European Journal of Haematology

WILEY

European Journal of Haematology

ORIGINAL ARTICLE OPEN ACCESS

Inferior Overall Survival After Haploidentical Donor Lymphocyte Infusions in Relapsed Myeloid Neoplasms

Tobias Matthieu Benoit[1] | Adrian Bachofner[1] | Nathan Wolfensberger[1] | Yvonne Zaugg-Berger[1] | Markus Gabriel Manz[1,2] | Dominik Schneidawind[1,2,3]

[1]Department of Medical Oncology and Hematology, University Hospital Zurich, Zürich, Switzerland | [2]Comprehensive Cancer Center Zurich, Zürich, Switzerland | [3]Department of Medicine II, University Hospital Tübingen and University of Tübingen, Tübingen, Germany

Correspondence: Tobias Matthieu Benoit (tobias.benoit@usz.ch)

Received: 18 July 2024 | **Revised:** 22 October 2024 | **Accepted:** 23 October 2024

Funding: The authors received no specific funding for this work.

Keywords: acute myeloid leukemia | allogeneic hematopoietic stem cell transplantation | donor lymphocyte infusion | haploidentical hematopoietic stem cell transplantation | myelodysplastic neoplasms

ABSTRACT

Objectives: Allogeneic hematopoietic stem cell transplantation (HSCT) effectively treats high-risk myeloid neoplasms, but relapses post-HSCT, particularly in acute myeloid leukemia (AML) and myelodysplastic neoplasms (MDS), pose significant challenges. Donor lymphocyte infusion (DLI) has been utilized, but its effectiveness, especially in haploidentical settings, remains insufficiently clarified, and graft-versus-host disease (GvHD) poses a substantial risk.

Methods: In this retrospective cohort study, 57 patients with AML or MDS who received DLI after allogeneic HSCT at our center from 2002 to 2023 were analyzed. Herein, only preemptively or therapeutically applied DLI were included, and endpoints included overall survival (OS), progression-free survival (PFS), and GvHD incidence post-DLI.

Results: Median OS after DLI was 517 days, with a 1-year OS of 62.5%. Factors associated with longer OS included patient age, HLA-identical donor, post-HSCT treatment naivety, and preemptive DLI indication. Haploidentical DLI was associated with inferior OS compared to HLA-identical DLI; however, PFS and GvHD incidence post-DLI did not differ significantly.

Conclusions: Our study findings indicate that OS rate is inferior in patients with relapsed AML or MDS treated with haploidentical DLI in comparison to those who received HLA-identical DLI. Given the limitations of haploidentical DLI, alternative strategies, such as higher cell doses or combination treatment approaches, warrant further investigation.

1 | Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is an effective therapy for the treatment of high-risk myeloid neoplasms, such as acute myeloid leukemia (AML) and myelodysplastic neoplasms (MDS) [1, 2]. By replacing the recipient's hematopoietic and immune system, allogeneic HSCT results in an alloreactive immunologic graft-versus-malignancy effect, also known as graft-versus-leukemia (GvL) effect, in which malignant cells that have survived prior induction therapies and conditioning can be destroyed by donor immune cells [3, 4]. However, relapse of AML and MDS after allogeneic HSCT consistently has a very poor outcome [5, 6].

Donor lymphocyte infusion (DLI) has been used in the management of AML and MDS relapse after allogeneic HSCT by increasing alloreactive immunologic activity of the graft [7–10]. Nowadays, the preemptive or therapeutic use of DLI is routine in the treatment of measurable residual disease (MRD) as well as overt relapses of myeloid neoplasms after allogeneic HSCT,

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2024 The Author(s). *European Journal of Haematology* published by John Wiley & Sons Ltd.

TABLE 1 | Characteristics of patients and DLI.

Variable	DLI, *n* (%)			*p*
	Total	HLA-matched transplantation	Haploidentical transplantation	
No. of patients (%)	57 (100)	48 (84.2)	9 (15.8)	
Patient's age, median, years (range)[a]	52.5 (17.4–67.0)	52.7 (17.4–67.0)	50.8 (18.1–58.6)	0.246
Patient's age[b]				0.428
<45 years	17 (29.8)	13 (27.1)	4 (44.4)	
≥45 years	40 (70.2)	35 (72.9)	5 (55.6)	
Gender[b]				**0.025**
Male	29 (50.9)	21 (43.8)	8 (88.9)	
Female	28 (49.1)	27 (56.3)	1 (11.1)	
Diagnosis[b]				1.000
AML[c]	49 (86)	41 (85.4)	8 (88.9)	0.111
Favorable	8 (16.3)	6 (14.6)	2 (25)	
Intermediate	15 (30.6)	13 (31.7)	2 (25)	
Adverse	26 (51)	22 (53.7)	4 (50)	
MDS[d]	8 (14)	7 (14.6)	1 (11.1)	0.767
Low	1 (12.5)	1 (14.3)	0 (0)	
Intermediate-1	0 (0)	0 (0)	0 (0)	
Intermediate-2	2 (25)	1 (14.3)	0 (0)	
High	5 (62.5)	4 (71.4)	1 (100)	
Conditioning regimen used[b]				1.000
MAC	26 (45.6)	22 (45.8)	4 (44.4)	
RIC	31 (54.4)	26 (54.2)	5 (55.6)	
Days from HSCT to first DLI, median (range)[a]	301 (98–2025)	309 (98–2025)	282 (161–1871)	0.768
Number of DLI cycles, median (range)[a]	2 (1–6)	2 (1–6)	2 (1–5)	0.819
Infused total $CD3^+$ DLI cell dose (/kg), median (range)[b]	8.75×10^6 (0.05×10^6–364×10^6)	11×10^6 (1×10^6–364×10^6)	6.25×10^6 (0.05×10^6–21.6×10^6)	0.172
DLI indication[b]				0.582
Preemptive DLI	7 (12.3)	7 (14.6)	0 (0)	
Therapeutic DLI	50 (87.7)	41 (85.4)	9 (100)	
Donor's age, median, years (range)[a]	42.5 (19.0–66.0)	39.2 (19.0–65.0)	49.9 (19.0–66.0)	0.237
Donor's age[b]				0.726
≥35 years	34 (59.6)	28 (58.3)	6 (66.7)	
<35 years	23 (40.4)	20 (41.7)	3 (33.3)	

