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ORIGINAL ARTICLE

DLI after haploidentical BMT with post-transplant CY

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Forty-two patients relapsing after an unmanipulated haploidentical BM transplant and post-transplant CY (PT-CY), were given 108 DLI, with median interval from transplant of 266 days (range, 67–1372). DLI were given at escalating doses, expressed as CD3+ cells/kg, without GVHD prophylaxis, and ranged from 1×10^3 to 1×10^7 cells/kg (median 5×10^5 cells/kg). The average number of DLI per patient was 2.6 (range, 1–6). The diagnosis was leukemias ($n = 32$) grafted with a myeloablative regimen and Hodgkin's disease ($n = 10$), grafted with a nonmyeloablative regimen. Leukemic patients with molecular relapse ($n = 20$), received DLI alone ($n = 17$) or in association with azacytidine ($n = 3$); leukemic patients with hematologic relapse ($n = 12$) received chemotherapy followed by DLI ($n = 11$) or DLI alone ($n = 1$); Hodgkin patients received DLI following 1–3 courses of chemotherapy. In these three groups the incidence of acute GVHD II–III was 15%, 17% and 10%; response rate was 45%, 33% and 70%; 2-year actuarial survival was 43%, 19% and 80% respectively. This study confirms that escalating doses of DLI can be given in the haploidentical setting with PT-CY, with a relatively low risk of acute GVHD. Response rates and survival are dependent on the underlying disease.

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INTRODUCTION

DLI in humans were first reported in 1990 as capable of inducing CRs in patients relapsing after an allogeneic hematopoietic SCT (HSCT):[1] this was further proof of a GVL effect, which had been shown in mice in 1973.[2] Since then, DLI have been tested in different disorders, and have been shown to have different efficacy depending on the underlying disease and on the phase of the disease itself.[3] One problem, which soon became apparent, was that DLI can also induce a potent, and sometimes lethal, GVHD,[3] with DLI-associated mortality as high as 50%.[4,5] Several factors influence GVHD developing after DLI. The first is the dose of T cells (CD3+) infused, with the highest risk of acute GVHD for patients receiving bulk DLI, that is the entire lympho-apheresis, as performed in the early nineties (~1×10^8 cells/kg CD3+ cells).[6] A way around this problem was devised with the use of escalated dose DLI,[7] starting with small doses (1×10^6 cells/kg) in the first DLI, followed by a gradual increase of ½ or 1 log, every 1–2 months.[7] With this approach one could identify early signs of GVHD[8] and stop the next higher dose of DLI. The second factor predicting GVHD is HLA compatibility between donor and recipients: the risk is lower in patients receiving DLI from an HLA-identical sibling, and higher in patients receiving DLI from an unrelated or mismatched donor.[5] The greater the disparity in HLA, the greater the risk of GVHD after DLI: in haploidentical T-cell-depleted setting, the upper limit of CD3+ cells that can be safely infused together with stem cells after myeloablative conditioning is 3×10^4 cells/kg.[9] The third factor influencing GVHD is the interval between SCT and DLI: in the canine model, lymphocyte infusions on days 1–2 or 21–22 led to severe GVHD, unlike infusion of the same amount of cells on day +60.[10]

In 2007, Huang *et al.*[11] reported 20 patients who were given an average of 0.61×10^8 cells/kg G-CSF-mobilized CD3+ cells from 1–3 Ag family mismatched donors as treatment of relapse following an unmanipulated haploidentical HSCT, the risk of acute grade III–IV GVHD (30%) and of chronic GVHD (64%) was significant but they suggested that infusion of large numbers of mismatched CD3+ cells was feasible. A more recent report, from the same authors, described haploidentical DLI administered to 124 patients after T cell-replete haploidentical SCT, confirming the high cumulative incidence of acute GVHD (53.2% for grade II–IV and 28.4% for grade III–IV).[12]

We now report 108 haploidentical DLI infused in 42 patients, following unmanipulated haploidentical BM transplant, with high-dose post-transplant CY (PT-CY) as GVHD prophylaxis.

