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Donor Lymphocyte Infusions for Chronic Myeloid Leukemia Relapsing after Allogeneic Stem Cell Transplantation: May We Predict Graft-versus-Leukemia Without Graft-versus-Host Disease?

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A B S T R A C T

Donor lymphocyte infusions (DLI) are an effective treatment for relapsed chronic myeloid leukemia (CML) after allogeneic stem cell transplantation (alloSCT). Leukemia resistance and secondary graft-versus-host disease (GVHD) are major obstacles to success with DLI. The aim of this study was to identify pre-DLI factors associated with prolonged survival in remission without secondary GVHD. We retrospectively analyzed 500 patients treated with DLI for CML relapse (16% molecular, 30% cytogenetic, and 54% hematological) after alloSCT. The overall probabilities of failure- and secondary GVHD–free survival (FGFS) were 29% and 27% at 5 and 10 years after DLI, respectively. The type of relapse was the major factor influencing FGFS (40% for molecular and/or cytogenetic relapse and 20% for hematological relapse at 5 years, $P < .001$). Chronic GVHD before DLI and an interval <1 year between alloSCT and first DLI were independently associated with inferior FGFS in patients with molecular and/or cytogenetic relapse. Consequently, FGFS was 13%, 35%, to 56% at 5 years in patients with 2, 1, and 0 adverse features, respectively. In patients with hematological relapse, independent adverse prognostic factors for FGFS were initial dose of $CD3^+$ cells $\geq 50 \times 10^6$/kg, donor-recipient sex mismatch, and chronic GVHD before DLI. FGFS was 0%, 17%, 33%, to 37% in patients with 3, 2, 1, and 0 adverse features, respectively. The probability of survival in remission without secondary GVHD was highest (>50% at 5 years) when DLI were given beyond 1 year from alloSCT for molecular and/or cytogenetic CML relapse that was not preceded by chronic GVHD.

INTRODUCTION

Despite the introduction of imatinib and other tyrosine kinase inhibitors (TKI), allogeneic stem cell transplantation (alloSCT) remains an important treatment option for chronic

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myeloid leukemia (CML) patients who do not respond adequately to TKI therapy [1]. Currently, alloSCT is recommended for eligible patients in advanced-phase CML and in instances of failure of and/or intolerance to TKI treatment [2]. Because of advances in supportive care and improvement of conditioning regimens, relapse of the original malignancy has become the most common cause of treatment failure and mortality after alloSCT [3].

Treatment with donor lymphocyte infusions (DLI) represents 1 of the most established therapeutic approaches to post-allograft relapse and has radically changed the prognosis of CML patients relapsing after alloSCT [4,5]. Responses achieved after DLI in relapsed CML are frequently durable, offering potential cure for the majority of patients [6-8]. CML relapse may be diagnosed at the molecular, cytogenetic, and hematological level, resulting in extreme heterogeneity of patient and disease status when treatment with DLI is applied [9-11]. This is even more complex nowadays with the availability of TKI, capable of restoring complete molecular remission (CMR) after relapse [12-14].

Major obstacles to success with DLI are represented by leukemia resistance and by the induction of graft-versus-host disease by the infused lymphocytes (secondary GVHD), the latter representing the most threatening side-effect of DLI treatment [4]. Response to DLI, incidence of secondary GVHD, and outcome of patients developing secondary GVHD have been reported in previous studies by the Chronic Malignancies Working Party of the European Group for Blood and Marrow Transplantation (EBMT) [15-17]. Occurring in up to 40% of patients, secondary GVHD was shown to be associated with a 2.3-fold increased risk of death compared with patients without secondary GVHD [17]. With regard to the availability of TKI, additional treatment modalities are available for preventing and treating relapse after alloSCT. However, since alloSCT is increasingly performed in TKI-resistant patients, DLI is an attractive and important treatment modality, especially if the resistant clone persists. It is evident that the best result is obtained when a patient treated with DLI achieves a durable molecular remission without experiencing secondary GVHD. In fact, CML response to DLI is frequently, but not always, separated from secondary GVHD, suggesting that the graft-versus-leukemia effect (GVL) may be independent of the development of secondary GVHD [6,9,16,18]. However, it remains unclear what the proportion of such patients is and which factors may predict for such a favorable outcome when DLI are administered for CML relapsing after alloSCT. The aim of this study was, therefore, to identify pre-DLI factors associated with probability of survival in remission without secondary GVHD. The information may be used to determine when treatment for CML relapse with DLI can be "optimal" (ie, high chance of achieving a prolonged survival and a durable remission without experiencing secondary GVHD).

PATIENTS AND METHODS

This study was based on the registry of the EBMT and conducted within the Chronic Malignancies Working Party. The study was approved by the review board of the Chronic Malignancies Working Party. EBMT member centers were asked to report and update their experience with patients treated with unmanipulated DLI for recurrent CML after the first alloSCT from an HLA-identical sibling or an HLA-matched volunteer unrelated donor. The reports included adequate information collected on disease response, secondary GVHD, and survival after DLI.