(Continues)

TABLE 1 | (Continued)

Variable	DLI, *n* (%)			*p*
	Total	HLA-matched transplantation	Haploidentical transplantation	
Other treatment lines post-HSCT (before DLI)[b]				0.561
No	15 (26.3)	13 (27.1)	2 (22.2)	
Yes	42 (73.7)	35 (72.9)	7 (77.8)	
HMA	27 (47.4)	22 (45.8)	5 (55.6)	0.43
HMA + Bcl2-inhibitor	5 (8.8)	3 (6.3)	2 (22.2)	0.173
FLT3-inhibitor	9 (15.8)	8 (16.7)	1 (11.1)	0.564
Classical chemotherapy	10 (17.5)	7 (14.6)	3 (33.3)	0.184

Note: Boldface representing $p < 0.05$.
Abbreviations: AML, acute myeloid leukemia; Bcl2, B-cell lymphoma 2; DLI, donor lymphocyte infusion; FLT3, FMS-related receptor tyrosine kinase 3; HMA, hypomethylating agents; HSCT, hematopoietic stem cell transplantation; MAC, myeloablative conditioning; MDS, myelodysplastic syndrome; RIC, reduced intensity conditioning.
[a]Mann–Whitney *U* test.
[b]Fisher's exact test (two-sided).
[c]Risk stratification according to ELN2022.
[d]Risk stratification according to IPSS-R.

respectively [7, 11]. However, the resulting risk of graft-versus-host disease (GvHD) is associated with high morbidity and mortality and limits the feasibility of DLI [10, 12]. Although more potential donors are available with the advent of allogeneic HSCT from related human leukocyte antigen (HLA) haploidentical donors [13–15], facilitating the treatment of AML and MDS, there is limited evidence of the efficacy of haploidentical DLI in the treatment of residual or relapsed disease after allogeneic HSCT [7]. In addition, there is concern that haploidentical DLI might have an increased risk for GvHD compared with HLA-identical DLI in patients with AML or MDS [7].

We therefore performed a retrospective cohort study of patients with AML or MDS who received DLI preemptively or with therapeutic intention after HSCT at our center and evaluated its efficacy and tolerability.

2 | Methods

2.1 | Patients

This is a retrospective, observational cohort study of adult patients > 16 years of age with AML or MDS who received DLI after allogeneic HSCT at our center from 2002 to 2023. All patients and, in the case of minors, also their legal guardians gave written informed consent. The study was conducted in accordance with the Declaration of Helsinki. In addition, the local ethics committee approved the retrospective study (BASEC-No 2023-01363).

2.2 | Definitions and Endpoints

In general, GvHD prophylaxis was chosen according to conditioning intensity and included ciclosporin A and methotrexate in the myeloablative setting or ciclosporin A and mycophenolate mofetil in the reduced-intensity conditioning setting, respectively. In the case of haploidentical transplantation, posttransplant cyclophosphamide was augmented according to the proposed protocol by Luznik and colleagues [15]. Unstimulated and unmanipulated donor lymphocytes were administered either preemptively in patients with MRD or in case of incomplete donor chimerism, or after systemic chemotherapy in patients with overt relapse with therapeutic intent. The use of DLI was contraindicated in patients who had previously developed or were currently experiencing active acute (grade 2–4) or chronic GvHD. The initial and subsequent DLI cell doses were selected according to the French guideline proposed by De Vos and colleagues [16] and similar to the recently published practice recommendations of the EBMT [17]: In the HLA-matched setting, the first DLI cell dose was 1×10^7 $CD3^+$ cells per kilogram of recipient body weight (kg) in therapeutic intent and 5×10^6 $CD3^+$ cells per kg for the preemptive indication, respectively. In the haploidentical setting, the anticipated first DLI cell dose was $0.5–1 \times 10^6$ $CD3^+$ cells per kg in case of hematological relapse and 1×10^5 $CD3^+$ cells per kg for preemptive purpose, respectively. DLI was usually administered fresh for the first infusion and cryopreserved for subsequent infusions. Generally, 4–6 weeks after the last DLI, further DLI cycles were administered with a 0.5 to 1 log higher cumulative $CD3^+$ cell dose if there were no signs of relevant GvHD or disease relapse. Prophylactic immunosuppressants or T-cell selection techniques were not regularly used to prevent GvHD after DLI, and no specific T-cell selection techniques post-collection were applied.

The primary endpoint of the study was overall survival (OS) after first DLI treatment. The secondary endpoints included progression-free survival (PFS) as well as the cumulative incidence of acute GvHD and chronic GvHD post-DLI. Relapse was defined as the hematologic recurrence of disease.