PATIENTS AND METHODS

Patients

Patients received transplants from haploidentical-related donors, after myeloablative conditioning ($n = 32$) or nonmyeloablative conditioning ($n = 10$), between November 2009 and February 2013 in our unit. Clinical characteristics are outlined in Table 1. All patients received T-cell-replete BM transplant from haploidentical-related donor: the number of mismatched HLA Ags was 4/8 (A, B, C, DR) in 39 patients and 3/8 in three patients. GVHD prophylaxis was based on high-dose CY post transplant in association with CsA and mycophenolate mofetil (MMF) as described for myeloablative conditioning[13] and for nonmyeloablative regimen.[14]

Molecular relapses: leukemia

Molecular relapses were defined[15] as the evidence of a pretransplant marker of disease using molecular probes, in the absence of hematological relapse (blast count $< 5\%$ in BM no evidence of extramedullary disease). Molecular evaluation was performed on BM blood; when positive molecular evaluation was repeated on the same specimen. In AML, RQ-PCR for Wilms tumor 1 (WT1) expression was used. Molecular positivity was considered when WT1 copy numbers every 10^4 copies of abelson (Abl) were > 100.[15] RQ-PCR was used to detect fusion transcripts AML1-ETO, PML-RARalpha or MLL-AF4. In ALL, a qualitative nested PCR was used to detect Ig gene rearrangement (IgH VDJ) and RQ-PCR for BCR-ABL

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Table 1. Patients' characteristics and DLI

	Leukemia molecular relapse	*Leukemia hematologic relapse*	*Hodgkin's disease*
Number of patients	20	12	10
Age (years) (range)	41 (18–66)	24 (17–63)	34 (26–58)
Gender (male/female)	10/10	6/6	7/3
Disease phase at transplant			
CR1	4 (20%)		
Further CR	6 (30%)	3 (25%)	3 (30%)
PR	—	—	2 (20%)
Active disease	10 (50%)	9 (75%)	5 (50%)
Previous autograft	1 (5%)	—	6 (100%)
Previous allograft	4 (20%)	5 (42%)	1 (10%)
Non myeloablative conditioning			
Fludarabine+cyclophosphamide + TBI 200 rads	—	—	10 (100%)
Myeloablative conditioning			
Thiotepa+busulfan+fludarabine	10 (50%)	9 (75%)	—
TBI+fludarabine	10 (50%)	3 (25%)	—
Chimerism status pre-DLI (%)			
Full donor chimerism	20 (100%)	7 (58%)	10 (100%)
Partial donor chimerism	—	5 (42%)	—
Chemotherapy pre-DLI		*10 (83%)*	*10 (100%)*
Gemcitabine	—	1 (8.3%)	3 (30%)
Bendamustine+rituximab	—	—	7 (70%)
Fludarabine+cytarabine+idarubcin	—	4 (33.3%)	—
Mitoxantron+etoposide+cytarabine	—	4 (33.3%)	—
Bortezomib	—	1 (8.3%)	—
Azacytidine	3 (15%)	—	—
Radiotherapy pre-DLI	—	*1 (8.3%)*	*1 (10%)*
DLI alone	17 (85%)	1 (8.3%)	—
Median interval BMT-relapse (days) (range)	—	208 (45–1312)	—
Median interval relapse-DLI (days) (range)	—	38 (6–339)	—
Median interval between sequential DLI (days) (range)	—	54 (15–195)	—

Abbreviations: CR = complete response; DLI = donor lymphocyte Infusions; PR = partial response; TBI = total body irradiation.

transcript. Molecular relapse was confirmed always with a second test, with a median interval of 35 days (range, 10–39).

AML patients had a median WT1 value of 229 copies/10^4 abl (range, 141–567). In total, 20 patients had a molecular relapse after BMT: 15 AML and 5 ALL. Patients received DLI alone ($n = 17$) or azacytidine followed by administration of DLI ($n = 3$).

Hematologic relapses: leukemia and myeloma

Hematologic relapses were defined by classic morphology criteria, with marrow containing $\geqslant 5\%$ of blasts in the smear or reappearance of blasts in the blood. Extramedullary relapse was defined as any manifestation of hematologic malignancies outside the hematopoietic system.

In total, 12 patients had a hematologic relapse after transplant: 7 AML, 4 ALL, and 1 patient with multiple myeloma.

Ten patients received one course of chemotherapy before DLI: anthracycline and cytarabine-containing regimens ($n = 8$), gemcitabine ($n = 1$) or bortezomib and dexametazone ($n = 1$, myeloma patient).

One AML patient received DLI in association with radiotherapy, because of extra hematologic relapse. One patient was treated with DLI alone, being considered not eligible to received chemotherapy.

