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ORIGINAL ARTICLE

Donor-lymphocyte infusion following haploidentical hematopoietic cell transplantation with peripheral blood stem cell grafts and PTCy

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Donor-lymphocyte infusion (DLI) for relapse following haploidentical hematopoietic cell transplantation (haploHCT) with post-transplant cyclophosphamide (PTCy) has been described in recipients of bone marrow grafts, but not recipients of G-CSF mobilized peripheral blood (PB) grafts. We retrospectively identified patients who underwent DLI following PB-haploHCT with PTCy for relapse, or loss of chimerism (LOC). Twelve patients (57%) received DLI for hematologic relapse/persistent disease, seven (33%) for extramedullary relapse and two (10%) for LOC. Sixteen (76%) received chemotherapy prior to DLI, which did not correlate with response. The most common first dose was 1×10^6 $CD3^+$ cells/kg. Two patients developed grade I aGvHD post DLI, one had grade II and two had grade III. One developed mild skin cGvHD 1361 days post DLI. Pre-DLI aGvHD predicted post-DLI aGvHD ($P = 0.025$). Six patients achieved CR after DLI for overt relapse, one achieved full donor chimerism after LOC. Patients with LOC or EM relapse had superior relapse-free survival following DLI ($P = 0.029$). DLI following PB-haploHCT with PTCy is a viable salvage therapy for overt relapse or LOC without a substantial increase in GvHD, and donor lymphocytes may be collected simultaneously with graft collection to facilitate availability in patients at high risk of relapse.

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INTRODUCTION

Donor-lymphocyte infusion (DLI) is an established immunotherapeutic option for disease relapse and loss of chimerism (LOC) following allogeneic hematopoietic cell transplantation (alloHCT).[1,2] Among HLA-matched related and unrelated alloHCT, DLI has been modestly effective at achieving CR in patients who would otherwise likely succumb to their disease.[3,4] In combination with cessation of immunosuppression, DLI is often associated with a significant incidence of GvHD.[3,5–7]

HLA-haploidentical hematopoietic cell transplantation (haploHCT) with post-transplantation cyclophosphamide (PTCy)-mediated ablation of alloreactive T cells yields comparable outcomes to HLA-matched alloHCT, regardless of whether the graft source is from bone marrow (BM) or G-CSF-stimulated PBSC.[8–10] Similarly, disease relapse remains a significant challenge among recipients of haploHCT with PTCy.[11–15]

PBSC grafts are preferred by many centers because of the ease by which they are obtained and the higher yield of $CD34^+$ cells which, in HLA-identical transplantation, has resulted in more rapid engraftment.[16] There is often a surplus of donor cells when collected from peripheral blood. While the higher number of $CD3^+$ T cells contained within PBSC grafts has been associated with a higher incidence of GvHD in HLA-identical alloHCT, PBSC grafts have not been associated with either more rapid engraftment or higher incidence of GvHD in PB-haploHCT with PTCy.[9,10,17,18] The surplus of donor lymphocytes from PB-derived grafts can be easily cryopreserved and reconstituted for the purpose of DLI if necessary, foregoing the process of obtaining donor lymphocytes via apheresis, which is necessary following BM donation.

There is limited experience with DLI following relapse in haploHCT. Huang *et al.*[19] reported a 55% incidence of overall acute GvHD (30% grades III–IV) following DLI for relapse after unmanipulated T-cell replete haploHCT without PTCy. The grafts had consisted of G-CSF mobilized BM cells plus PBSC, and they had used fresh or cryopreserved G-CSF mobilized PB lymphocytes for DLI. Among a larger cohort with the same transplant characteristics, the group demonstrated that DLI led to a 58% incidence of aGvHD (28% grades III–IV), with higher grades resulting in significant non-relapse mortality (NRM).[20]

Zeidan *et al.*[21] studied the effect of DLI, derived from apheresis of fresh, unprimed donor lymphocytes, in 40 patients who relapsed following haploHCT with PTCy and BM-derived grafts (haploBMT). They demonstrated a 25% incidence of aGvHD, and higher rates of CR among those treated with chemotherapy prior to DLI. A subsequent study by Ghiso *et al.*[22] of unprimed, apheresis-derived DLI for relapse in 42 recipients of haploBMT with PTCy demonstrated a 33% incidence of aGvHD (14% grades II–III, no grade IV), and higher CR among those with only molecular relapse compared to hematologic relapse (45% vs 33%). Remission and GvHD outcomes among recipients of haploBMT with PTCy were comparable to those who received DLI following HLA-matched transplants.