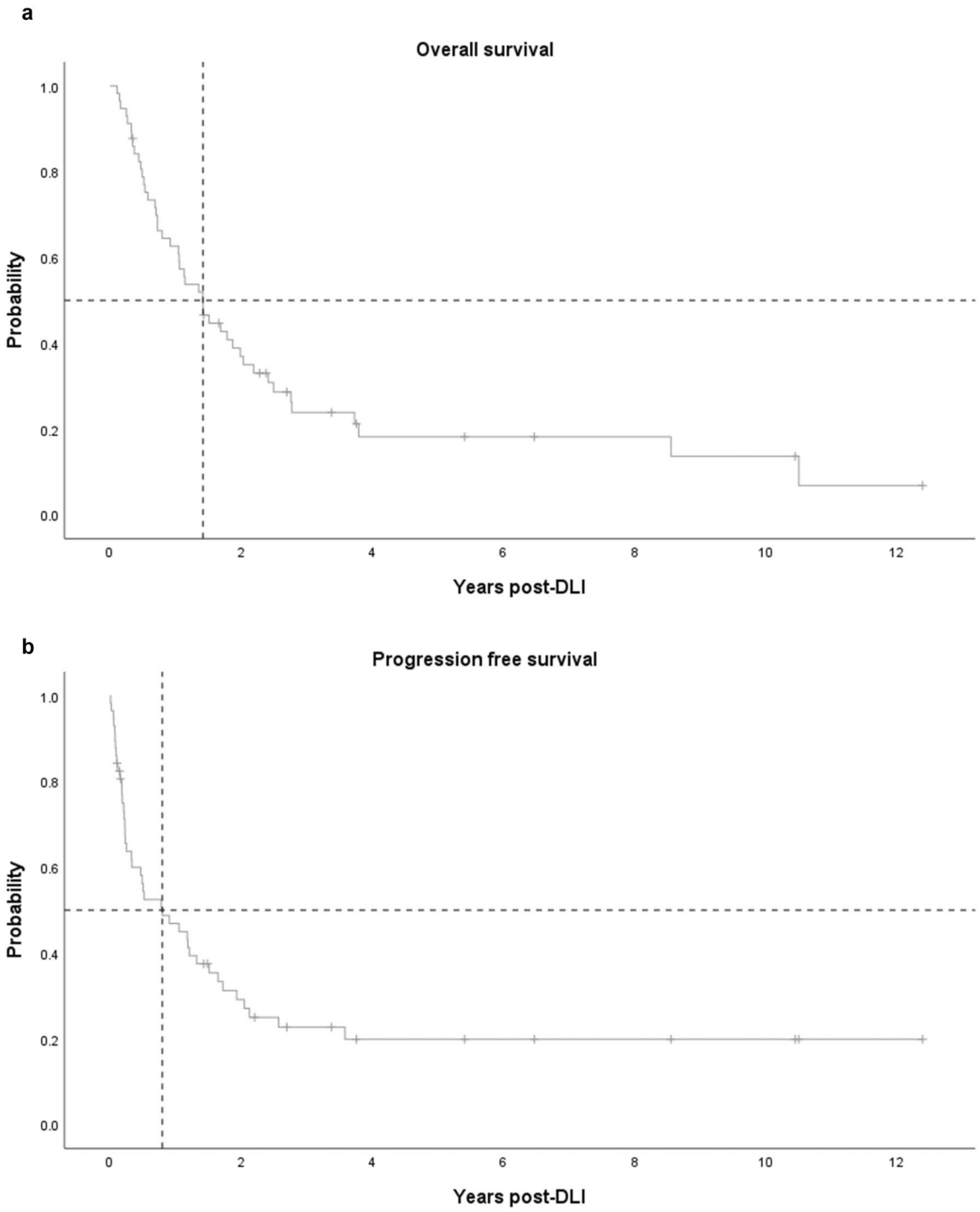

FIGURE 1 | Overall survival (a) and progression-free survival (b) of the entire cohort ($n = 57$). (a) Median progression free survival (SD; 95%-CI): 0.8 years (0.34; 0.132–1.463). (b) Median overall survival (SD; 95%-CI): 1.4 years (0.235, 0.955–1.877). CI, confidence interval; DLI, donor lymphocyte infusion; SD, standard deviation.

2.3 | Statistical Analysis

All statistical analyses were performed using SPSS Statistics version 29.0.0.0 (SPSS, IBM Corp., Armonk, NY, USA). Patient and DLI characteristics were compared using Pearson's chi-square and Fisher's exact test for categorical variables and Mann–Whitney *U* test for continuous variables. The overall response to DLI was compared using Fisher's exact test for the univariate analysis and the logistic regression model for the multivariate analysis. The Kaplan–Meier method was used to estimate the probability of OS. For the multivariate survival analysis, Cox proportional hazard regression models with assumed influencing factors were constructed and the hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated. Two-tailed $p < 0.05$ was considered statistically significant. The endpoint of follow-up for all surviving subjects was June 30, 2023.

TABLE 2 | Univariate analysis for the risk factors of transplant outcomes in DLI recipients ($n = 57$).

	Grade 2–4 acute GvHD post-DLI		Limited chronic GvHD post-DLI		Extended chronic GvHD post-DLI		PFS			OS		
Variable	**Incidence (%)**	***p***	**Incidence (%)**	***p***	**Incidence (%)**	***p***	**Median (years)**	**95%-CI**	***p****	**Median (years)**	**95%-CI**	***p****
Patient's age		0.751*		0.464*		1.00*			0.086			**0.028**
< 45 years	29.4		23.5		17.6		0.332	0.129–0.534		1.044	0.565–1.523	
≥ 45 years	25		15		15		1.211	0.335–2.087		1.786	1.037–2.535	
Gender		0.55*		1.00*		0.297*			0.439			0.167
Male	32.1		17.2		10.3		0.49	0–1.555		0.918	0.33–1.505	
Female	21.4		17.9		21.4		0.797	0.147–1.447		1.685	0.997–2.373	
Conditioning regimen used		0.236*		0.16*		1.00*			0.698			0.627
MAC	34.6		26.9		15.4		0.904	0.466–1.342		1.51	0.499–2.521	
RIC	19.4		9.7		16.1		0.49	0–1.698		1.353	0.528–2.179	
Donor		0.106**		0.799**		0.783**			0.083			**0.005**
Matched sibling donor	12.5		20.8		16.7		1.211	0–2.834		1.871	0.476–3.267	
Matched unrelated donor	33.3		16.7		12.5		0.781	0.284–1.277		1.419	0.865–1.973	
Haploidentical sibling relative	44.4		11.1		22.2		0.181	0.057–0.305		0.468	0.38–0.557	
Donor HLA-match		0.223*		1.00*		0.623*			0.089			**0.003**
HLA-identical donor	22.9		18.8		14.6		0.904	0.148–1.661		1.685	1.107–2.262	
Haploidentical donor	44.4		11.1		22.2		0.181	0.057–0.305		0.468	0.38–0.557	
DLI indication		0.172*		0.333*		1.00*			**0.002**			**< 0.001**
Preemptive	0		0		14.3		10.633	7.442–13.824		10.504	0–23.912	
Therapeutic	30		20		16		0.504	0–1.018		1.142	0.718–1.567	
Donor's age		0.76*		1.00*		0.288*			0.364			0.46
< 35 years	30.4		17.4		8.7		0.332	0–0.677		1.414	0.658–2.17	

