early death ($n=4$), grade 3 or 4 acute GVHD ($n=3$), relapse ($n=2$) or inadequate donor collection ($n=1$). At a median follow-up of almost 20 months, the DFS for the four patients receiving the planned second PBSC infusion was 57%, although all had chronic GVHD requiring immunosuppressive therapy. The outcomes of this study using PBSC are similar to those reported with patients receiving DLI.

Two other studies have analyzed outcomes of pDLI after RIC.[27,28] Barge *et al*.[27] reported on 18 patients, 12 of whom, (AML = 3; CML blast crisis = 1; CLL = 4; NHL = 3 and MM = 1) were given planned DLI at 6 months after RIC and sibling *in vitro* TCD PBSC HCT. The DLI dose, given as unselected mononuclear cells, was based on disease status. Patients with relapse or progression at 6 months received $10–100 \times 10^6$ MNC/kg plus IFN-α compared with only 10×10^6 MNC/kg for patients with stable disease or mixed chimerism. For the 11 patients receiving DLI, 5 responded (CR = 3; PR = 2) and 1 patient had stable disease. Acute GVHD developed in six patients, chronic GVHD in four patients and GVHD accounted for the death of one patient. In the report from de Lima *et al*.,[28] 12 patients with anticipated life expectancy of less than 6 months received fludarabine–melphalan conditioning and sibling PBSC allogeneic HCT for active hematologic malignancies, including AML ($n=4$), myelodysplasia (MDS, $n=1$), ALL ($n=3$), CML ($n=3$) and MM ($n=1$). All patients were scheduled to receive non-mobilized DLI at days +30, +60 and +90. Six patients did not receive any DLI. Of these, four patients achieved a CR, only one of whom was in CR at 14 months after HCT. The other three patients had either died due to TRM or due to relapse.

In the one study that compared outcomes of patients receiving DLI with those patients not receiving DLI, relapse rates were lower resulting in improved LFS.[21] Furthermore, the incidence of acute and chronic GVHD and the risk of TRM do not seem to differ from expected outcomes after conventional transplants without DLI. Consequently, pDLI remains an intriguing approach that may allow tailored DLI for patients with high risks of relapse or lower risks of GVHD.

Myeloid disease

CML

Multiple analyses have reported the outcomes of patients with CML who have received tDLI.[29–38] Although many of the reports include only small numbers of patients, four large series have been reported and are summarized in Table 3.[29,33,36,37] These analyses comprise over 500 patients in whom nearly half had only molecular or cytogenetic relapse at the time of DLI. None of these patients received salvage therapy prior to DLI with the exception of hydroxyurea for the control of hematologic relapse. The cell doses infused varied, with some patients receiving multiple DLI infusions based either on lack of achieving a molecular remission or on planned dose escalation in the absence of GVHD. Despite these variations, an mCR was obtained by 405 of 527 patients (77%). OS at 3+ years ranged from 53 to 95% depending on remission obtained after DLI. Other smaller studies during this time period report similar outcomes regardless of employing novel strategies such as depletion of $CD8^+$ cells,[31,39] use of unselected mononuclear cells at relapse following a TCD BM graft[34] or the addition of IFN-α to the DLI.[35]

CML remains the ideal setting for DLI with the majority of patients achieving an mCR. The experience with DLI in CML has led to a better understanding of the DLI role after allogeneic transplantation. The optimal cell dose, however, remains unclear. The studies reviewed found that responses are dose-dependent, but lower doses can affect an mCR in the setting of using an URD DLI. Similarly, higher doses of cells lead to increased incidence of GVHD, but this risk can be attenuated using escalating dose regimens with multiple DLI infusions. Responses are improved in patients with lower tumor burden (that is, molecular or cytogenetic relapse vs hematologic relapse) and in patients experiencing a longer remission after transplant. The role of tyrosine kinase inhibitors after DLI is unknown.

AML and MDS

DLI is significantly less effective in managing post-transplant relapse of AML and MDS than for CML. Only a single analysis has focused on DLI for relapsed AML.[40] This large retrospective analysis from the European Blood and Marrow Transplant Group (EBMT) compared OS in