Although these studies describe DLI following haploBMT with PTCy and haploHCT with combined PBSC/BM grafts without PTCy, there are no data pertaining to DLI following haploHCT

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with PTCy and PBSC grafts (PB-haploHCT). Here we conducted a retrospective analysis of all patients at our institution who received DLI for disease relapse or LOC following PB-haploHCT with PTCy.

METHODS AND MATERIALS

Study population

The Institutional Review Board approved the study. We retrospectively identified all patients who received DLI following T-cell replete PB-haploHCT with PTCy at the Washington University School of Medicine between 1 August 2010 and 30 August 2016. Patients received DLI for either hematologic relapse (⩾5% blasts in BM smear or any peripheral blasts), extramedullary (EM) relapse, or relapse prophylaxis upon detection of progressive LOC (evaluated by short tandem repeat analysis of peripheral $CD3^+$ or unfractionated BM cells) in those who had achieved CR with full-donor chimerism.

DLI

Infusate was obtained via cryopreserved specimens, which had been collected at the same time as the initial haploidentical PBSC grafts. Dose calculation was based on $CD3^+$ cells/kg of the recipient's body weight. Initial dosing of DLI for PB-haploHCT recipients factored the theoretical increased risk of GvHD, and was started at one to two orders of magnitude lower than conventional DLI in matched transplant recipients.[21] This followed similar logic to the dosing strategies in studies conducted on DLI in haploBMT recipients, which were published midway through our study period, to which subsequent DLI administrations were adherent.[21,22] Dose escalation was only conducted in patients who tolerated initial dosing without development of GvHD. Patients receiving DLI were off immunosuppression, and no patient had evidence of active GvHD at the time of administration.

Response and GvHD monitoring

All surviving patients underwent BM biopsies between 30 and 60 days following DLI to assess clinical response, or earlier if clinically warranted. Patients who had achieved CR following DLI had $<5\%$ blasts by BM biopsy. Those who had EM relapse prior to DLI were required to be without evidence of disease by exam, imaging and BM biopsy at the time of post-DLI assessment in order to have achieved CR. Acute GvHD was graded using the Keystone criteria and cGvHD was graded according to the NIH consensus criteria.[23,24]

Statistical analysis

All statistical analyses were performed using statistical package SAS 9.4 (Cary, NC, USA). Occurrence of GvHD and clinical remission were treated as dichotomous variables and the association with patient characteristics was assessed using Fisher's exact test. Overall survival (OS) and relapse-free survival (RFS) were calculated from the date of first DLI administration using the Kaplan–Meier method. The distribution of time-to-death across responders and non-responders was also summarized using the Kaplan–Meier estimator where the response status was treated as a time-dependent covariate.[25]

RESULTS

Baseline characteristics

Twenty-one patients received DLI for relapse or LOC following PB-haploHCT with PTCy. Demographic and baseline characteristics are presented in Table 1.

Fourteen (67%) of the patients had a diagnosis of AML. Twelve (57%) patients received DLI for hematologic relapse/disease persistence, seven (33%) for EM relapse and two (10%) for progressive LOC. Mean time to relapse from PB-haploHCT was 233 days (s.d.: 154 days).

Nineteen (90%) were initially on tacrolimus and mycophenolate mofetil for GvHD prophylaxis (the other two were on sirolimus and mycophenolate mofetil). Prior to relapse, seven (33%) patients had acute GvHD (three grade I, three grade II and one grade III), and six (29%) had mild or moderate chronic GvHD (cGvHD), with no severe cGvHD. All GvHD had resolved prior to administration of initial DLI.