Lymphocytes were collected from the donors by apheresis on 1 or more occasions and administered as single or multiple infusions. Infusions given on multiple days had to be at least 7 days apart to be counted as separate infusions. The phase of CML was classified in accordance with the criteria proposed by the Center for International Blood and Marrow Transplant Research [19]. Relapse was classified as molecular, cytogenetic, or hematological in accordance with previous reports [16,17]. Patients treated with DLI for CML relapse in blast crisis were excluded. Relapse stage at DLI was defined as molecular, cytogenetic, and hematological assessed on the date of first DLI infusion after relapse or closest date before this infusion. Acute and chronic GVHD occurring after DLI was reported according to the standard clinical criteria and in accordance with previous reports [16,17]. A total of 500 patients treated with DLI for CML relapse at 68 EBMT centers between 1988 and 2004 had complete data for analysis. None of the patients had received imatinib before transplantation. All patients received DLI for relapse of CML after alloSCT in absence of GVHD and/or its treatment.

Survival was calculated from the date of the first infusion of donor lymphocytes until death or last follow-up evaluation. Failure-free survival (FFS) was calculated from the date of the first infusion of donor lymphocytes until death, last follow-up evaluation, or occurrence of an event such as unresponsiveness to DLI or relapse after response to DLI. Failure- and secondary GVHD–free survival (FGFS) was calculated from the date of the first infusion of donor lymphocytes until death, last follow-up evaluation, or occurrence of an event, such as unresponsiveness to DLI, relapse after response to DLI, or secondary GVHD.

Survival curves were calculated according to the method of Kaplan and Meier; the log-rank test was used to compare survival curves; a proportional hazard regression model (Cox model) was used for survival probabilities [20]. We studied the following possible risk factors (categorization criteria): patient gender (0 = male, 1 = female), patient age at DLI (0 = <40 years, 1 = ≥40 years), donor type (0 = HLA-identical sibling, 1 = unrelated), donor gender (0 = male, 1 = female), sex mismatch with the donor (0 = matched, 1 = mismatched, ie, female donor/male recipient and male donor/female recipient), phase at alloSCT (0 = first chronic phase [CP1], 1 = beyond CP1), stem cell source (0 = bone marrow, 1 = peripheral blood), total body irradiation in the conditioning regimen (0 = no, 1 = yes), T cell depletion (0 = no, 1 = yes), acute GVHD before DLI (0 = no, 1 = yes), chronic GVHD before DLI (0 = no, 1 = yes), interval from alloSCT to DLI (0 = ≥1 year, 1 = <1 year), type of relapse (0 = molecular and/or cytogenetic, 1 = hematological), initial cell dose (ie, donor CD3 + cells/kg recipient body weight of first transfusion) (0 = <median value, 1 = ≥median value). Factors significantly associated with shorter FGFS in univariate analysis were tested for their predictive value on FGFS in multivariate Cox regression models. Hazard ratios were estimated with 95% confidence interval (95% CI). Values of $P < .05$ were considered statistically significant.

RESULTS

Patient Characteristics

Fifty-nine percent of patients were males and the median patient age at time of DLI was 39 (range, 4 to 64) years with 15% older than 50 years. The donor was an HLA-identical sibling in 73% and unrelated in 27%. The donor was female in 37% of the cases and 44% of patients were sex mismatched with the donor. AlloSCT was performed in CP1 in 410 patients (82%), whereas 89 (18%) underwent transplantation in more advanced phases of the disease. Stem cell source was bone marrow in 408 (86%), peripheral blood in 64 (14%), and information was not available in 28 patients. All patients were conditioned with a standard regimen, including total body irradiation in 77% of the cases. GVHD prophylactic measures included in vivo T cell depletion in 241 patients (51%). A total of 316 patients (69%) had GVHD before relapse: 124 acute GVHD only, 66 chronic GVHD only, 114 acute and chronic GVHD, and 12 GVHD unclassified. Median follow-up time of surviving patients was 56 months (range, 1 to 168).

DLI Characteristics

DLI was started at a median interval of 23 months from alloSCT (range, 1 to 146 months) in 132 patients (26%) within 12 months from alloSCT. Relapse type was molecular in 80 (16%), cytogenetic in 150 (30%), and hematological in 270 (54%) cases. DLI was started with a cell dose of $\leq 20 \times 10^6$ $CD3^+$ cells/kg recipient body weight in 62% of patients; 207 patients (41%) received 2 or more additional infusions of donor cells. Cumulative cell dose ranged from 1×10^5 to 1.4×10^9 $CD3^+$ cells/kg recipient body weight (median, 70×10^6

$CD3^+$ cells/kg recipient body weight). Data on donor chimerism were available in a total of 44 patients. At the time of DLI, a total of 11 patients were taking immunosuppressive medication (ie, cyclosporine A). A total of 27 patients (5%) received concomitant imatinib therapy with DLI.