Table 3 Therapeutic DLI for management of CML relapse after alloHCT

Study/follow-up	*N*	*Donor source*	*Cell dose ($\times 10^6$) cells are CD3+ T-cells unless noted*	*aGVHD grade II-IV*	*cGVHD*	*Response*	*OS*	*TRM*
Simula[29] 77 months	81	Related (31) URD (50)	ESD repeated q20 weeks if no molecular CR Sibs 10/kg start URD 1/kg start	12/81 (15%)	12/81 (15%)	MCR 71/88 (88%)	Median follow-up of 77 months 92%	NR
Dazzi[33] 29 months (5–89)	66	Related (35) URD (31)	BD: 150/kg (40—530) ESD (Sib): 10/kg; 50/kg; 100/kg ESD (URD): 1/kg; 10/kg; 50/kg; 100/kg	BD: 43% ESD: 10%	BD: 41% ESC: 11%	MCR 44/66 (67%)	At 3 years If mCR: 95% If no mCR: 53%	NR
Guglielmi[36] 48 months	298	Related (238) URD (60)	Group A: 50 MNC/kg Group B: 150 MNC/kg Group C: 380 MNC/kg	Group A: 16% Group B: 38% Group C: 49%	Group A: 21% Group B: 30% Group C: 20%	M/c CR Group A: 76/98 (78%) Group B: 78/107 (73%) Group C: 65/93 (70%)	At 3 years Group A: 84% Group B: 63% Group C: 58%	At 3 years Group A: 5% Group B: 20% Group C: 22%
Fozza[37] NR	82	Related (31) URD (51)	ESD (Sib): 10/kg; 50/kg; 100/kg ESD (URD): 1/kg; 10/kg; 50/kg; 100/kg	12/82 (15%)	12/79 (15%)	mCR 71/82 (87%)	NR	NR

Abbreviations: aGVHD = acute GVHD; cGVHD = chronic GVHD; OS = overall survival; TRM = treatment-related mortality URD = unrelated donor; ESD = escalated dosing; BD = bulk dosing; mCR = molecular CR; M/c CR = molecular/cytogenetic CR; NR = not reported.

399 AML patients who did ($n=171$) or did not ($n=228$) receive DLI for the management of post-transplant relapse. Prior to DLI, 124 (73%) patients received chemotherapy, although 84 still had active AML at the time of DLI. Patients receiving DLI had an improved 2-year OS compared with those not treated with DLI (DLI: $21\pm3\%$ vs no DLI: $9\pm2\%$; $P<0.001$). For the DLI cohort, multivariate analysis demonstrated that patients with low tumor burden (<35% blasts), remission at the time of DLI and favorable cytogenetics had improved survival.

In contrast, the MDS literature provides only 30 patients from two analyses.[41,42] Campregher *et al*.[41] described 16 patients who received DLI for relapsed MDS post transplant between 1993 and 2004. Prior to DLI, three patients were treated with chemotherapy and four patients received IFN-α. Patients received either G-CSF-mobilized DLI ($n=6$) or non-mobilized leukaphereses products ($n=10$) with a median cell dose infused of 100×10^6 $CD3^+$ cells/kg (range: 10–150). Twelve patients received multiple DLI. Two patients were in CR prior to DLI, and of the remaining 14, 3 achieved a CR. Underlying MDS was the cause of death in 14 patients, and 2 of the patients with CR ultimately died due to pulmonary complications just over 5 years from DLI. A French multicenter retrospective analysis of 14 patients found an estimated median OS of 17 months with 8 patients dying due to progressive disease.[42] Only two patients achieved a CR after DLI. Acute GVHD developed in half the patients—including the two patients who obtained CR—with the majority having grade II disease. Again, both cytotoxic agent treatment prior to DLI and the cell dose given varied.

The small sample sizes, inconsistent pre-DLI therapy and the varied DLI cell doses limit recommendations for the effectiveness of DLI for patients with AML and MDS. It appears, however, that some patients attain durable remissions after post-transplant relapse. Although it remains unclear if there is a survival advantage for those patients who respond to pre-DLI chemotherapy, the CML experience demonstrates improved responses in patients with only molecular relapse, suggests a benefit for disease reduction prior to DLI.

Other myeloproliferative diseases

Few data are reported using DLI therapy for either Juvenile Myelomonocytic Leukemia (JMML) ($n=23$) or idiopathic myelofibrosis (MF) ($n=1$).[43–46] The European Working Group of MDS in Childhood reported on 21 patients with JMML who received DLI for either mixed chimerism ($n=7$) or relapse ($n=14$) after allogeneic HCT.[43] No patient received chemotherapy prior to DLI and again cell doses infused varied widely (range: 0.01–240×10^6 CD3/kg). Sixteen patients received multiple (median 3) lymphocyte infusions. Only 6 of 21 patients achieved a CR; 4 responders developed acute GVHD and 2 responders developed chronic GVHD. On univariate analysis, factors associated with response to DLI included abnormal karyotype at diagnosis, administration of a total of $>10\times10^6$ $CD3^+$ cells/kg and the development of acute GVHD after DLI.