(Continues)

TABLE 2 | (Continued)

Variable	Grade 2–4 acute GvHD post-DLI		Limited chronic GvHD post-DLI		Extended chronic GvHD post-DLI		PFS			OS		
	Incidence (%)	*p*	Incidence (%)	*p*	Incidence (%)	*p*	Median (years)	95%-CI	*p***	Median (years)	95%-CI	*p****
≥ 35 years	23.5		17.6		20.6		1.178	0.401–1.955		1.416	0.554–2.279	
Other treatment lines post-HSCT (and pre-DLI)		0.162*		0.189*		0.561*			**0.018**			**0.008**
Yes	30.9		21.4		16.7		0.518	0.121–0.914		1.142	0.677–1.608	
No	13.3		6.7		13.3		6.725	1.565–3.657		8.553	0–19.177	

Note: Boldface representing $p < 0.05$.
Abbreviations: DLI, donor lymphocyte infusion; GvHD, graft-versus-host disease; HLA, human leukocyte antigen; MAC, myeloablative conditioning; OS, overall survival; PFS, progression-free survival; RIC, reduced intensity conditioning.
*Fisher's exact test (two-sided).
**Pearson's chi-square test.
***Log rank test (Mantel-Cox).

3 | Results

3.1 | Baseline Characteristics of Patients and DLI

Patient and DLI characteristics are summarized in Table 1. A total of 57 patients with AML ($n = 48$) or MDS ($n = 9$) who received DLI after allogeneic HSCT between 2002 and 2023 were analyzed. 48 patients received allogeneic HSCT from an HLA-matched donor (24 matched sibling donors, 24 matched unrelated donors) and nine from a haploidentical donor. Although there was no significant sex difference across the entire cohort, significantly more male ($n = 8$) than female ($n = 1$) patients had a haploidentical HSCT ($p = 0.025$). The median age of recipients at HSCT was 52.5 years (range, 17.4 to 67 years). All DLIs were performed with donor lymphocytes obtained by unstimulated leukapheresis. The median time to first DLI after allogeneic HSCT was 301 days (range, 98 to 2025 days), and the median age of the DLI donor was 42.5 years (range, 19 to 66 years). The median number of DLI cycles was two, and the median total DLI cell dose was 8.75×10^6 $CD3^+$ cells per kilogram of body weight. Fifty patients received DLI with therapeutic intent in overt relapse and seven patients were treated pre-emptively (three with incomplete donor chimerism and four with MRD). Forty-two patients had received one or more treatment lines before their DLI, mostly with hypomethylating agents (47.4%). No significant differences were observed between the two groups regarding patient age, diagnosis, type of conditioning regimen, additional lines of treatment before DLI use, and donor characteristics. The median follow-up time of the whole cohort (survivors and non-survivors) was 516 days (range, 39 to 4523 days).

3.2 | Efficacy Analysis of DLI

First, we examined the outcome after DLI. The median OS after DLI in the entire cohort was 517 days (95%-CI: 349 to 685 days), and the 1-year OS after DLI application was 62.5% (Figure 1). The main causes of death after DLI included disease progression (62.8%), infections (20.9%), and GvHD (4.7%). In univariate analysis, older patient age, HLA-identical donor, post-HSCT treatment naivety, and indication for preemptive DLI were significantly associated with longer OS. In addition, indication for preemptive DLI and absence of other lines of treatment before DLI were associated with significantly better PFS (Table 2). Median OS after DLI, stratified by HLA match, was 1.7 years for non-haploidentical and 0.5 years for haploidentical donors ($p = 0.003$), but no significant difference in PFS was observed (Figure 2). When analyzing only therapeutically intended DLI, haploidentical DLIs were still associated with worse OS compared to non-haploidentical DLIs (0.5 years vs. 1.4 years; $p = 0.022$; Figure S1). Moreover, multivariate Cox regression analysis revealed that an HLA-identical donor was a significantly favorable factor for OS after DLI (HR: 2.318; 95%-CI: 1.032–5.534; $p = 0.048$). On the other hand, a therapeutic DLI indication (HR: 0.050; 95%-CI: 0.006–0.397; $p = 0.005$) and patient age below 45 years (HR: 0.438; 95%-CI: 0.01–0.889; $p = 0.029$) were statistically significant unfavorable factors for OS after DLI (Table 3). When comparing DLI dose stratified by median total cell dose, no significant difference in OS or PFS was observed (Figure S2).

3.3 | GvHD Incidence and Risk Factors

We then analyzed the incidence of acute and chronic GvHD induced by DLI. The incidence of grade II-IV acute GvHD and chronic GvHD after DLI was 26.3% and 24.6%, respectively. The cumulative incidence of extensive chronic GvHD was 15.8%. In univariate analysis, neither patient age, sex, conditioning regimen chosen for allogeneic HSCT, donor HLA match, DLI indication, nor DLI donor age were significantly correlated with the incidence of acute or chronic GvHD in our study cohort (Table 2). Furthermore, there was no significant difference in PFS or OS when stratified for acute or chronic GvHD (Figures S3–S5). Finally, multivariate analysis revealed no significant association between GvHD and OS in our study population (Table 3).