Outcome

After DLI administration, response to DLI was assessed in a total of 480 patients (20 patients had missing information). Cytogenetic complete remission and/or CMR were achieved in 341 patients (71%) in a median of 7.5 months (within 41 months in 95% of the cases) from first DLI. The median duration of response was 35 (range, 0 to 150) months. A total of 139 patients showed no response after DLI treatment. Twenty-three patients experienced a recurrence at a median of 11 months from the maximal response (range, 2 to 38).

A total of 222 (44%) patients developed secondary GVHD a median of 3 (range, .2 to 77) months from first DLI (within 32 months in 95% of the cases). Secondary acute GVHD occurred in 191 (38%) patients at a median of 2 (range, .2 to 42) months after first DLI (within 29 months in 95% of the cases) with 61, 70, 40, and 20 patients being diagnosed with secondary acute GVHD grade 1, 2, 3, and 4, respectively. Secondary chronic GVHD occurred in 87 (17%) patients (limited and extensive chronic GVHD in 50 and 37 patients, respectively) at a median of 7 (range, 1 to 77) months after first DLI (within 41 months in 95% of the cases). A total of 29 patients experienced secondary chronic GVHD without preceding secondary acute GVHD.

Estimated probability of survival was 64% (95% CI, 62% to 66%) and 59% (95% CI, 56% to 62%) at 5 and 10 years after DLI, respectively. Estimated probability of FFS was 57% (95% CI, 55% to 59%) and 54% (95% CI, 51% to 57%) at 5 and 10 years after DLI, respectively. Estimated probability of FGFS was 29% (95% CI, 27% to 31%) and 27% (95% CI, 24% to 30%) at 5 and 10 years after DLI, respectively (Figure 1).

For the purpose of this analysis, patients treated in molecular relapse and those treated in cytogenetic relapse were grouped together because they had similar FFS (68% and 73% at 5 years after DLI, respectively, $P = .50$) and FGFS (40% and 39% at 5 years after DLI, respectively, $P = .80$). Estimated probability of FFS at 5 years after DLI was 71% in molecular and/or cytogenetic relapses and 46% in hematological relapses ($P < .001$) (Figure 2A). Estimated probability of FGFS at 5 years after DLI was 40% in molecular and/or cytogenetic relapses and 20% in hematological relapses ($P < .001$) (Figure 2B). It should be noted that for the purpose of this study, secondary acute and chronic GVHD of all severity grades were considered in the composite endpoint of FGFS. Because type of relapse was a major factor influencing FGFS, prognostic factors were analyzed separately, according to the type of relapse.

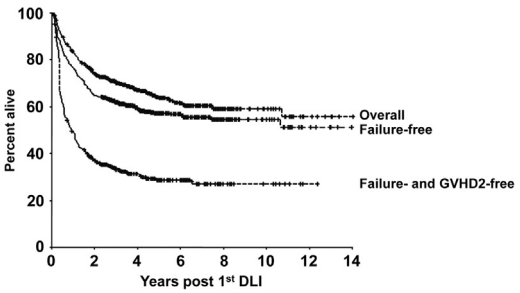

Figure 1. Overall survival, FFS, and FGFS in 500 patients treated with DLI for relapse of CML after alloSCT. Probability of survival at 5 and 10 years after DLI was 64% (95% CI, 62% to 66%) and 59% (95% CI, 56% to 62%), respectively. Probability of FFS was 57% (95% CI, 55% to 59%) and 54% (95% CI, 51% to 57%) at 5 and 10 years after DLI, respectively. Probability of FGFS was 29% (95% CI, 27% to 31%) and 27% (95% CI, 24% to 30%) at 5 and 10 years after DLI, respectively.

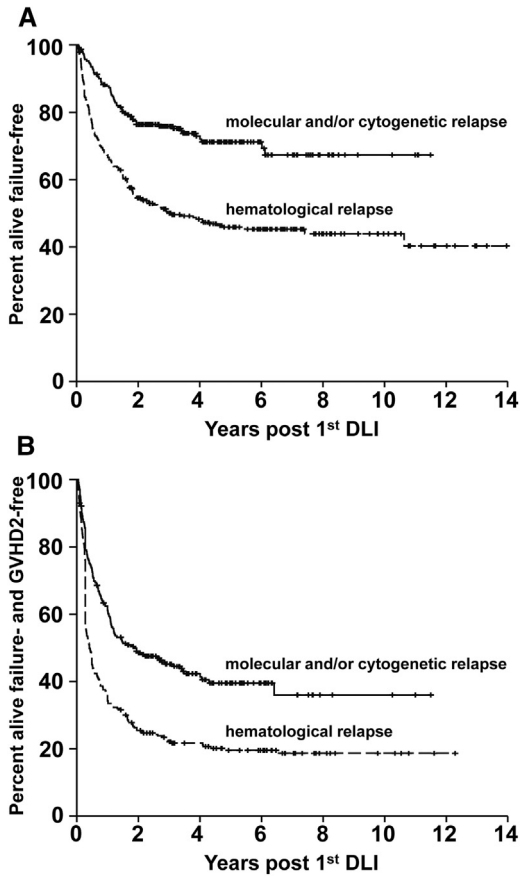

Figure 2. FFS (A) and FGFS (B) in 500 patients treated with DLI for relapse of CML after alloSCT according to the type of relapse (molecular and/or cytogenetic relapse, n = 230; hematological relapse, n = 270). (A) Probability of FFS at 5 years after DLI was 71% in molecular and/or cytogenetic relapses and 46% in hematological relapses ($P < .001$). (B) Probability FGFS at 5 years after DLI was 40% in molecular and/or cytogenetic relapses and 20% in hematological relapses ($P < .001$).