Hodgkin's disease (HD)

Patients with HD were monitored clinically and by positron emission tomography (PET) scans: surgical biopsies were performed in case of dubious PET scans. Relapse was identified as a positive PET, with or without clinically detectable nodes. All patients ($n = 10$) were treated with chemotherapy before DLI: gemcitabine ($n = 3$) or bendamustine in association with rituximab ($n = 7$). The median number of chemotherapy courses before DLI is 2.5 (range, 1–4), with a median interval between chemotherapy and DLI of 9 days (range, 5–21). One patient was treated with radiotherapy in association with chemotherapy, because of a vertebral lesion.

Donor chimerism

Donor chimerism in unfractionated BM cells and in CD3+ selected peripheral blood cells, before and after DLI, was evaluated by STR analysis. Five patients in hematologic relapse showed loss of donor chimerism: donor chimerism was, respectively, 37%, 43%, 78%, 86%, and 88% at the time of relapse. Four of these patients received chemotherapy before administration of DLI and one patient obtained full donor chimerism after chemotherapy, the others had mixed donor chimerism at DLI.

DLI

DLI were collected from the original donor. The proportion of CD3+ cells was assessed by flow cytometry. The dose of DLI infused was calculated on the basis of CD3+ cells/kg of recipient's body wt. Donors did not receive priming medications or priming agents before the lymphocyte apheresis. All donors and patients signed an informed consent form. Immunosuppressive agents were discontinued when relapse was diagnosed. Patients were required to have adequate organ function and a Karnofsky performance status $>60\%$. Patients with active central nervous system leukemia or active infections were excluded from this program. Patients were followed post DLI for any signs of GVHD. Response to DLI was assessed with BM aspirate/biopsy or CT scan imaging or PET when indicated, once a month post DLI in acute leukemias and every 2–3 months post DLI Hodgkin's disease.

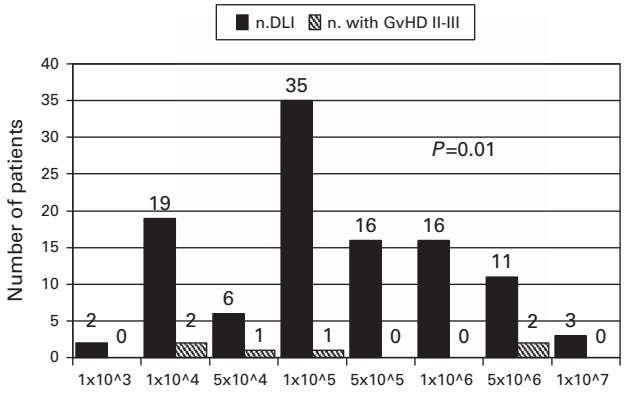

Figure 1. Risk of acute grade II–III GVHD, stratified by escalating levels of DLI. Dark bars represent number of infusions; light bars represent number of patient developing acute GVHD at that cell dose. No correlation is seen between CD3+ cell dose and acute GVHD, with similar risk at 5×10^4 or 5×10^6 CD3+ cells/kg.

The median overall interval from transplant to DLI was 266 days (range, 67–1372); the median interval from relapse to DLI was 38 days (range, 6–339). The dose of DLI is outlined in Figure 1. The starting dose was 1×10^4 CD3+ T cells per kg or 1×10^5 CD3+ cells/kg, therefore a dose escalation of ½ or 1 log was performed in the subsequent administrations, in absence of GVHD. The average number of DLI per patient is 2.6 (range, 1–6). Sixteen patients received one DLI, six patient received two DLI, eight patients received three DLI; five patients received four DLI, five patients received five DLI and two patients received six DLI. The median interval between sequential doses of DLI was 54 days (range, 15–195).

Statistics

OS was determinate using the Kaplan–Meier method and was calculated from date of administration of first DLI. Cumulative incidence was used to estimate acute and chronic GVHD. Responses were defined as best response achieved, and patients were classified as no response/death, PR or CR. Duration of CR was defined as interval from documented CR post DLI and relapse. The follow-up was calculated from the first infusion of DLI. The NCSS 2007 software for Windows (NCSS, Kaysville, UT, USA) was used for chi-square tables, cumulative incidence (CI) rates and actuarial survival.