4 | Discussion

In our retrospective cohort study of patients with high-risk myeloid neoplasms treated with DLI, we observed a significantly inferior survival outcome with DLI from a haploidentical donor compared to that from an HLA-matched donor. Previously, some retrospective studies have indicated that the outcomes of haploidentical DLI in patients with hematologic

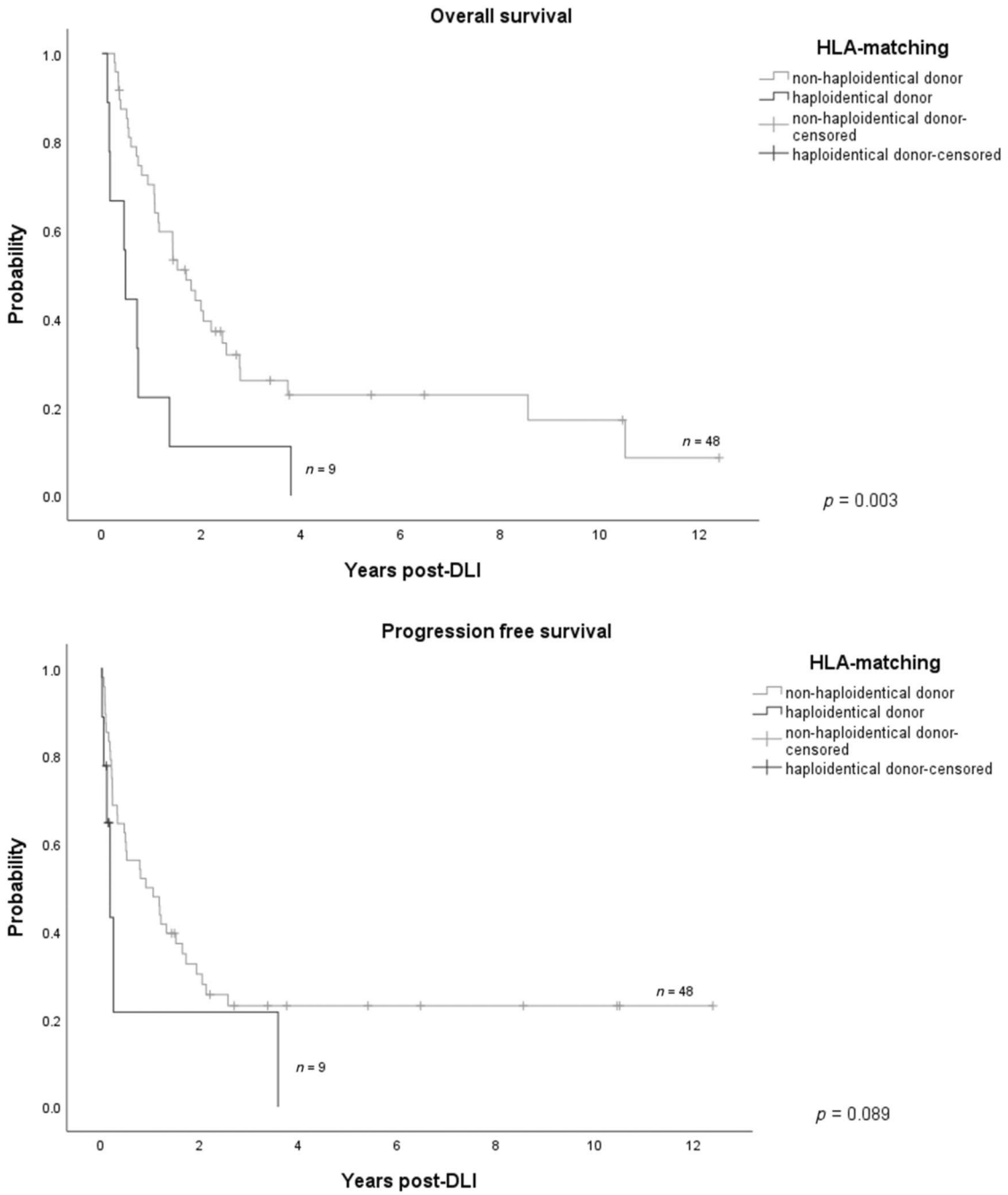

FIGURE 2 | Overall survival and progression-free survival stratified according to HLA matching. Non-haploidentical donor—median OS (SD; 95%-CI): 1.7 years (0.295; 1.107–2.262) Haploidentical donor—median OS (SD; 95%-CI): 0.5 years (0.045; 0.38–0.557). Non-haploidentical donor—median PFS (SD; 95%-CI): 0.9 years (0.386; 0.148–1.661). Haploidentical donor—median PFS (SD; 95%-CI): 0.2 years (0.063; 0.057–0.305).

TABLE 3 | Multivariate Cox regression analysis for overall survival ($n = 57$).

Variable	HR	95%-CI	*p*
Sex (female vs. male)	0.949	0.473–1.906	0.883
Patient's age (< 45 years vs. ≥ 45 years)	**0.438**	**0.209–0.919**	**0.029**
HLA-identical vs. haploidentical donor	**2.474**	**1.029–5.95**	**0.043**
Type of conditioning (MAC vs. RIC)	1.596	0.841–3.031	0.153
Indication of DLI (therapeutic vs. preemptive)	**0.094**	**0.01–0.889**	**0.039**
Other treatment lines post-HSCT before DLI (no vs. yes)	1.995	0.785–5.072	0.147
aGvHD ≥°II post-DLI	1	0.517–1.933	1
Extensive cGvHD post-DLI	0.636	0.256–1.576	0.328

Note: Bold values have a significance level < 0.05.

Abbreviations: aGvHD, acute graft-versus-host disease; cGvHD, chronic graft-versus-host disease; CI, confidence interval; DLI, donor lymphocyte infusion; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; MAC, myeloablative conditioning; RIC, reduced intensity conditioning.

relapse are comparable to non-haploidentical DLI [18, 19]. However, a study by Harada et al. investigating DLI after haploidentical HSCT revealed a 1-year survival rate of only 13.5%, indicating that haploidentical DLI may be less effective in AML patients [20]. The unfavorable outcomes observed in our study following haploidentical DLI may be, at least in part, attributable to the characteristics of our patient population, as all haploidentical DLIs were administered with a therapeutic indication. Nevertheless, even when only therapeutically intended DLIs were analyzed, haploidentical DLI demonstrated inferior OS. Furthermore, our multivariate analysis revealed that DLI from a haploidentical donor remained a predictive risk factor for inferior OS. One potential explanation for this finding is that immune escape mechanisms, such as impaired HLA expression, have been demonstrated to be prevalent in relapsed myeloid neoplasms following HSCT [21–23]. The frequency of HLA loss has been shown to correlate with the donor source, with an inverse relationship to the degree of donor-recipient mismatch [24]. Moreover, the cell dose and timing of DLI after HSCT may also influence the response. The initial DLI cell dosage is typically lower than that for HLA-matched DLI [16], although dose–response correlations for OS or GvHD are not yet well established in the literature [7, 11, 18, 19, 25]. Nevertheless, further investigation is required to substantiate this observation.

