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

Matthias Eefting,[1] Peter A von dem Borne,[1] Liesbeth C de Wreede,[2] Constantijn JM Halkes,[1] Sabina Kersting,[1] Erik WA Marijt,[1] Hendrik Veelken,[1] and JH Frederik Falkenburg[1]

[1]Department of Hematology, Leiden University Medical Center; and [2]Department of Medical Statistics and Bioinformatics, Leiden University Medical Center, the Netherlands

ABSTRACT

The prognosis of patients with relapsed acute myeloid leukemia after allogeneic transplantation is poor. We hypothesized that initial disease control by effective cytoreduction, followed by rapid induction of a profound allo-immune response by donor-lymphocyte infusion during the neutropenic phase, is essential for long-term survival. Additional interferon-α was administered when no acute graft-*versus*-host-disease occurred within 3 weeks after donor-lymphocyte infusion. Overall, 44 patients with relapsed acute myeloid leukemia were assessed; 26 had relapsed after myeloablative conditioning and 18 after reduced-intensity conditioning. Of these 44 patients, seven were not eligible for cytoreductive treatment because of poor performance status (n=3) or severe graft-*versus*-host-disease (n=4) at the time of relapse. Patients with smoldering relapses (n=5) received donor-lymphocyte infusion only. Thirty-two patients received cytoreductive treatment, followed by donor-lymphocyte infusion in 22 patients. Reasons for not receiving donor-lymphocyte infusion were chemotherapy-related death (n=1) and chemotherapy-refractory disease (n=9). The 2-year overall survival rate after donor-lymphocyte infusion was 36% (95% confidence-interval: 16-57%). The impact of acute graft-*versus*-host-disease on survival was calculated with a Cox-regression model including onset of acute graft-*versus*-host-disease as a time-dependent variable. Development of grade 1-3, but not grade 4, acute graft-*versus*-host-disease was associated with superior survival as compared to absence of graft-*versus*-host-disease (hazard ratio 0.22, *P*=0.03). In conclusion, efficient cytoreduction followed by donor-lymphocyte infusion and subsequent interferon-α leading to limited acute graft-*versus*-host-disease represents a potentially curative option for patients with relapsed acute myeloid leukemia after allogeneic transplantation.

Introduction

Allogeneic stem cell transplantation (SCT) as treatment for patients with acute myeloid leukemia (AML) aims to combine high-dose chemotherapy with immunotherapy by donor T cells mediating a graft-*versus*-leukemia (GvL) response. The curative potential of the donor-derived allo-immune effect is illustrated by the survival advantage of patients with AML after allogeneic SCT as compared to survival after autologous SCT or high-dose chemotherapy.[1,2] The importance of the GvL response is further supported by donor *versus* no donor comparisons in patients who are potential candidates for allogeneic SCT, favoring the outcome of patients with a stem cell donor.[3] However, the T-cell-mediated alloimmune response also leads to the occurrence of graft-*versus*-host disease (GvHD), which negatively influences outcome after allogeneic SCT.[4,5]

GvHD after allogeneic SCT can be reduced by T-cell depletion of the stem cell graft, but this has been associated with an increased incidence of relapses.[6-10] However, a recent study by Pasquini *et al.* showed that the reduction of T cells in the graft does not necessarily have a negative impact on the relapse rate of AML after myeloablative allogeneic SCT as compared to T-cell-replete allogeneic SCT.[11] This finding suggests that even a limited GvL response may contribute to the cure of patients with AML. Furthermore, a reduced GvL response of T-cell-depleted allogeneic SCT may be restored by post-transplant immunological interventions, such as low-dose, prophylactic donor lymphocyte infusions (DLI) to prevent relapse. This postponed infusion of donor T cells after T-cell-depleted allogeneic SCT is associated with a lower incidence of severe GvHD.[12]

Although AML can be cured by allogeneic SCT, approximately 30% of transplanted patients with AML develop a relapse after the transplant, and these relapsed patients have a dismal prognosis.[13-15] Salvage re-induction chemotherapy leads to clinical remissions in a substantial number of patients, but these remissions are usually of short duration. A retrospective analysis by the EBMT Acute Leukemia Working Party indicated that long-term survival of patients with relapsed AML is almost exclusively achieved by induction of complete remission followed by DLI.[16] Apparently, additional control from a GvL immune response by donor lymphocytes is essential to consolidate chemotherapy re-induced remissions. In contrast, Pollyea *et al.* did not observe an advantage for cellular therapy compared to chemotherapy for patients with relapsed AML.[17]

Although low doses of DLI can result in GvL reactivity, the interval between the clinical response and the administration of the T cells may be several months.[18] To achieve an effective

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Correspondence: j.h.f.falkenburg@lumc.nl

GvL response in relapsed AML, a rapid, large amplitude immune response is likely to be required, since the fast proliferation rate of an acute leukemia can lead to a high tumor burden, which will suppress the immune response.[19,20] A rapid and profound immune response is, however, likely to come at the cost of GvHD.