The lone MF patient was treated with G-CSF-mobilized PBSC for the management of a post-HCT relapse.[46] No chemotherapy was given prior to stem cell infusion. The patient received 297×10^6 $CD3^+$ cells/kg as a single infusion, developed chronic GVHD and was alive in CR at 20 months after DLI.

The literature addressing myeloproliferative diseases other than CML is small. Although there appears to be some benefit in this group, the small sample size and heterogeneity make it difficult to determine an optimal approach.

Lymphoid diseases

ALL

The GVT effect is thought to be less robust in ALL. Three reports describe seven patients who were treated after receiving chemotherapy in combination with DLI and six patients responded.[47–49] Three of these responders, however, subsequently relapsed. No definitive recommendations can be made regarding DLI for post-transplant relapse of ALL but it remains a viable area of investigation.

Hodgkin's lymphoma

Similar to ALL, there are few reports of DLI for relapsed Hodgkin's lymphoma (HL).[50,51] Six of the 10 patients treated attained either stable disease ($n=2$) or CR undetermined ($n=4$). Four of 10 patients received chemotherapy prior to DLI and G-CSF-mobilized peripheral blood progenitors were given to 4 patients. Some patients received multiple DLI, and the impact of DLI for relapsed HL remains unclear.

NHL

Although slightly larger numbers of NHL patients given DLI have been published, there are too few patients in any single lymphoma histology sub-category to give adequate information for a specific lymphoma subtype.[52–55] Two larger series limited to NHL have been published.[52,55] Russell *et al*.[52] reported 17 patients who received DLI after allogeneic HCT (related donor = 15; URD = 2) between 1996 and 2005 for either disease relapse ($n=10$) or refractory disease ($n=7$). Patients had CLL ($n=4$), mantle cell lymphoma (MCL; $n=4$), follicular lymphoma (FL; $n=3$), diffuse large cell lymphoma (DLCL; $n=4$) or Richter's transformation ($n=1$). Fifteen patients had received a Campath-containing reduced intensity allogeneic HCT. Nine patients (MCL, DLCL and Richter's) received chemotherapy prior to DLI, although only four showed some evidence of disease response. The intent was to use an escalated DLI dose regimen, although only eight patients received more than one DLI infusion. The starting DLI cell dose was 20×10^6 $CD3^+$/kg for related donors and about 1 log lower for patients receiving an URD DLI product. In total, 11 of 17 patients achieved a CR (FL = 3, CLL = 3 and MCL = 5), leading to a 3-year PFS and OS of 52 and 58%, respectively. Only 1 patient died due to treatment-related causes, although 7 of 16 evaluable patients developed acute GVHD grades II–IV.

Bloor *et al*.[55] recently reported their 28 patient series (related donor = 24; URD = 4), 26 of whom had received a RIC regimen. Again, lymphoid histologies varied (CLL = 6; MCL = 3; FL = 14; and transformed FL = 5). DLI was given to 17 patients for progressive disease (PD), whereas the remaining 11 were treated solely for mixed chimerism. For the patients with PD, seven patients received pre-DLI treatment with either rituximab ($n=5$) or chemotherapy ($n=2$) with the addition of radiation in two patients. The intent was for patients to receive escalated DLI doses every 3 months with the starting dose of 1×10^6 $CD3^+$/kg and subsequent doses of 3×10^6, 10×10^6, 30×10^6 and 100×10^6/kg. Patients received a median of two (range: 1–5) DLI infusions. A CR was obtained in 13 of 17 PD patients with a median time to response of 12 months. This effect translated into an estimated 5-year PFS and OS of 76% (55–96) and 88% (72–100). For the patients with mixed chimerism, 92% achieved full donor chimerism by 6.7 months. The incidence of GVHD was low (acute: 15% (6–36); chronic: 31% (17–56)), although three patients died due to infection.

For NHL patients, it appears that the GVT effect works best in the setting of the indolent lymphomas. Unanswered questions include determining the need for pre-DLI therapy, benefit of DLI for patients with progressive and/or bulky disease, cell dose and the impact of GVHD on response.