Molecular and/or Cytogenetic Relapse

Univariate analysis showed that 3 factors (ie, donor type, chronic GVHD before relapse, and interval from alloSCT to DLI) were significantly correlated to FGFS from DLI (Table 1).

Multivariate analysis showed that interval from SCT to DLI <1 year (hazard ratio [HR], 2.2; 95% CI, 1.5 to 3.2; $P < .001$) and a history of chronic GVHD before DLI (HR, 1.8; 95% CI, 1.3 to 2.5; $P = .001$) were independent adverse prognostic factors for FGFS (Table 2A). Forty-three percent, 44%, and 13% of patients had 0, 1, and 2 adverse features, respectively. Consequently, FGFS at 5 years was 13%, to 35%, and 56% in patients with 2, 1, and 0 adverse features, respectively ($P < .001$) (Figure 3A).

Hematological Relapse

Univariate analysis showed that 6 factors (ie, donor gender, sex mismatch with the donor, phase at alloSCT, chronic GVHD before relapse, interval from alloSCT to DLI,

Table 1
Clinical Features and FGFS in 500 Patients Treated with DLI for CML Relapsing after alloSCT According to the Type of Relapse (Univariate Analysis)

Clinical Features	Molecular and/or Cytogenetic Relapse				Hematological Relapse			
	Year of DLI, median (range) 1999 (1990-2004)				1996 (1988-2002)			
	Total No.	Total %	FGFS % at 5 Years	*P* Value	Total No.	Total %	FGFS % at 5 Years	*P* Value
Patient gender								
Male	125	54	38	.95	169	63	21	.56
Female	105	46	41		101	37	18	
Patient age								
<40 yr	115	50	40	.71	149	55	22	.14
≥40 yr	115	50	39		120	45	16	
NA	0				1			
Donor type								
HLA-identical sibling	157	68	45	.02	207	77	21	.30
Unrelated	73	32	29		63	23	16	
Donor gender								
Male	139	61	37	.78	174	65	23	.04
Female	89	39	43		92	35	14	
NA	2				4			
Donor-recipient sex Match								
Matched	128	56	42	.19	148	56	23	.03
Mismatched	100	44	35		118	44	16	
NA	2				4			
CML phase at alloSCT								
CP1	202	88	41	.22	208	77	23	.03
Beyond CP1	27	12	29		62	23	8	
NA	1							
Stem cell source								
BM	176	82	40	.50	232	90	24	.67
PB	38	18	43		26	10	14	
NA	16				12			
TBI								
No	53	23	37	.88	62	23	20	.98
Yes	174	77	41		204	77	20	
NA	3				4			
T cell depleted								
No	94	43	42	.38	139	54	19	.36
Yes	123	57	36		118	46	19	
NA	13				13			
Acute GVHD before DLI								
No	109	48	38	.35	134	50	23	.54
Yes	116	52	43		132	50	17	
NA	5				4			
Chronic GVHD before DLI								
No	130	59	49	.003	156	63	26	.02
Yes	89	41	29		93	37	13	
NA	11				21			
Time from alloSCT to DLI								
≥1 yr	160	70	48	<.001	207	77	22	.02
<1 yr	70	30	21		62	23	11	
NA	0				1			
Initial cell dose								
<Median*	78	39	43	.88	87	51	30	.003
≥Median	120	61	43		82	49	18	
NA	32				101			
Total	230	100	40		270	100	20	

NA indicates not assessed; BM, bone marrow; PB, peripheral blood; TBI, total body irradiation.
* Median value was of 10×10^6 $CD3^+$ cells/kg in patients with molecular and/or cytogenetic relapse, and of 50×10^6 $CD3^+$ cells/kg in patients with hematological relapse.

and initial cell dose) were significantly correlated to FGFS from DLI (Table 1).

Multivariate analysis showed that sex mismatch with the donor (HR, 1.6; 95% CI, 1.1 to 2.4, $P = .01$), a history of chronic GVHD before DLI (HR, 1.7; 95% CI, 1.2 to 2.5, $P = .006$), and an initial cell dose $\geq 50 \times 10^6$/kg (HR, 1.8; 95% CI, 1.2 to 2.7, $P = .002$) were independent adverse prognostic factors for FGFS (Table 2B). Seventeen percent, 40%, 33%, and 9% of patients had 0, 1, 2, and 3 adverse features, respectively. Survival in remission without secondary GVHD at 5 years was 0%, 17%, 33%, and 37% in patients with 3, 2, 1, and 0 adverse features, respectively ($P < .001$) (Figure 3B).