In accordance with previously published data [18, 20, 26–34], our findings demonstrated that preemptively administered DLI yielded considerably superior outcomes compared to therapeutically indicated DLI. These findings indicate that overt relapse in myeloid neoplasms may not be adequately managed with DLI as a sole therapeutic modality. It has been consistently demonstrated that the combination of chemotherapy plus DLI is more effective than either DLI [35] or chemotherapy alone [36] in the treatment of overt AML relapses following HSCT. Promising DLI combination strategies, for example with epigenetic modulators, tyrosine kinase inhibitors, BCL2 inhibitors, or in combination with immunomodulatory drugs, have been described elsewhere [7, 33, 37–40]. While these strategies may prove beneficial, further investigation is required to determine the most efficacious approach [17]. Moreover, our findings lend support to the practice of regular monitoring for MRD and the implementation of preemptive DLI in instances of molecular relapse as a potential therapeutic option for patients exhibiting no signs of higher-grade GvHD [30, 33, 41, 42].

In consideration of toxicity, the incidence of higher-grade acute and chronic GvHD in our cohort was comparable with other published data. However, there was a discrepancy in the reported incidence and risk of haploidentical DLI-induced GvHD, as evidenced by reports [18, 19, 25, 43, 44]. For example, EBMT has recently published the outcomes of a cohort of 192 adult patients with acute leukemia who received preemptive DLI after HLA-matched donor transplants. The cumulative incidence of clinically relevant acute GvHD or chronic GvHD at 5 years was 33.7% [12]. Our univariate analyses revealed no significant correlation between the incidence of GvHD and several key factors, including donor HLA match, DLI donor age, conditioning regimen selected for HSCT, patient age, or patient sex. Furthermore, the presence or absence of acute or chronic GvHD did not significantly impact OS or PFS in our study population. Consequently, we were unable to corroborate the assertion that acute GvHD following non-haploidentical DLI confers superior survival outcomes in patients with relapsed AML as proposed by Eefting and colleagues [45].

In addition to the inherent limitations of a single-center retrospective analysis, this study is further constrained by the relatively small number of patients who received haploidentical DLI and the absence of comprehensive MRD data for all patients surveyed. Furthermore, while preemptive DLI was typically administered as monotherapy, therapeutic DLI was frequently preceded by other therapies, such as hypomethylating agents, which may limit the ability to fully assess its impact on outcome.

In conclusion, the findings of this study indicate that non-haploidentical DLI yields superior outcomes compared to haploidentical DLI. Furthermore, the data demonstrated that preemptively applied DLI elicited markedly more favorable responses than therapeutically administered DLI in patients with high-risk myeloid neoplasms. It is possible that the dosage regimens of haploidentical DLI are not optimal for the treatment of relapsed AML or MDS following HSCT. One potential avenue for further investigation could be the administration of haploidentical DLI at higher cell doses or as a combination strategy. In light of the suboptimal outcomes observed in our study cohort, the use of DLI alone may be limited to preemptive indications only. Moreover, distinct immune escape mechanisms, such as HLA loss, which play a particular role in haploidentical transplantation, may contribute to a

decreased efficacy. Nevertheless, further research is required to substantiate our findings and gain a deeper mechanistic understanding.

Author Contributions

Tobias Matthieu Benoit: conceptualization, data curation, formal analysis, investigation, project administration, visualization, and writing – original draft. **Dominik Schneidawind:** conceptualization, methodology, supervision, writing – review & editing. **Yvonne Zaugg-Berger:** resources, data curation. **Adrian Bachofner, Nathan Wolfensberger, and Markus Gabriel Manz:** supervision, validation, writing – review & editing. All authors read and approved the submitted manuscript.

Acknowledgements

The authors would like to thank all participating patients and their families for their support, without which this study would not have been possible.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The materials described in the manuscript, including all relevant raw data, are available from the corresponding author upon reasonable request.

References

1. B. Dholaria, B. N. Savani, B. K. Hamilton, et al., "Hematopoietic Cell Transplantation in the Treatment of Newly Diagnosed Adult Acute Myeloid Leukemia: An Evidence-Based Review From the American Society of Transplantation and Cellular Therapy," *Transplantation and Cellular Therapy* 27, no. 1 (2021): 6–20.

2. J. A. Snowden, I. Sanchez-Ortega, S. Corbacioglu, et al., "Indications for Haematopoietic Cell Transplantation for Haematological Diseases, Solid Tumours and Immune Disorders: Current Practice in Europe, 2022," *Bone Marrow Transplantation* 57, no. 8 (2022): 1217–1239.

3. A. Takami, "Hematopoietic Stem Cell Transplantation for Acute Myeloid Leukemia," *International Journal of Hematology* 107, no. 5 (2018): 513–518.

4. V. Sumbly, I. Landry, C. Sneed, et al., "Leukemic Stem Cells and Advances in Hematopoietic Stem Cell Transplantation for Acute Myeloid Leukemia: A Narrative Review of Clinical Trials," *Stem Cell Investigation* 9 (2022): 10.

5. N. Bejanyan, D. J. Weisdorf, B. R. Logan, et al., "Survival of Patients With Acute Myeloid Leukemia Relapsing After Allogeneic Hematopoietic Cell Transplantation: A Center for International Blood and Marrow Transplant Research Study," *Biology of Blood and Marrow Transplantation* 21, no. 3 (2015): 454–459.

6. R. Zeiser, D. W. Beelen, W. Bethge, et al., "Biology-Driven Approaches to Prevent and Treat Relapse of Myeloid Neoplasia After Allogeneic Hematopoietic Stem Cell Transplantation," *Biology of Blood and Marrow Transplantation* 25, no. 4 (2019): e128–e140.