Table 1. Baseline characteristics of patients who received DLI following haploHCT with PBSC grafts and PTCy

Characteristics	*Number* (N = *21*)
Gender	
M	13
F	8
Age at haploDLI	
⩽50	13
>50	8
Disease	
AML	14
Non-Hodgkin's lymphoma	1
Myelodysplastic syndrome	2
ALL	2
CML	2
Conditioning	
Myeloablative	8
Nonmyeloablative	13
Type of relapse	
Hematologic	12
Loss of chimerism	2
Extramedullary	7
Time from haploHCT to relapse (month)	
⩽3	4
>3–6	7
>6–12	6
>12–24	4
aGvHD pre-DLI	
Grades I–II	6
Grades III–IV	1
cGvHD pre-DLI	
Mild/moderate	6
Severe	0

DLI characteristics

During the follow-up period, 21 patients received 28 doses of DLI. Mean time to DLI from relapse was 33 days (s.d.: 26 days), and from initial haploHCT was 266 days (s.d.: 154 days). Mean follow-up post DLI for all recipients was 233 days (s.d.: 332 days), and for those still alive at follow-up was 491 days (s.d.: 424 days). Thirteen patients (61.9%) received an initial DLI dose greater than or equal to 1×10^6 $CD3^+$ cells/kg. The most common initial dose was 1×10^6 $CD3^+$ cells/kg, which was the first dose given to 10 (48%) patients. Eight patients (38%) received an initial dose less than 1×10^6 $CD3^+$ cells/kg. The majority of patients (18 patients, 86%) received only one dose of DLI. Three patients underwent dose escalation with subsequent rounds given at an average interval of 42 days (s.d.: 17 days). One patient received two rounds (1×10^6 and 3×10^6 $CD3^+$ cells/kg), one received three rounds (1×10^5, 3×10^5 and 1×10^6 $CD3^+$ cells/kg) and one received five rounds (1×10^6, 3×10^6, 5×10^6, 1×10^7 and 3×10^7 $CD3^+$ cells/kg). Sixteen patients (76%) received cytotoxic chemotherapy prior to DLI, with FLAG-Ida (fludarabine, high-dose cytosine arabinoside, G-CSF, idarubicin) given most frequently.

Table 2. Patient characteristics of responders

Patient no.	*Sex*	*Disease*	*DRI*	*HCT to relapse (day)*	*Type of relapse*	*Age at first DLI (year)*	*Relapse to first DLI (day)*	*Date of first DLI*	*Dose of first DLI ($CD3^+$/kg)*	*Total number of DLI doses*	*aGvHD Post DLI (Grade)*	*cGvHD post DLI*	*Duration of response from date of response documentation (month)*	*Survival from date of first DLI (month) and vital status at end of follow-up*
1	M	AML	High	198	LOC	73	18	4 June 13	1×10^5	1	0	Mild	44	46 (A)
2	F	NHL	High	82	EM	43	57	7 May 14	3×10^6	1	0	None	1.0	3.3 (D)
3	M	MDS	Intermediate	496	Morph	70	17	16 July1 5	2×10^6	1	0	None	0.1	0.8 (D)
4	M	AML	Low	511	EM	50	22	4 May 16	1×10^6	2	0	None	6.5	11 (A)
5	M	T-ALL	High	280	EM	27	37	11 November 15	1×10^6	1	0	None	16	17 (A)
6	F	AML	Intermediate	125	EM	43	26	14-March 16	1×10^5	1	0	None	3.0	13 (A)
7	M	AML	Low	138	Morph	19	22	26 April 16	1×10^6	1	3	None	8.8	9.3 (A)

Abbreviations: EM = extramedullary; LOC = loss of chimerism; MDS = myelodysplastic syndrome; morph = overt hematologic relapse; NHL = non-Hodgkin lymphoma.