MM

Several published series relate the data using DLI for the management of MM patients who have relapsed after allogeneic HCT (Table 4).[56–61] Observational data suggest that there is a definite GVT effect in MM as the development of GVHD correlates with response in three different analyses.[56–58] Lokhorst[56] reported 54 patients receiving a partially T-cell-depleted ($n=50$) or unmanipulated ($n=4$) related marrow graft after myeloablative conditioning. Forty (74%) patients received pre-DLI chemotherapy and 18 patients achieved a PR. The DLI cell dose infused varied and nine patients received multiple DLI. For patients who responded, 80% developed acute GVHD and 73% developed chronic GVHD. Multivariate analysis found that a T-cell dose of more than 100×10^6 CD3/kg and CR at the time of allogeneic HCT predicted response to DLI. A multicenter analysis of 25 patients similarly found that the development of GVHD was associated with response to DLI.[58] A European analysis of 63 patients who had relapsed ($n=48$) or had PD ($n=15$) after a non-myeloablative allogeneic HCT again demonstrated that response correlated with the development of GVHD.[57] In all of these analyses, the TRM was fairly low with only 13 of 142 (9%) patients reportedly dying due to complications of DLI.

Two studies evaluated pDLI to prevent MM relapse after allogeneic HCT have been reported.[60,61] Alyea *et al*.[61] reported on 24 patients receiving a myeloablative CD6-depleted, related BM transplant with planned CD4-selected DLI to be given at 6–9 months post transplant for patients of all immune-suppressive therapy. In all, 14 patients—11 with residual MM—received DLI at a cell dose of 30×10^6

Table 4 DLI for management of MM after allo-HCT

Study/ Follow-up	*n*	*Donor source*	*Cell dose ($x10^6$) cells are CD3+ T cells unless noted*	*aGVHD grade II–IV*	*cGVHD*	*Response*	*OS*	*PFS*	*TRM*
Lokhorst[56] NR	54	Related	1/kg (1) 5–10/kg (7) 10–50/kg (15) 50–100/kg (16) >100/kg (13)	31/54	25/54	28/54 CR = 9 PR = 19	Median 23 months (2–118)	Median: 19 months (3–116)	3/54
Salama[58] 56 weeks	25	Related (24) URD (1)	Varied	12/25	11/21	5/25 (3/3 with chemotherapy before DLI)	NR At 56 weeks: 12/25 alive with 2 in CR	NR	3/25
Van de Donk[57] 14 months	63[a]	Related (46) URD (17)	1–300/kg	14/63	27/63	24/63 CR = 12 PR = 12	Responders: not reached Non-responders: 23.6 months (1–51)	Responders 27.8 months (1–46) Non-responders NR	7/63

Abbreviations: aGVHD = acute GVHD; cGVHD = chronic GVHD; ESD = escalated dosing; MC = mixed chimerism; MM = multiple myeloma; NR = not reported; OS = overall survival; PD = progressive disease; TRM = treatment–related mortality; URD = unrelated donor.
[a]All patients received a non-myeloablative allo-HCT.

($n = 11$) or 10×10^6 CD4/kg ($n = 3$). After DLI, six (55%) patients obtained a CR and an additional four (36%) achieved a PR at a median time to best response of 6.4 months. Peggs *et al*.[60] reported on 20 patients who received a non-myeloablative *in vivo* T-cell-depleted allogeneic HCT (related = 12 and URD = 8). DLI at 6 months and then every 3 months as an escalated dose was planned if patients demonstrated residual disease or mixed chimerism without active GVHD. The 2-year PFS was low at 25%, although all patients with GVHD (5/5) responded compared with only two of nine patients without GVHD after DLI.

The above data suggest that there is an effective GVT component against MM. The published data thus far, however, do not provide sufficient information to indicate the need for pre-DLI chemotherapy, benefit of pDLI vs tDLI, or the appropriate cell dose to be administered.

Novel strategies

Several unique methods explore the dual goals of improving the response to DLI without increasing TRM.[6,15,62–64] One strategy is to infuse a selected cell product. Alyea *et al*.[6] prospectively evaluated the role of $CD8^+$-depleted DLI for the management of relapsed disease (CML = 25; AML/MDS = 5; ALL = 1; NHL = 1; CLL = 1; MM = 7) after allogeneic-related donor BM transplant (TCD = 39 and non-TCD = 1). Patients were taken off immune suppression or chemotherapy for a minimum of 1 week prior to DLI. The infused $CD4^+$ cell dose differed based on the study cohorts ($150 \times 10^6 = 7$; $100 \times 10^6 = 5$; and $30 \times 10^6 = 28$). Acute GVHD developed in 32% (90% CI: 19–46) and all 12 patients with GVHD responded. Nine patients without GVHD, however, also responded. The response rate varied with better responses for patients with CML (79% (90% CI: 58–92)) and MM (83% (42–99)), when compared with other diseases (14% (1–52)).