RESULTS

GVHD

Eight patients (19%) had acute GVHD grade I (Table 2), which resolved spontaneously in five, two required treatment with steroids, and one required steroids and CsA. Six patients developed grade II–III GVHD with a cumulative incidence of 14% at a median interval of 17 days (range, 7–47) following DLI, and were treated with steroids alone ($n=3$), steroids and CsA ($n=2$), and steroids, CsA and extracorporeal photopheresis ($n=1$). Four patients had grade II and two patients had grade III GVHD (Table 2). The occurrence of GVHD was not related to DLI dose (Figure 1). The risk was 10% at 1×10^4 cells/kg, 16% at 5×10^4 cells/kg, 3% at 1×10^5 cells/kg, 0% at 5×10^5 and 1×10^6 cells/kg, 18% at 5×10^6 cells/kg and 0% at 1×10^7 cells/kg.

The CI of acute GVHD grade II–III in the three groups of patients is shown in Table 2: it was 15%, 17% and 10% (*P* not significant), respectively, for molecular relapse of leukemias, hematologic relapse of leukemias and HD.

Chronic GVHD was not observed in evaluable patients. The characteristics of GVHD are summarized in Table 2.

None of the known risk factor for GVHD was a significant predictor for GVHD post DLI: female donor (43% vs 46%), prior acute GVHD of any grade (36% vs 36%), prior acute GVHD grade II–III (7% vs 7%), prior limited chronic GVHD (21% vs 17%), median interval DLI transplantation (290 days vs 252 days), prior chemotherapy (50% vs 57%). Concerning the number and kind of HLA differences, the population was homogenous: 39 donor–recipient pair with 4/8 mismatched Ags, 2 with 3/8 mismatched Ags and sharing locus C, 1 with 3/8 mismatched Ags and sharing locus DR.

Table 2. Characteristics of GVHD post DLI

	Leukemia molecular relapse	*Leukemia hematologic relapse*	*Hodgkin's disease*	*Total*
Number of patients	20	12	10	42
aGVHD (%)				
Grade I (%)	3 (15%)	4 (33%)	1 (10%)	8 (19%)
Grade II (%)	2 (10%)	1 (8,3%)	1 (10%)	4 (9,5%)
Grade III (%)	1 (5%)	1 (8,3%)	—	2 (4,7%)
Grade IV (%)	—	—	—	—
Grade II-III %	3 (15%)	2 (17%)	1 (10%)	6 (14%)
No of organs involved in aGVHD				
One organ (skin only)	5	6	2	13
Two organs	—	—	—	—
Three organs	1	—	—	1
Organs involved in aGVHD				
Skin	6	6	2	14
Liver	1	—	—	1
Intestine	1	—	—	1
Treatment of aGVHD after DLI				
Steroids	1	2	2	5
CsA+steroids	1	1	—	2
CsA+steroids+ECP	1	—	—	1
Chronic GVHD	0	0	0	0
Median onset of acute GVHD after DLI (days) (range)	18 (13–66)	16 (14–17)	17 (15–19)	17 (7–47)

Abbreviations: aGVHD = acute graft vs host disease; ECP = extracorporeal photopheresis.

Toxicity

Severe aplasia following haploidentical DLI was not seen; infective complications developed in five patients (12%): CMV reactivation ($n=2$), Herpes Zoster reactivation ($n=1$), and sepsis ($n=2$).

Molecular relapse: leukemias

Twenty patients received DLI because of a molecular relapse: nine obtained CR (normalized WT1 or negativity of transcript) (Table 3) and six are in continuous CR. Three patients have stable disease (persistent molecular positivity) and eight patients progressed to a hematologic relapse despite DLI. The overall response rate was 45% with median response duration of 7 months (range, 3–25). The median follow-up from first DLI is 8 months (range, 3–23). The median survival from DLI and from transplant is, respectively, 9 months (range, 4–23) and 17 months (range, 7–35). The actuarial 2-year survival is 43% (Figure 2).

Three patients relapsed at 4, 7 and 8 months, respectively, after achieving remission from DLI. Thirteen patients were alive (65%).