GvHD

Overall, five (24%) of the 21 patients developed aGvHD after DLI. In these five patients, median time to DLI from initial haploHCT and from relapse, respectively, were 224 days (s.d.: 77 days) and 26 days (s.d.: 20 days). In comparison, mean time to DLI from initial haploHCT and from relapse in the 16 patients who did not developed aGvHD after DLI were 204 days (s.d.: 171 days) and 23 days (s.d.: 28 days), respectively. Two (10%) patients developed grade I aGvHD, one (5%) developed grade II and two (10%) had grade III. One patient developed mild, biopsy-proven skin cGvHD 1361 days after DLI. No patients died as a consequence of GvHD and none of the patients receiving multiple doses developed GvHD. Of the eight patients older than 50 years at first DLI, one developed aGvHD and one developed cGvHD.

Patients who previously had aGvHD prior to DLI were significantly more likely to develop aGvHD following DLI ($P = 0.025$). However, DLI dose, treated as a dichotomous variable ($< 1\times10^6$ $CD3^+$ cells/kg or $\geqslant 1\times10^6$ $CD3^+$ cells/kg) and ordinal variable, was not associated with a significant difference in aGvHD ($P = 0.61$). Pretreatment with chemotherapy ($P = 0.55$), type of relapse ($P = 0.77$) and prior cGvHD ($P = 0.11$) were also not significantly associated with aGvHD.

Survival and response

DLI was successful in seven (33%) patients, six who achieved CR after overt relapse, one who achieved full donor chimerism after LOC (Table 2).

Three patients died of sepsis at 11, 12 and 24 days after DLI, and one patient went home on hospice within 24 h of DLI. These patients had no peripheral blasts but did not survive to response assessment by BM biopsy. Three patients had persistent disease with elevated circulating blasts and did not undergo BM biopsy. Seven patients had persistent disease on BM biopsy after DLI. Mean follow-up for responders was 374 days (s.d.: 478 days). None of the DLI, patient or disease characteristics analyzed were statistically associated with CR. The majority (five out of nine, 56%) of those who received a higher initial dose ($\geqslant 1\times10^6$ $CD3^+$ cells/kg) and survived to assessment achieved CR, whereas only two of eight patients (25%) receiving a lower initial dose ($< 1\times10^6$ $CD3^+$ cells/kg) achieved CR, however this did not reach statistical significance ($P = 0.20$). Pretreatment with chemotherapy, type of relapse, time to relapse from transplant, time from relapse to DLI, time off immunosuppression and prior GvHD were not predictive of response. Pre-DLI chimerism $>50\%$ donor was also not associated with CR after DLI.

Of the eight patients over age 50 at first DLI, three were alive at the end of the follow-up period at a mean survival of 803 days (s.d.: 581 days), however only one had a complete response to DLI. Mean time-to-death for those five patients over 50 who did not survive to follow-up was 22 days (s.d.: 19 days), with only one who had complete response to DLI.

One responder developed grade III aGvHD, and another developed mild cGvHD at 1361 days post DLI. Three responders are alive, relapse-free at 279, 498 and 1380 days post DLI. Two responders died in remission and two are alive but relapsed at 121 and 212 days post DLI.

Five patients among the entire cohort underwent subsequent transplants. Two of the non-responders received subsequent matched-unrelated donor alloHCT, but only one survived to the end of follow-up. One patient initially achieved CR but then relapsed 212 days post DLI and underwent subsequent PB-haploHCT with active disease. One responder underwent another PB-haploHCT after DLI while in CR. One of the patients with LOC developed graft failure for which he underwent a successful matched-unrelated donor alloHCT.

Relapse-free survival post DLI for the overall cohort and stratified by type of relapse is presented in Figures 1a and b, respectively, and was significantly different between those who received DLI for LOC, hematologic relapse and EM relapse ($P = 0.029$).