7. B. Dholaria, B. N. Savani, M. Labopin, et al., "Clinical Applications of Donor Lymphocyte Infusion From an HLA-Haploidentical Donor: Consensus Recommendations From the Acute Leukemia Working Party of the EBMT," *Haematologica* 105, no. 1 (2020): 47–58.

8. F. Kreidieh, I. Abou Dalle, N. Moukalled, et al., "Relapse After Allogeneic Hematopoietic Stem Cell Transplantation in Acute Myeloid Leukemia: An Overview of Prevention and Treatment," *International Journal of Hematology* 116, no. 3 (2022): 330–340.

9. R. Guieze, G. Damaj, B. Pereira, et al., "Management of Myelodysplastic Syndrome Relapsing After Allogeneic Hematopoietic Stem Cell Transplantation: A Study by the French Society of Bone Marrow Transplantation and Cell Therapies," *Biology of Blood and Marrow Transplantation* 22, no. 2 (2016): 240–247.

10. G. Orti, P. Barba, L. Fox, O. Salamero, F. Bosch, and D. Valcarcel, "Donor Lymphocyte Infusions in AML and MDS: Enhancing the Graft-Versus-Leukemia Effect," *Experimental Hematology* 48 (2017): 1–11.

11. N. Santoro, J. E. Mooyaart, R. Devillier, et al., "Donor Lymphocyte Infusions After Haploidentical Stem Cell Transplantation With PTCY: A Study on Behalf of the EBMT Cellular Therapy & Immunobiology Working Party," *Bone Marrow Transplantation* 58, no. 1 (2023): 54–60.

12. C. Schmid, M. Labopin, N. Schaap, et al., "Long-Term Results and GvHD After Prophylactic and Preemptive Donor Lymphocyte Infusion After Allogeneic Stem Cell Transplantation for Acute Leukemia," *Bone Marrow Transplantation* 57, no. 2 (2022): 215–223.

13. J. R. Passweg, H. Baldomero, P. Bader, et al., "Use of Haploidentical Stem Cell Transplantation Continues to Increase: The 2015 European Society for Blood and Marrow Transplant Activity Survey Report," *Bone Marrow Transplantation* 52, no. 6 (2017): 811–817.

14. S. H. C. Baumeister, B. Rambaldi, R. M. Shapiro, and R. Romee, "Key Aspects of the Immunobiology of Haploidentical Hematopoietic Cell Transplantation," *Frontiers in Immunology* 11 (2020): 191.

15. L. Luznik, P. V. O'Donnell, H. J. Symons, et al., "HLA-Haploidentical Bone Marrow Transplantation for Hematologic Malignancies Using Nonmyeloablative Conditioning and High-Dose, Posttransplantation Cyclophosphamide," *Biology of Blood and Marrow Transplantation* 14, no. 6 (2008): 641–650.

16. J. De Vos, E. Baudoux, J. O. Bay, et al., "Donor Lymphocyte Infusions (DLI): Guidelines From the Francophone Society of Bone Marrow Transplantation and Cellular Therapy (SFGM-TC)," *Bulletin du Cancer* 106, no. S1 (2019): S35–S39.

17. S. Pagliuca, C. Schmid, N. Santoro, et al., "Donor Lymphocyte Infusion After Allogeneic Haematopoietic Cell Transplantation for Haematological Malignancies: Basic Considerations and Best Practice Recommendations From the EBMT," *Lancet Haematology* 11, no. 6 (2024): e448–e458.

18. A. M. Zeidan, P. M. Forde, H. Symons, et al., "HLA-Haploidentical Donor Lymphocyte Infusions for Patients With Relapsed Hematologic Malignancies After Related HLA-Haploidentical Bone Marrow Transplantation," *Biology of Blood and Marrow Transplantation* 20, no. 3 (2014): 314–318.

19. A. Ghiso, A. M. Raiola, F. Gualandi, et al., "DLI After Haploidentical BMT With Post-Transplant CY," *Bone Marrow Transplantation* 50, no. 1 (2015): 56–61.

20. K. Harada, S. Mizuno, S. Yano, et al., "Donor Lymphocyte Infusion After Haploidentical Hematopoietic Stem Cell Transplantation for Acute Myeloid Leukemia," *Annals of Hematology* 101, no. 3 (2022): 643–653.

21. R. Zeiser and L. Vago, "Mechanisms of Immune Escape After Allogeneic Hematopoietic Cell Transplantation," *Blood* 133, no. 12 (2019): 1290–1297.

22. T. Sauerer, G. F. Velazquez, and C. Schmid, "Relapse of Acute Myeloid Leukemia After Allogeneic Stem Cell Transplantation: Immune Escape Mechanisms and Current Implications for Therapy," *Molecular Cancer* 22, no. 1 (2023): 180.

23. M. J. Christopher, A. A. Petti, M. P. Rettig, et al., "Immune Escape of Relapsed AML Cells After Allogeneic Transplantation," *New England Journal of Medicine* 379, no. 24 (2018): 2330–2341.

24. L. Vago, C. Toffalori, M. Ahci, et al., "Incidence of HLA Loss in a Global Multicentric Cohort of Post-Transplantation Relapses: Results From the Hlaloss Collaborative Study," *Blood* 132, no. S1 (2018): 818.

25. S. R. Goldsmith, M. Slade, J. F. DiPersio, et al., "Donor-Lymphocyte Infusion Following Haploidentical Hematopoietic Cell Transplantation With Peripheral Blood Stem Cell Grafts and PTCy," *Bone Marrow Transplantation* 52, no. 12 (2017): 1623–1628.

26. C. Schmid, M. Labopin, A. Nagler, et al., "Donor Lymphocyte Infusion in the Treatment of First Hematological Relapse After Allogeneic Stem-Cell Transplantation in Adults With Acute Myeloid Leukemia: A Retrospective Risk Factors Analysis and Comparison With Other Strategies by the EBMT Acute Leukemia Working Party," *Journal of Clinical Oncology* 25, no. 31 (2007): 4938–4945.

27. Y. Tan, K. Du, Y. Luo, et al., "Superiority of Preemptive Donor Lymphocyte Infusion Based on Minimal Residual Disease in Acute Leukemia Patients After Allogeneic Hematopoietic Stem Cell Transplantation," *Transfusion* 54, no. 6 (2014): 1493–1500.