DISCUSSION

Response of CML to DLI is sometimes, but not always, accompanied by secondary GVHD that may be fatal in some cases. With escalating dose regimens, responses could be achieved without secondary GVHD and such outcome was not infrequent, particularly when DLI were given to treat molecular and/or cytogenetic relapse [16,21,22]. However, measures of such a phenomenon and factors associated with such positive outcomes have not yet been provided by previous studies. We retrospectively studied a large population of 500 patients treated with DLI for CML relapsing after alloSCT. Overall FFS and FGFS survival curves show that

Table 2
Cox Regression Analysis of Prognostic Factors for FGFS after DLI in Patients with Molecular and/or Cytogenetic CML Relapse and Patients with Hematological CML Relapse (Multivariate Analysis)

A. Molecular and/or Cytogenetic CML Relapse			
Parameter	HR	95% CI	*P* Value
Donor type			
HLA-identical sibling	1		
Unrelated	1.2	.8-1.7	.42
Chronic GVHD before DLI			
No	1		
Yes	1.8	1.3-2.5	.001
Time from alloSCT to DLI			
≥1 yr	1		
<1 yr	2.2	1.5-3.2	<.001
B. Hematological CML Relapse			
Parameter	HR	95% CI	*P* Value
Donor gender			
Male	1		
Female	1.3	.9-2.0	.14
Donor-recipient sex match			
Matched	1		
Mismatched	1.6	1.1-2.4	.01
CML phase at alloSCT			
CP1	1		
Beyond CP1	1.1	.7-1.7	.77
Chronic GVHD before DLI			
No	1		
Yes	1.7	1.2-2.5	.006
Time from alloSCT to DLI			
≥1 yr	1		
<1 yr	1.0	.6-1.7	.87
Initial cell dose			
$<50 \times 10^6$/kg	1		
$\geq 50 \times 10^6$/kg	1.8	1.2-2.7	.002

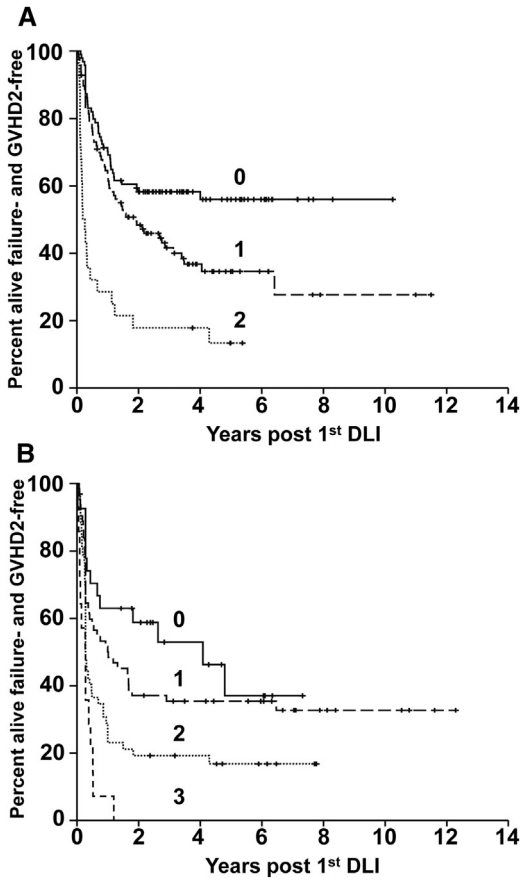

Figure 3. FGFS according to the cumulative number of adverse features in patients treated with DLI for molecular and/or cytogenetic CML relapse (n = 219) (A) and in patients treated with DLI for hematological CML relapse (n = 156) (B) after alloSCT. (A) Ninety-four (43%), 97 (44%), and 28 (13%) patients showed 0, 1, and 2 adverse features (ie, chronic GVHD before relapse, and interval from alloSCT to DLI <1 year), respectively. FGFS at 5 years was 13%, 35%, and 56% in patients with 2, 1, and 0 adverse features, respectively ($P < .001$). (B) Twenty-seven (17%), 63 (40%), 52 (33%), and 14 (9%) patients had 0, 1, 2, and 3 adverse features (ie, sex mismatch with the donor, chronic GVHD before relapse, and initial cell dose $\geq 50 \times 10^6$ $CD3^+$ cells/kg), respectively. FGFS at 5 years was 0%, 17%, 33%, and 37% in patients with 3, 2, 1, and 0 adverse features, respectively ($P < .001$).

approximately one half of responding patients escaped GVHD after DLI. The type of relapse was the major factor influencing such an outcome, with patients in molecular and/or cytogenetic relapse doing far better than patients in hematological relapse. Thus, and with regard to the differing treatment periods, our analysis was conducted separately in the 2 groups according to type of relapse: molecular and/or cytogenetic relapse (230 patients) and hematological relapse (270 patients).