Overall survival for the entire cohort and stratified by type of relapse, as presented in Figures 2a and b, was not statistically different between type of relapse ($P = 0.11$). For those with overt hematologic relapse, 30-, 60- and 120-day RFS was 50%, 25% and 8%, respectively, and 30-, 60- and 120-day OS was 58%, 39% and 29%, respectively. For those with EM relapse, 30-, 60- and 120-day RFS was 86%, 86% and 43%, respectively, and 30-, 60- and 120-day OS was 86%, 86% and 71%, respectively. Both patients treated with DLI for LOC were alive, relapse-free at 813 days and 1380 days post DLI at the point of last follow-up, although one developed graft failure requiring a second transplantation. Overall survival comparing responders to DLI to non-responders is presented in Figure 2c.

DLI dose was not associated with a significant difference in RFS (HR: 0.86, 95% CI: 0.33–2.29; $P = 0.77$) or OS (HR: 1.05, 95% CI: 0.34–3.23; $P = 0.93$). Neither were pretreatment with chemotherapy, time to relapse from transplant, time from relapse to DLI, time off immunosuppression, $>50\%$ donor chimerism pre-DLI or GvHD prior to DLI.

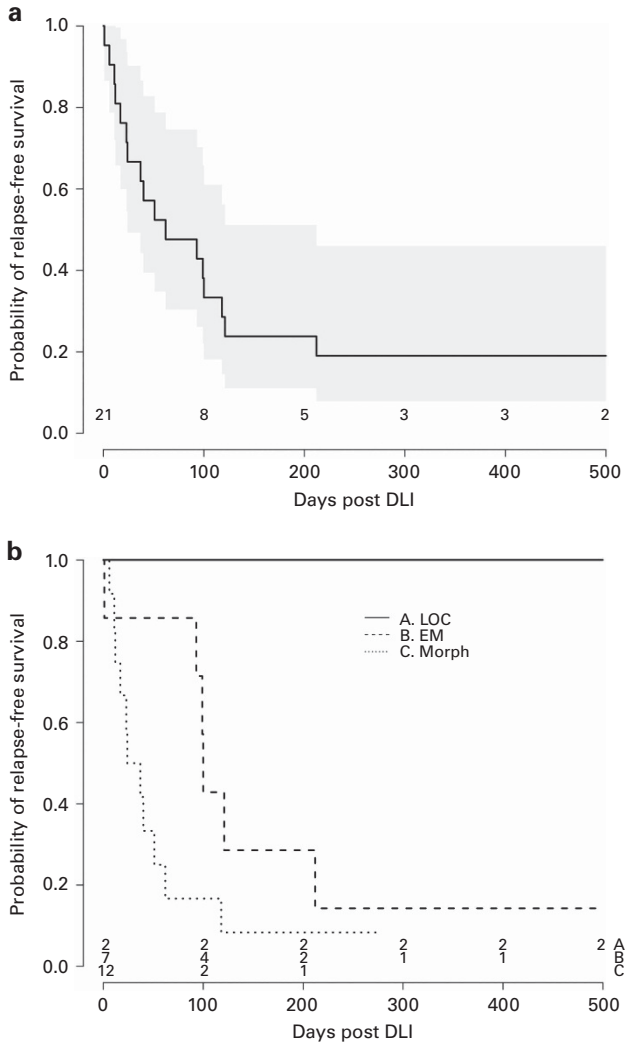

Figure 1. Relapse-free survival with number at risk following donor lymphocyte infusion for disease relapse after PB-haploHCT with post-transplant cyclophosphamide: (**a**) entire cohort; (**b**) stratified by type of disease relapse. EM, extramedullary; LOC, loss of chimerism; morph, overt hematologic relapse.