28. A. Dominietto, S. Pozzi, M. Miglino, et al., "Donor Lymphocyte Infusions for the Treatment of Minimal Residual Disease in Acute Leukemia," *Blood* 109, no. 11 (2007): 5063–5064.

29. X. D. Mo, X. H. Zhang, L. P. Xu, et al., "Haploidentical Hematopoietic Stem Cell Transplantation for Myelodysplastic Syndrome," *Biology of Blood and Marrow Transplantation* 23, no. 12 (2017): 2143–2150.

30. A. R. Rettig, G. Ihorst, H. Bertz, et al., "Donor Lymphocyte Infusions After First Allogeneic Hematopoietic Stem-Cell Transplantation in Adults With Acute Myeloid Leukemia: A Single-Center Landmark Analysis," *Annals of Hematology* 100, no. 9 (2021): 2339–2350.

31. L. E. Caldemeyer, L. P. Akard, J. R. Edwards, A. Tandra, D. R. Wagenknecht, and M. J. Dugan, "Donor Lymphocyte Infusions Used to Treat Mixed-Chimeric and High-Risk Patient Populations in the Relapsed and Nonrelapsed Settings After Allogeneic Transplantation for Hematologic Malignancies Are Associated With High Five-Year Survival if Persistent Full Donor Chimerism Is Obtained or Maintained," *Biology of Blood and Marrow Transplantation* 23, no. 11 (2017): 1989–1997.

32. A. W. Loren and D. L. Porter, "Donor Leukocyte Infusions for the Treatment of Relapsed Acute Leukemia After Allogeneic Stem Cell Transplantation," *Bone Marrow Transplantation* 41, no. 5 (2008): 483–493.

33. A. Biederstadt and K. Rezvani, "How I Treat High-Risk Acute Myeloid Leukemia Using Preemptive Adoptive Cellular Immunotherapy," *Blood* 141, no. 1 (2023): 22–38.

34. T. Guillaume, S. Porcheron, F. Audat, et al., "Prophylactic, Preemptive and Curative Use of Donor Lymphocyte Infusion in Patients Undergoing Allogeneic Stem Cell Transplantation: Guidelines of the SFGM-TC," *Pathologie-Biologie* 62, no. 4 (2014): 193–196.

35. J. E. Levine, T. Braun, S. L. Penza, et al., "Prospective Trial of Chemotherapy and Donor Leukocyte Infusions for Relapse of Advanced Myeloid Malignancies After Allogeneic Stem-Cell Transplantation," *Journal of Clinical Oncology* 20, no. 2 (2002): 405–412.

36. C. H. Yan, J. Z. Wang, D. H. Liu, et al., "Chemotherapy Followed by Modified Donor Lymphocyte Infusion as a Treatment for Relapsed Acute Leukemia After Haploidentical Hematopoietic Stem Cell Transplantation Without In Vitro T-Cell Depletion: Superior Outcomes Compared With Chemotherapy Alone and an Analysis of Prognostic Factors," *European Journal of Haematology* 91, no. 4 (2013): 304–314.

37. N. R. Mathew, F. Baumgartner, L. Braun, et al., "Sorafenib Promotes Graft-Versus-Leukemia Activity in Mice and Humans Through IL-15 Production in FLT3-ITD-Mutant Leukemia Cells," *Nature Medicine* 24, no. 3 (2018): 282–291.

38. O. Amit, Y. B. On, G. Perez, L. Shargian-Alon, M. Yeshurun, and R. Ram, "Venetoclax and Donor Lymphocyte Infusion for Early Relapsed Acute Myeloid Leukemia After Allogeneic Hematopoietic Cell Transplantation. A Retrospective Multicenter Trial," *Annals of Hematology* 100, no. 3 (2021): 817–824.

39. T. Schroeder, A. Czibere, U. Platzbecker, et al., "Azacitidine and Donor Lymphocyte Infusions as First Salvage Therapy for Relapse of AML or MDS After Allogeneic Stem Cell Transplantation," *Leukemia* 27, no. 6 (2013): 1229–1235.

40. J. Steinmann, H. Bertz, R. Wasch, et al., "5-Azacytidine and DLI Can Induce Long-Term Remissions in AML Patients Relapsed After Allograft," *Bone Marrow Transplantation* 50, no. 5 (2015): 690–695.

41. S. Park, T. Y. Kim, J. H. Lee, et al., "Differential Effects of Donor Lymphocyte Infusion Upon Treatment Response and GVHD According to Relapse Level and Donor Sources in Patients With Myelodysplastic Syndrome," *Therapeutic Advances in Hematology* 12 (2021): 20406207211043748.

42. A. Spyridonidis, "How I Treat Measurable (Minimal) Residual Disease in Acute Leukemia After Allogeneic Hematopoietic Cell Transplantation," *Blood* 135, no. 19 (2020): 1639–1649.

43. X. J. Huang, D. H. Liu, K. Y. Liu, L. P. Xu, H. Chen, and W. Han, "Donor Lymphocyte Infusion for the Treatment of Leukemia Relapse After HLA-Mismatched/Haploidentical T-Cell-Replete Hematopoietic Stem Cell Transplantation," *Haematologica* 92, no. 3 (2007): 414–417.

44. T. Miyamoto, T. Fukuda, M. Nakashima, et al., "Donor Lymphocyte Infusion for Relapsed Hematological Malignancies After Unrelated Allogeneic Bone Marrow Transplantation Facilitated by the Japan Marrow Donor Program," *Biology of Blood and Marrow Transplantation* 23, no. 6 (2017): 938–944.

45. M. Eefting, P. A. von dem Borne, L. C. de Wreede, et al., "Intentional Donor Lymphocyte-Induced Limited Acute Graft-Versus-Host Disease Is Essential for Long-Term Survival of Relapsed Acute Myeloid Leukemia After Allogeneic Stem Cell Transplantation," *Haematologica* 99, no. 4 (2014): 751–758.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.