Our univariate analyses revealed that in both types of relapse, prior chronic GVHD and a shorter interval between alloSCT and first DLI were significantly associated with inferior FGFS. For patients relapsed at the molecular and/or cytogenetic level both factors independently predicted a 2-fold increased risk of treatment failure and secondary GVHD in multivariate analysis. Consequently, estimated leukemia-free survival in absence of secondary GVHD was significantly improved, reaching 56% at 5 years when DLI were given beyond 1 year from alloSCT for a molecular and/or cytogenetic CML relapse that was not preceded by chronic GVHD. For patients with hematological CML relapse, besides occurrence of chronic GVHD before relapse, sex mismatch with the donor and an initial cell dose $\geq 50 \times 10^6$ $CD3^+$ cells/kg were confirmed as independent prognostic factors predicting worse FGFS. When stratified according to the cumulative number of these 3 adverse pre-DLI factors, survival in remission without secondary GVHD decreased from 37% to 0% at 5 years in patients presenting with no and all 3 adverse features, respectively.

Previous studies show that advanced stage of CML relapse was a major factor predicting leukemia-free survival after DLI without influencing secondary GVHD incidence or DLI-related mortality [16,23]. Confirming the impact of relapse stage on failure-free survival, in our study the "pure GVL" effect (durable remission after DLI treatment in absence of secondary GVHD) could be similarly observed in nearly one half of the responding patients receiving DLI for any type of CML relapse. We are aware that the expression "pure GVL" is not established. But given the current limitations in separating GVL from GVHD, we believe that it may be used to characterize outcomes when regarding the composite endpoint of FGFS in our study. Early relapse after alloSCT has been shown to be a risk factor predicting worse treatment response and survival in patients treated with DLI [16,24]. In addition, a longer interval between alloSCT and first DLI was associated with less secondary GVHD in several studies [15,17,24]. Although controversial, some form of tolerance induced by the previous transplantation, of which the exact mechanisms remain to be identified, was suggested for this observation [17]. Because relapse type is a major risk factor for treatment failure but not for secondary GVHD, factors affecting both treatment response and secondary GVHD incidence may be of higher prognostic relevance in patients

with less advanced stages of CML relapse. Accordingly, in our study, a shorter interval between alloSCT and first DLI (<1 year) was associated with worse FGFS in patients with molecular and/or cytogenetic relapse but not in patients with hematological CML relapse. In a previous study, sex mismatch with the donor and higher cell dose at first DLI resulted in similar response rates but greater secondary GVHD incidence and increased DLI-related mortality, leading to reduced overall survival [16]. In the present study, both factors emerged as independent predictors of worse FGFS in patients with hematological CML relapse. However, it is important to note that in the molecular and/or cytogenetic subgroup, there was no correlation between the initial cell dose and FGFS.

The correlation between increasing initial $CD3^+$ cell dose and incidence of secondary GVHD has been well reported [16,17,21,24]. In addition, CML disease stage at relapse was demonstrated to be a major factor influencing the effective cell dose (ie, $CD3^+$ cell dose required to achieve remission), suggesting that although DLI treatment is highly effective even at lower cell doses, the larger the tumor burden, the greater the number of cells required to restore remission [25]. In our study, the median initial $CD3^+$ cell dose was lower in patients treated for molecular and/or cytogenetic relapse compared with those treated for hematological relapse. Therefore, it could be that relapse rates were higher at the lower initial dose, and so the composite endpoint of FGFS in our study washes out the difference. Furthermore, it has to be mentioned that for the parameter sex mismatch with the donor all mismatched donor-recipient sex combinations were included, which is analogous with the previous study [16]. Although Y-chromosome minor antigens are known to contribute to the allo-reactive immunogenicity in male recipients from female donors, in our study, the constellation female donor/male recipient was not associated with shorter FGFS compared with other donor-recipient combinations.

Our study of 500 CML patients revealed that, regardless of the relapse type, occurrence of chronic GVHD after transplantation and before relapse was a major factor predicting worse FGFS after DLI. This is in contrast to previous reports by Raiola et al. [23] and Bar et al. [24] demonstrating no impact of prior chronic GVHD on secondary GVHD incidence, treatment response, and overall mortality after DLI treatment in 100 CML patients and 225 patients with different hematological malignancies, respectively. In addition to a larger patient number, another reason for our observation is probably the composite endpoint of FGFS used in our study. The potential impact of prior chronic GVHD on increasing risk of treatment failure and/or increasing risk of subsequent GVHD could then be explained, as both risks contribute additively to shorter FGFS.

It is obvious that this study has several limitations including its retrospective nature, multicentric approach, and the treatment period addressed. Consequently, the majority of patients in our study received bone marrow transplantation, which is different from the current practice. In addition, the median follow-up at the time of the analysis was 56 months, which may be too short to assess late occurring events, such as chronic GVHD, that may develop many years after DLI. Furthermore, although the patients evaluated in our study received DLI in absence of any GVHD and/or its treatment, it is important to note that GVHD developing after DLI administration may be de novo, but in patients who had experienced GVHD before relapse, it may also represent reactivation of a pre-existing GVHD.