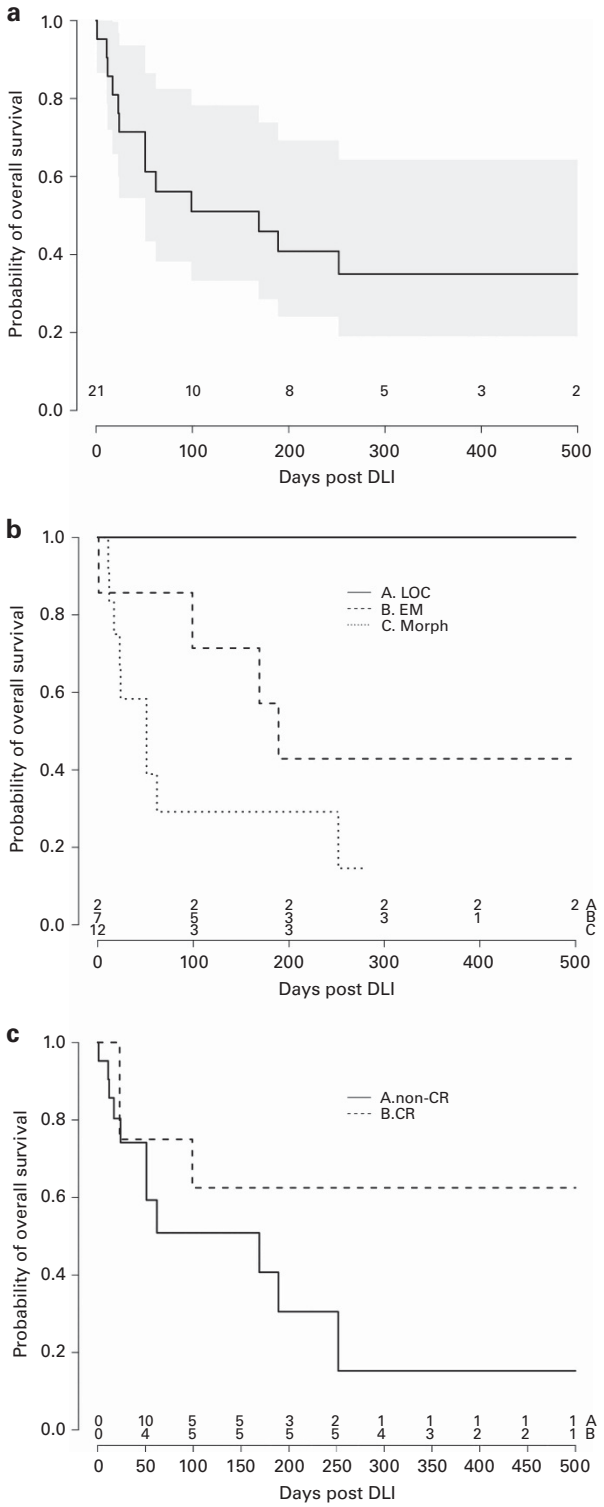

Figure 2. Overall survival with number at risk following DLI for disease relapse after PB-haploHCT with post-transplant cyclophosphamide: (**a**) entire cohort; (**b**) stratified by type of disease relapse. EM, extramedullary; LOC, loss of chimerism; morph, overt hematologic relapse; (**c**) stratified by response.

DISCUSSION

To our knowledge, this is the first study to report the efficacy and safety of DLI in recipients of PB-haploHCT with PTCy. In a cohort of 21 such patients, seven (33%) achieved CR at a mean follow-up of 374 days (s.d.: 478 days), with relapse of two responders, death in remission of two responders, and repeat PB-haploHCT in another. This rate of CR is similar to that which has been demonstrated following DLI among recipients of haploBMT with PTCy, haploHCT with BM/PBSC-derived grafts without PTCy and HLA-matched related and unrelated alloHCT.[3,6,19–22]