To maximize the effect of combined cytoreductive treatment and DLI administration, we adopted a therapeutic strategy for post-allogeneic SCT relapsed AML based on disease control by salvage re-induction chemotherapy followed by DLI administration in the neutropenic phase. The pro-inflammatory milieu after chemotherapy might favor the induction of the immune response, whereas the expansion of infused donor cells is promoted by lymphopenia-induced homeostatic proliferation.[21,22] If no acute GvHD was observed within 3 weeks after DLI, the immune response was further stimulated by treatment with interferon-α.[23] We hypothesized that the combination of efficient cytoreduction by re-induction therapy for initial disease control, with DLI administered in rapid succession under circumstances favoring the development of an early and profound immune response may be essential to eradicate relapsed leukemia, but likely at the cost of GvHD.[24]

Methods

Data collection

Between January 2000 and January 2010, 44 patients with relapsed AML after allogeneic SCT were treated. The patients were categorized according to their pre-transplant disease characteristics as having intermediate-risk, poor-risk, or very poor-risk AML (see *Online Supplementary Methods*). The study was approved by Leiden University Medical Center Research Ethics Committee. Informed consent was obtained from the patients prior to data collection. Data were analyzed as of November 2012.

Transplantation protocol

Details about the transplantation protocols, T-cell depletion, donor matching, and diagnosis and treatment of GvHD are provided in the *Online Supplementary Methods* section.

Relapse

Relapse after allogeneic SCT was defined as an increase of morphologically determined blasts in the bone marrow to 5% or more, and/or by the presence of more than 1% blasts in peripheral blood, and/or by the reappearance of molecular and/or cytogenetic markers. Further details and definitions of smoldering relapse and high tumor burden relapse are provided in the *Online Supplementary Methods* section.

Treatment strategies for relapse

Patients with a poor performance status, defined as WHO performance status 3 or higher, and/or with severe GvHD requiring systemic immunosuppression at the time of relapse were unlikely to benefit from an intensive DLI-based strategy and received palliative treatment only.

Patients with high tumor burden relapse received salvage re-induction therapy prior to infusion of donor lymphocytes. If a patient responded to re-induction therapy, with response being defined as absence of circulating leukemic blasts, unmanipulated donor lymphocytes collected from the original donor were administered 3 weeks after the start of re-induction therapy (during the neutropenic phase). In the case of a smoldering relapse, patients received DLI without prior cytoreductive treatment.

DLI-dosing depended on the time point after allogeneic SCT, administration of pre-emptive DLI prior to relapse and donor type (see *Online Supplementary Methods* section).

The occurrence and impact of GvHD on outcome after relapse were evaluated in all patients receiving DLI for relapse. If no acute GvHD was observed within 3 weeks after DLI, interferon-α was administered subcutaneously at a daily dose of 3 million units until GvHD occurred. Escalating doses of DLI were administered if acute GvHD failed to develop by 3 months after starting treatment.

Statistical analysis

For different analyses, time was measured since relapse, initiation of re-induction therapy or infusion of donor lymphocytes. Definitions of survival are provided in the *Online Supplementary Methods* section. Probabilities of overall survival with associated 95% confidence intervals (95% CI) were calculated by the Kaplan-Meier method; differences between groups were compared using a long-rank test. The cumulative incidence of acute GvHD after DLI was calculated using R2.15.0 software, library 'cmprsk' (*http://www.r-project.org/foundation*).

To investigate the impact of a rapid immune response associated with acute GvHD on long-term survival and disease recurrence after DLI, extended Cox regression models, as proposed by Andersen & Gill, were constructed for overall survival, relapse-free survival and relapse incidence from the starting point of DLI (see the *Online Supplementary Methods* section for details).[25] All P values <0.05 were considered statistically significant. The statistical software used was SPSS, PASW Statistics 20, release 20.0.0 (2011).

Results

The baseline characteristics of the entire cohort of 44 patients with relapsed AML after allogeneic SCT are presented in Table 1, and a treatment flowchart of the cohort is shown in Figure 1. Seven of the 44 patients (16%) had poor performance status (n=3) or severe GvHD (n=4) at the time of relapse, and received palliative treatment only. They all died from primary disease.