Data on the comparison between TKI and DLI are limited and the exact role of TKI in the treatment of post-allograft relapse in CML remains to be defined. In an early study by Weisser et al. [26], DLI was superior to treatment with imatinib in terms of leukemia-free survival, suggesting that TKI alone might not provide definite cure for relapsed CML after alloSCT. In contrast, a more recent report demonstrated that imatinib treatment resulted in higher overall and disease-free survival compared with those after DLI [27]. Moreover, concomitant use of imatinib was not associated with an increased risk of secondary GVHD [17], and in a small series, imatinib was shown to synergize with DLI to achieve rapid CMR of CML relapsing after alloSCT [28]. Unfortunately, in our study the number of patients on concomitant TKI treatment was very low; thus, precluding further analysis in the context of the pre-DLI factors identified.

Nevertheless, our data might prove extremely important when deciding the best therapy for CML relapse after alloSCT. Although there is clearly a lack of reliable data on the use of TKI for the treatment of relapse, many centers are utilizing imatinib or second generation TKIs (dasatinib or nilotinib) for these patients. The advantages of TKI are the low incidence of secondary GHVD (including patients with prior GHVD), the relatively low toxicity profile (apart from hematological toxicity), and the significant response rate [29,30]. The disadvantages include on and off target side effects, pancytopenia, the potential for selection of mutant variants of Bcr-Abl, and the high incidence of relapse if treatment is withdrawn [3,30].

For a given patient, the presence of relapse at the hematological level, prior GVHD, and an interval from transplantation of less than 1 year may argue in favor of using TKI (as the probability of FGFS is very low) and for a patient relapsing in molecular or cytogenetic relapse, with no prior history of GVHD and more than 12 months from the transplantation, the probability of long term FGFS of over 50% would make the use of DLI the preferred option. Moreover, similar studies are warranted for other diseases that share a number of characteristics with CML: chronic, slow type of relapse after alloSCT, good responses to DLI, evidence of GVL without GVHD, and the availability of modern targeted therapies that could be used for the management of relapse after alloSCT. Examples of these are chronic idiopathic myelofibrosis and JAK2 inhibitors, such as ruxolitinib, chronic lymphocytic leukemia and idelalisib/ibrutinib, multiple myeloma and lenalidomide/bortezomib, and to a lesser extent, myelodysplastic syndrome and azacitidine/decitabine.

In conclusion, this study shows that a “pure GVL” effect was present in approximately one half of responding patients treated with DLI. The probability of survival in durable remission without secondary GVHD was higher when DLI was given at molecular and/or cytogenetic than at hematological stage of CML relapse. The chances of exploiting the “pure GVL” effect were best when DLI were given beyond 1 year from alloSCT for molecular and/or cytogenetic CML relapse that was not preceded by chronic GVHD.