Optimal dosing of DLI following haploHCT with PTCy is uncertain because of the theoretical increased risk of GvHD in such a population. Zeidan *et al.*[20,21] demonstrated that an initial dose of 1×10^6 $CD3^+$ cells/kg was generally well tolerated among recipients of haploBMT with PTCy, with a lower incidence of GvHD than had been reported at higher doses in a population that had not received PTCy. Neither they nor Ghiso *et al.*[22] determined a statistically significant dose-dependent relationship between DLI and GvHD, nor was dose associated with a significant difference in response. The small initial sample size, as well as a significant proportion of death prior to response assessment, limited our analysis of response. However, despite not reaching statistical significance, among those who survived to response assessment in our study, 56% of those who received an initial dose of $\geqslant 1 \times 10^6$ $CD3^+$ cells/kg achieved CR compared to only 25% of those with an initial dose $< 1 \times 10^6$ $CD3^+$ cells/kg. Furthermore, there were no

differences in GvHD outcomes between these initial dosage groups, suggesting that a higher initial dose may allow for increased efficacy without increased toxicity.

Overall, GvHD occurred infrequently in our cohort after DLI and the manifestations were relatively mild. Only two patients developed grade III aGvHD, and this was responsive to steroids. There were not any deaths attributable to GvHD. The incidence of aGvHD was similar to that which had been reported among recipients of haploBMT with PTCy, and was markedly lower than those who had received G-CSF mobilized PBSC DLI following haploHCT without PTCy.[20–22] The only statistically significant predictor of post-DLI aGvHD among our cohort was prior development of aGvHD before DLI. This may be attributable to a combination of withdrawal of immunosuppression and fueling preexisting alloreactivity with infusions of donor lymphocytes. Nevertheless, such a predictive factor may be important to weigh in the decision to pursue DLI.

Although limited by the small cohorts within each relapse type, we observed improved RFS among patients who received DLI for progressive LOC and EM relapse. The two patients with LOC who received DLI achieved the longest RFS among the cohort. Similarly, Zeidan *et al.* and Ghiso *et al.* both demonstrated higher CR among those receiving DLI for MRD and LOC as compared to overt hematologic relapse. A substantial proportion of our cohort (33%) manifested EM relapse without marrow involvement and had better RFS than those with overt hematologic relapse. Although these results are preliminary, such data may support use of DLI preemptively in a setting of early relapse and low disease burden.

An interesting concept unique to haploidentical transplantation, initially identified in non-PTCy haploHCT by Vago *et al.*[26–28] and subsequently in a few cases of haploHCT with PTCy, is the potential for leukemic cells to escape immunosurveillance through loss of the mismatched HLA. This undoubtedly has implications for the use of DLI for relapse following PB haploHCT with PTCy, as leukemic cells that have uniparental HLA would also escape the immunotherapeutic effect of DLI. While we are limited in that we do not routinely monitor for HLA loss in recurrent disease, in future studies we intend to investigate various mechanisms potentially responsible for relapse, including loss of heterozygosity. This will potentially allow for more targeted utilization of DLI and possibly improve its overall efficacy.

Relapse of hematologic malignancies following hematopoietic cell transplantation portends a poor prognosis, regardless of the graft source or HLA matching. We have demonstrated through preliminary retrospective analysis of our single center experience that, in recipients of PB-haploHCT with PTCy who relapse, DLI is likely as efficacious as it is in recipients of haploBMT with PTCy and matched alloHCT, with a similar and acceptable safety profile. Furthermore, the novelty and importance of studying DLI in our population of PB-haploHCT recipients is that donor lymphocyte collection is able to occur simultaneously with the initial graft collection. Combined with cryopreservation, this facilitates the availability of DLI for patients at high risk of relapse without the need to subject a donor to subsequent apheresis.

Though limited by small cohort size, our data support further study as to appropriate DLI dosing following PB-haploHCT with PTCy, as ours suggested that a higher dose of $\geqslant 1 \times 10^6$ $CD3^+$ cells/kg may be more efficacious for achieving CR without increased risk of GvHD. Additionally, further investigation into preemptive use of DLI in PB-haploHCT with PTCy recipients following identification of MRD, LOC or EM relapse is necessary to determine whether disease burden and relapse types are important determinants of DLI success. Our study was limited by small sample size, however, the results support continued investigation in both retrospective and prospective fashion.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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