Hematologic relapse: leukemias, myeloma

Of the 12 patients, 4 obtained CR, 6 developed progressive disease, and 2 are not evaluable. The overall response rate was 33% with median response duration of 4 months (range, 2–10). The median follow-up from first DLI is 6 months (range, 1–11). The median survival from DLI and from transplant is, respectively,

Table 3. Results

	Leukemia molecular relapse	*Leukemia hematologic relapse*	*Hodgkin's disease*
Number of patients	20	12	10
Response to DLI			
CR	9 (45%)	4 (33%)	4 (40%)
PR	—	—	3 (30%)
Stable disease	3 (15%)	1 (10%)	—
Progression	8 (40%)	6 (50%)	2 (20%)
Non evaluable	—	2 (17%)	—
Median response duration (months) (range)	7 (3–25)	4 (2–10)	9 (3–28)
Median follow-up from DLI (months) (range)	8 (3–23)	6 (1–11)	14 (4–34)
Median survival from DLI (months) (range)	9 (4–23)	7 (1–15)	18 (4–34)
Median survival from BMT (months) (range)	17 (7–35)	17 (7–28)	33 (11–51)
Relapse following CR post DLI	*3 (33%)*	*2 (50%)*	*2 (50%)*
Alive	13 (65%)	5 (42%)	8 (80%)
DFS	6 (30%)	2 (17%)	4 (40%)
Median DFS (months) (range)	7 (3–23)	4 (2–10)	4 (3–28)

Abbreviations: CR = complete response; DLI = donor lymphocyte Infusions; PR = partial response.

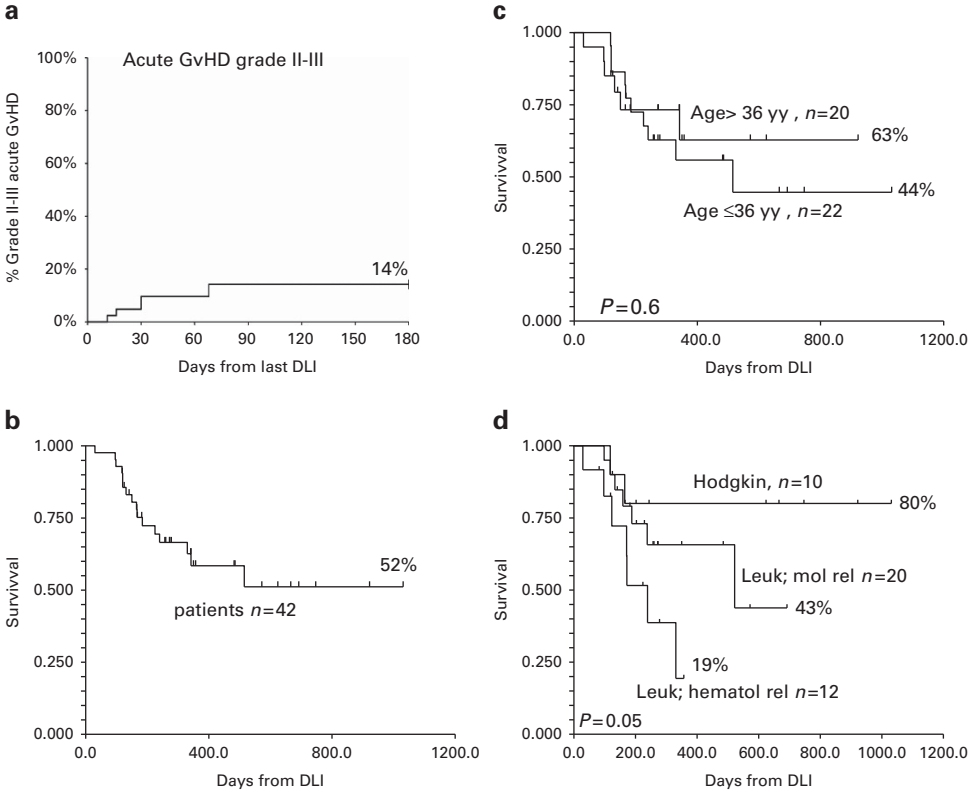

Figure 2. Risk of acute GVHD and survival. (**a**) Cumulative risk of acute grade II–III GVHD (14%): (**b**) OS for all 42 patients (52%). (**c**) OS in patients stratified for median age, not significantly different. (**d**) OS in patients with HD (80%). Patients with leukemia are stratified according to type of relapse: molecular (43% OS) or hematologic (19% OS).

7 months (range, 1–15) and 17 months (range, 7–28). Two-year actuarial survival is 19% (Figure 2). Five patients (42%) are alive, two are disease-free (17%), two patients relapsed at 2 and 10 months from CR, seven died of disease relapse, one has ongoing GVHD.