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REFERENCES

1. Gratwohl A, Heim D. Current role of stem cell transplantation in chronic myeloid leukaemia. *Best Pract Res Clin Haematol*. 2009;22:431-443.
2. Baccarani M, Deininger MW, Rosti G, et al. European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013. *Blood*. 2013;122:872-884.
3. Barrett AJ, Battiwalla M. Relapse after allogeneic stem cell transplantation. *Expert Rev Hematol*. 2010;3:429-441.
4. Dazzi F, Fozza C. Disease relapse after haematopoietic stem cell transplantation: risk factors and treatment. *Best Pract Res Clin Haematol*. 2007;20:311-327.
5. Deol A, Lum LG. Role of donor lymphocyte infusions in relapsed hematological malignancies after stem cell transplantation revisited. *Cancer Treat Rev*. 2010;36:528-538.
6. Collins RH Jr, Shpilberg O, Drobyski WR, et al. Donor leukocyte infusions in 140 patients with relapsed malignancy after allogeneic bone marrow transplantation. *J Clin Oncol*. 1997;15:433-444.
7. Porter DL, Collins RH Jr, Shpilberg O, et al. Long-term follow-up of patients who achieved complete remission after donor leukocyte infusions. *Biol Blood Marrow Transplant*. 1999;5:253-261.
8. Dazzi F, Szydlo RM, Cross NC, et al. Durability of responses following donor lymphocyte infusions for patients who relapse after allogeneic stem cell transplantation for chronic myeloid leukemia. *Blood*. 2000;96:2712-2716.
9. van Rhee F, Lin F, Cullis JO, et al. Relapse of chronic myeloid leukemia after allogeneic bone marrow transplant: the case for giving donor leukocyte transfusions before the onset of hematologic relapse. *Blood*. 1994;83:3377-3383.
10. Arnold R, Bartram CR, Heinze B, et al. Evaluation of remission state in chronic myeloid leukemia patients after bone marrow transplantation using cytogenetic and molecular genetic approaches. *Bone Marrow Transplant*. 1989;4:389-392.
11. Guglielmi C, Arcese W, Hermans J, et al. Risk assessment in patients with Ph+ chronic myelogenous leukemia at first relapse after allogeneic stem cell transplant: an EBMT retrospective analysis. The Chronic Leukemia Working Party of the European Group for Blood and Marrow Transplantation. *Blood*. 2000;95:3328-3334.
12. Olavarria E, Craddock C, Dazzi F, et al. Imatinib mesylate (STI571) in the treatment of relapse of chronic myeloid leukemia after allogeneic stem cell transplantation. *Blood*. 2002;99:3861-3862.
13. Hess G, Bunjes D, Siegert W, et al. Sustained complete molecular remissions after treatment with imatinib-mesylate in patients with failure after allogeneic stem cell transplantation for chronic myelogenous leukemia: results of a prospective phase II open-label multicenter study. *J Clin Oncol*. 2005;23:7583-7593.
14. Apperley JF. Managing the patient with chronic myeloid leukemia through and after allogeneic stem cell transplantation. *Hematology Am Soc Hematol Educ Program*. 2006;226-232.
15. Kolb HJ, Schattenberg A, Goldman JM, et al. Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. *Blood*. 1995;86:2041-2050.
16. Guglielmi C, Arcese W, Dazzi F, et al. Donor lymphocyte infusion for relapsed chronic myelogenous leukemia: prognostic relevance of the initial cell dose. *Blood*. 2002;100:397-405.
17. Chalandon Y, Passweg JR, Schmid C, et al. Outcome of patients developing GVHD after DLI given to treat CML relapse: a study by the Chronic Leukemia Working Party of the EBMT. *Bone Marrow Transplant*. 2010;45:558-564.
18. Kolb HJ, Schmid C, Barrett AJ, Schendel DJ. Graft-versus-leukemia reactions in allogeneic chimeras. *Blood*. 2004;103:767-776.
19. Speck B, Bortin MM, Champlin R, et al. Allogeneic bone-marrow transplantation for chronic myelogenous leukaemia. *Lancet*. 1984;1:665-668.
20. Matthews DE, Farewell VT. *Using and understanding Medical Statistics*. Basel, Switzerland: Karger; 1996.
21. Mackinnon S, Papadopoulos EB, Carabasi MH, et al. Adoptive immunotherapy evaluating escalating doses of donor leukocytes for relapse of chronic myeloid leukemia after bone marrow transplantation: separation of graft-versus-leukemia responses from graft-versus-host disease. *Blood*. 1995;86:1261-1268.
22. Dazzi F, Szydlo RM, Craddock C, et al. Comparison of single-dose and escalating-dose regimens of donor lymphocyte infusion for relapse after allografting for chronic myeloid leukemia. *Blood*. 2000;95:67-71.
23. Raiola AM, Van Lint MT, Valbonesi M, et al. Factors predicting response and graft-versus-host disease after donor lymphocyte infusions: a study on 593 infusions. *Bone Marrow Transplant*. 2003;31:687-693.
24. Bar M, Sandmaier BM, Inamoto Y, et al. Donor lymphocyte infusion for relapsed hematological malignancies after allogeneic hematopoietic cell transplantation: prognostic relevance of the initial CD3+ T cell dose. *Biol Blood Marrow Transplant*. 2013;19:949-957.
25. Simula MP, Marktel S, Fozza C, et al. Response to donor lymphocyte infusions for chronic myeloid leukemia is dose-dependent: the importance of escalating the cell dose to maximize therapeutic efficacy. *Leukemia*. 2007;21:943-948.
26. Weisser M, Tischer J, Schnittger S, et al. A comparison of donor lymphocyte infusions or imatinib mesylate for patients with chronic myelogenous leukemia who have relapsed after allogeneic stem cell transplantation. *Haematologica*. 2006;91:663-666.
27. Jin H, Xiong Y, Sun J, et al. Is Imatinib Maintenance Required for Patients with Relapse Chronic Myeloid Leukemia Post-Transplantation Obtaining CMR? A Pilot Retrospective Investigation. *PLoS One*. 2013;8:e65981.
28. Savani BN, Montero A, Kurlander R, et al. Imatinib synergizes with donor lymphocyte infusions to achieve rapid molecular remission of CML relapsing after allogeneic stem cell transplantation. *Bone Marrow Transplant*. 2005;36:1009-1015.
29. DeAngelo DJ, Hochberg EP, Alyea EP, et al. Extended follow-up of patients treated with imatinib mesylate (gleevec) for chronic myelogenous leukemia relapse after allogeneic transplantation: durable cytogenetic remission and conversion to complete donor chimerism without graft-versus-host disease. *Clin Cancer Res*. 2004;10:5065-5071.
30. Olavarria E, Ottmann OG, Deininger M, et al. Response to imatinib in patients who relapse after allogeneic stem cell transplantation for chronic myeloid leukemia. *Leukemia*. 2003;17:1707-1712.