Five of the remaining 37 patients (14%) met criteria for smoldering relapse (Table 2), and they received DLI without preceding cytoreductive treatment. Four of these patients developed overall grade 2-4 acute GvHD. Two of these patients achieved long-term overall survival, whereas the other two patients died from GvHD-related complications (Figure 2). One patient did not develop any GvHD after DLI and interferon-α and persistence of bone marrow blasts (<10%) was observed. This patient ultimately died from infectious complications.

Thirty-two patients with relapsed AML were treated with re-induction chemotherapy. The median time from relapse to start of treatment was 13 days (range, 0-49 days). One patient with a molecular relapse of BCR-ABL p190-positive AML (phenotype positive for CD34, CD133, cyMPO, CD117, HLA-DR, CD13, CD33, CD4 and CD7) received DLI on the day of relapse, followed by targeted therapy with imatinib. Another patient received DLI 4 days after relapse, followed by gemtuzumab-ozogamycin, after which the patient developed acute GvHD. The remaining 30 patients were treated with gemtuzumab-ozogamycin (n=6), intensive chemotherapy

(n=17) or both (n=7) before DLI was planned. One patient died from chemotherapy-related toxicity. Nine patients did not respond to salvage re-induction therapy and died from primary disease. The remaining 20 patients received DLI after re-induction chemotherapy. The median time from the start of chemotherapy to DLI in these 20 patients was 23 days (range, 13-91 days).

The relapse and treatment characteristics of all 22 patients receiving re-induction chemotherapy and DLI are presented in Table 3. At 3 weeks after DLI, four of these 22

Table 1. Patients' baseline characteristics.

Variable	Entire cohort (44 patients)
Gender (%)	26 Male (59%), 18 Female (41%)
Median age at alloSCT (range)	53 years (18-68)
Initial diagnosis prior to alloSCT (%)	41 AML (93%), 3 MDS (7%)
AML risk-group at diagnosis (%)	4 Int-risk (10%), 27 Poor-risk (66%), 10 MK (24%)
HCT-CI pre-alloSCT (%)	18 Score 0 (41%), 20 Score 1-2 (45%), 6 Score ≥3 (14%)
Median EBMT risk score prior to alloSCT (range)	3 (2-6)
Conditioning regimen alloSCT (%)	26 Myeloablative (59%), 18 Reduced-intensity (41%)
Stem cell source (%)	6 Bone marrow (14%), 38 G-CSF stimulated peripheral blood (86%)
T cell depletion (%)	41 'Campath in the bag' (93%), 3 CD34-selection (7%)
Donor type (%)	30 Sibling donor (68%; 28 fully matched), 14 Unrelated donor (32%; 11 fully matched)
Median time alloSCT to relapse (range)	191 days (65-1117)
Relapse within 6 months after alloSCT (%)	21 (48%)
Type of relapse (%)	39 High tumor burden (89%), 5 Smoldering (11%)
Median % BM blasts / PB blasts (range) at relapse	40 (0-90) / 0 (0-94)
<30% BM blasts and <10% PB blasts (%) at relapse	15 (34%)
Extramedullary relapse (%)	4 (9%)
Prophylactic DLI prior to relapse (%)	11 (25%)
Maximum grading of acute GvHD prior to relapse (%)	16 No acute GvHD (36%), 24 Grade 1-2 (55%), 4 Grade 3-4 (9%)

AlloSCT: allogeneic stem cell transplantation; Int-risk: intermediate risk AML; MK: monosomal karyotype AML; HCT-CI: hematopoietic cell transplantation comorbidity index; CR1: first complete remission; CR2: second complete remission; CD34-selection: collection of MACS-sorted CD34⁺cells collection; relapse indicates first relapse after alloSCT; BM: bone marrow; PB: peripheral blood; MDS: myelodysplastic syndrome; G-CSF: granulocyte colony-stimulating factor.