Hodgkin's disease

Of the 10 patients with HD, 4 (40%) achieved CR and 3 (30%) obtain PR, with a median response duration of 9 months (range, 3–28). Two patients were refractory to DLI and died of progressive disease, after 4 and 5 months from first DLI. One patient relapsed at 3 months from CR and one patient progressed at 10 months from PR; they received further chemotherapy followed by one DLI and obtained, respectively, a complete and a PR. Eight patients are alive (80%), four are disease-free (40%). The median follow-up from first DLI is 14 months (range, 4–34), the median survival is 18 months from first DLI (range, 4–34) and 33 months from BMT (range, 11–51). Two-year actuarial survival is 80% (Figure 2).

Results according to disease and relapse type are summarized in Table 3.

DISCUSSION

GVHD is a frequent complication of DLI and is predicted by the dose of CD3+ cells infused and the degree of HLA mismatch.[16,17] In this study we show that HLA haploidentical escalating doses of DLI, can be infused in patients who received high-dose post transplant CY, with a CI of grade II–III acute GVHD of 14%. We initially started with small doses of DLI (CD3+ 1×10^3 cells/kg), anticipating a high risk of GVHD. This was not seen in the majority of patients and we have gradually increased the initial dose to 1×10^5 cells/kg for molecular relapse and 1×10^6 cells/kg for hematologic relapse and are escalating the highest dose to 1×10^7 cells/kg.

This is in contrast to what is described for haploidentical CD34 selected transplants, in whom the limit of CD3+ cells, above which GVHD occurs, has been set at 3×10^4 cells/kg.[9] The reasons for this significant difference are not clear. One possible explanation is that myeloablative conditioning creates both lymphopenia and an inflammatory milieu, with gut damage, and both conditions are favorable for the activation and expansion of alloreactive donor T cells. It is also possible that regulatory T cells circulate in the patients post high-dose CY and prevent the activation of newly infused mature T cells. The level of safety of haploidentical DLI, based on these 108 infusions, is such that one could also design a study looking at their prophylactic use, particularly in high-risk acute leukemias. A recent report has been published, including 40 patients receiving haploidentical DLI after haploidentical T-cell-replete BMT with post-transplantation CY.[18] In that study the starting dose was typically 1×10^6 cells/kg CD3+ cells and the risk of acute GVHD grade II–IV was reported to be 20%, in keeping with results from the present study.

Response to DLI greatly depends on the underlying disease. In Hodgkin patients we have always used haploidentical DLI following one or three courses of chemotherapy, with bendamustine or gemcitabine: we have seen an overall response rate of 70%, with 40% PET-negative CRs. The median duration of response was 9 months (range, 3–28), and 8/10 patients survive with a median follow-up of 20 months from DLI. These data compare favorably with reports of DLI in patients with HD relapsing after an allogeneic transplant, with response rates in the range of 40–50%.[19–21] It should also be noted that the recent availability of brentuximab vedotin, is an additional therapeutic option that can be effectively combined with haploidentical DLI. In the present study, no patient received brentuximab before DLI. The extremely low transplant mortality[14] and the possibility of using DLI at relapse make HLA haploidentical transplants, very attractive for patients with advanced Hodgkin's lymphoma.

Acute leukemias are of course very different in terms of response, and several reports have shown that DLI are unlikely to be effective when given alone for hematologic relapse.[22,23] However, in a retrospective analysis of 399 patients with AML in first hematologic relapse after transplant, DLI ($n = 171$) were compared with non-DLI ($n = 228$). The actuarial 2-year survival was 21% vs 9% at a median follow-up of 27 and 40 months, respectively, for DLI and non-DLI patients.[24]

In the present study 33% of leukemias with hematologic relapse entered a remission with the combination of chemotherapy followed by DLI, maintaining the response for a median of 4 months. The proportion of leukemias responding when given DLI for molecular relapse was slightly higher (45%), and survival superior compared with hematologic relapse (43% vs 19%, $P = 0.07$). Hematologic relapse, in particular associated with a loss of donor chimerism, remains a group of patients with a very poor prognosis, often refractory to chemo- and cellular therapy.

These data highlight the difficulties in treating relapsed acute leukemia, whether hematologic or molecular. It may be that, for high-risk patient one will have to use prophylactic cellular therapy. The safety data provided in this study, with little acute GVHD, will allow us to design a prophylactic protocol with escalating doses of DLI, given early post-transplant.

In conclusion, we have shown that haploidentical DLI can be safely administered in patients receiving unmanipulated family mismatched marrow and post-transplant CY, and these data may be used to design trials of cellular therapy.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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