Another interesting approach is to combine chemotherapy with a cellular infusion. One study reported using chemotherapy plus mobilized PBSC rather than DLI in patients with relapsed disease (AML = 6; CML = 3; MDS = 1) after allogeneic HCT (related = 3; URD = 7).[15] The chemotherapy regimens varied, but all patients received GM-CSF after infusion. The median cell doses infused were 6×10^6 $CD34^+$/kg (3.5–13.6) and 260×10^6 $CD3^+$ cells/kg (180–370). Eight patients achieved a CR, whereas all 10 patients developed acute GVHD and 7 patients died due to treatment-related causes. Miller *et al*.[62] administered fludarabine and CY for lymphocyte depletion effect prior to DLI to promote *in vivo* T-cell expansion in 15 patients (AML = 6; ALL = 1; MDS = 2; NHL = 1; CLL = 2; MM = 1; JMML = 1; and MF = 1) and compared the results with patients receiving DLI without preceding chemotherapy (CML = 28 and non-CML = 35). Patients with preceding chemotherapy and all CML patients received 100×10^6 CD3/kg, but higher doses were given to the non-CML patients who did not receive chemotherapy. Patients receiving chemotherapy had a significantly higher incidence of acute GVHD (60 vs 24%; $P = 0.01$). OS, however, did not differ between the chemotherapy group and the non-CML non-chemotherapy group. The increased TRM due to GVHD in the chemotherapy group appeared to offset the GVT benefit, whereas PD reduced survival rates for the other patients.

Another novel approach is the use of *ex vivo*-activated DLI as described by Porter *et al*.[63] All patients had relapsed disease (ALL = 7; AML = 4; CML blast crisis = 1; CLL = 1; NHL = 3; HL = 1; and MM = 1) after allogeneic HCT and 14 patients received chemotherapy before DLI. Patients received 'standard' DLI with median cell dose of 150×10^6 MNC/kg (range 90–350); 12 days later activated donor T cells, co-stimulated with anti-CD3- and anti-CD28-coated beads, were given. The activated cell doses were escalated from $1–100 \times 10^6$ CD3/kg. This strategy led to a CR in eight patients and an estimated 2-year OS of 51%. Acute GVHD grades II–IV and chronic GVHD each occurred in four patients but no patients died due to complications of the DLI procedure.

Finally, the group from Italy has explored using genetically modified DLI.[64] Lymphocytes engineered to express the suicide gene thymidine kinase of herpes simplex

virus were infused into 23 hematologic malignancy patients (CML = 4; AML = 9; ALL = 2; NHL = 4; HL = 2; and MM = 2) who relapsed after allogeneic HCT. Six patients were not evaluable due to death from PD within 4 weeks of infusion. Eleven patients demonstrated disease response (CR = 6 and PR = 5), with three patients alive in CR at a median of 471 days. Only 12 patients were considered evaluable for GVHD, of which 3 developed acute or chronic GVHD treated successfully with ganciclovir.

Small numbers of patients have been treated with each of these novel strategies. These methods, however, appear promising and deserve further exploration to improve the efficacy of DLI.

Conclusions

DLI clearly remains a viable option for patients who relapse after allogeneic HCT. Despite a thorough review of published literature, several questions remain unanswered, including the optimal timing and cell dose infused, the type of cells infused, use of a mobilized vs steady-state lymphopheresis, the need for pre-DLI chemotherapy and the appropriate indications for DLI. Diseases of more indolent nature (that is, CML, MM and some types of NHL) appear to respond to the GVT effect provided by DLI. On the other hand, slow-growing diseases such as HL do not clearly respond. The rapidly proliferating diseases such as acute leukemia likely will benefit only if cytoreductive therapy is added and novel strategies to reduce TRM are employed.

To answer these questions, collaborative multicenter prospective trials are needed. Until such time as these trials are completed, however, clinicians must rely on the available data. Several recommendations regarding timing can be made based on the literature summarized above. First, patients should be considered for DLI early after relapse, such as in the setting of molecular or cytogenetic relapse of CML. This approach also may be effective in the setting of MM patients demonstrating early recurrence of the M-protein or possibly in lymphomas with early positron emission tomography scan recurrence. Secondly, patients receiving TCD grafts appear to benefit from a pDLI strategy in the absence of GVHD. Third, patients with bulky or rapidly progressive disease may benefit from cytoreductive therapy prior to DLI. Finally, it appears that results are similar using either escalated dose or bulk dose infusions with the limitations on cell dose actually being the total dose infused. There is an increase in GVHD with doses more than 100×10^6 CD3/kg, although GVHD can be seen at lower doses. Doses of 1 log lower should be used if patients are receiving cells from an URD.

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