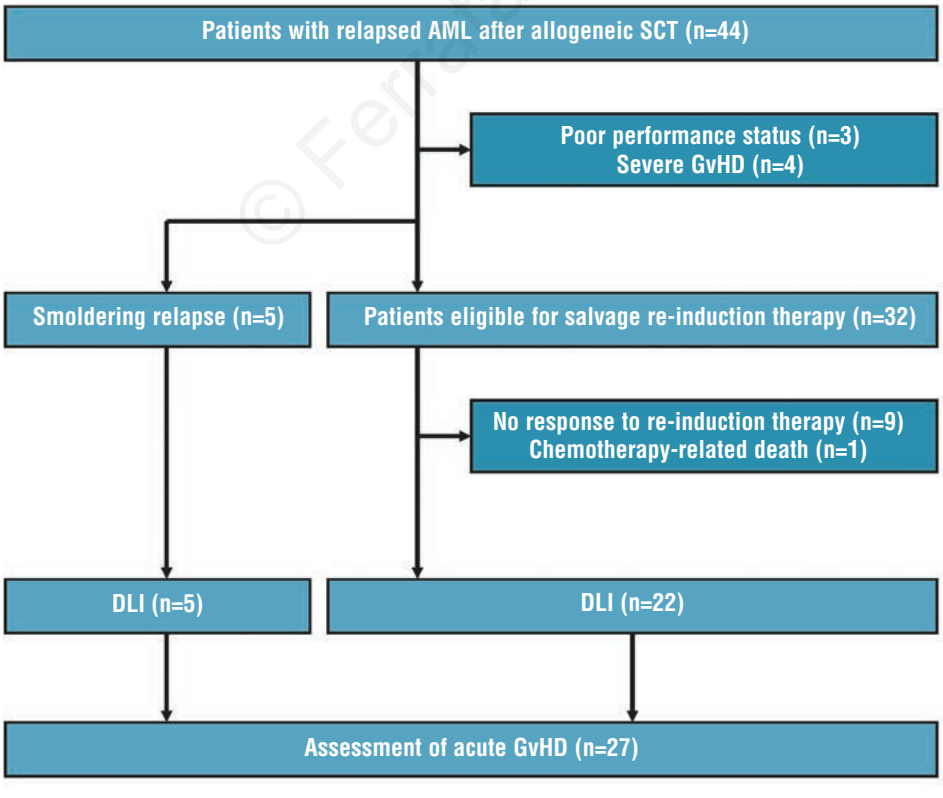

Figure 1. Flowchart of 44 relapsed AML patients.

patients had developed signs of acute GvHD. Five patients without signs of acute GvHD did not receive interferon-α due to chemotherapy-related toxicity (n=2) or severe infectious complications (n=3). The remaining 13 patients received interferon-α.

Overall survival

Median follow-up from relapse of surviving patients of the entire cohort was 63 months (range, 24-128 months). The estimated 2-year overall survival rate calculated from day of relapse of the entire cohort of 44 patients was 23% (95% CI 10-35%). The estimated 2-year overall survival rate calculated from start of treatment in the 37 patients eligible for re-induction treatment and/or DLI was 27% (95% CI 13-41%), while that in the 32 patients with high tumor burden relapse starting re-induction treatment was 25% (95% CI 10-40%). Finally, the estimated 2-year overall survival rate calculated from DLI was 36% (95% CI 16-57%) in the group of 22 patients who received re-induction therapy followed by DLI (Figure 2).

The overall outcome of AML patients with a monosomal karyotype was particularly poor. The main reason for their poor outcome appeared to be poor primary disease control, and only four of these ten patients ultimately received DLI. The only surviving patient with monosomal karyotype had a molecular relapse of BCR-ABL p190-positive AML which was treated with imatinib and DLI (Table 3).

Assessment and impact of acute graft-versus-host disease on long-term survival after relapse

All 27 patients who received DLI (see Tables 2 and 3) were evaluated for the presence of acute GvHD after infusion of the lymphocytes. The cumulative incidence of acute GvHD at 3 months after DLI was 56% (95% CI: 36-75%) (Figure 3).

Eleven patients developed grade 3-4 acute GvHD, of whom nine required systemic immunosuppression for GvHD until death or for more than 1 year after relapse. Only one patient with signs of acute GvHD developed a second relapse. No relapses were observed in six patients who developed grade 4 acute GvHD. However GvHD-associated mortality in these patients was high: five patients died due to GvHD-associated complications, including infections and multiple organ failure. In total, seven of 27 patients (26%) receiving DLI died due to GvHD-related complications. We did not observe a clear difference in distribution pattern (i.e. skin or liver involvement) or severity of GvHD between patients who developed GvHD within 3 weeks after DLI and patients who developed GvHD after administration of interferon-α (see *Online Supplementary Table S1*).

The impact of an immune response leading to acute GvHD on long-term survival after relapse was estimated by time-dependent Cox regression models (Table 4). Compared to the absence of acute GvHD, development of grade 1-3 acute GvHD was associated with superior overall survival [hazard ratio (HR)=0.22; 95% CI: 0.06-0.86; *P*=0.03)] and reduced relapse incidence (HR=0.09; 95% CI: 0.01-0.87; *P*=0.04). Development of grade 4 acute GvHD did not result in an improved overall survival (HR=1.61;

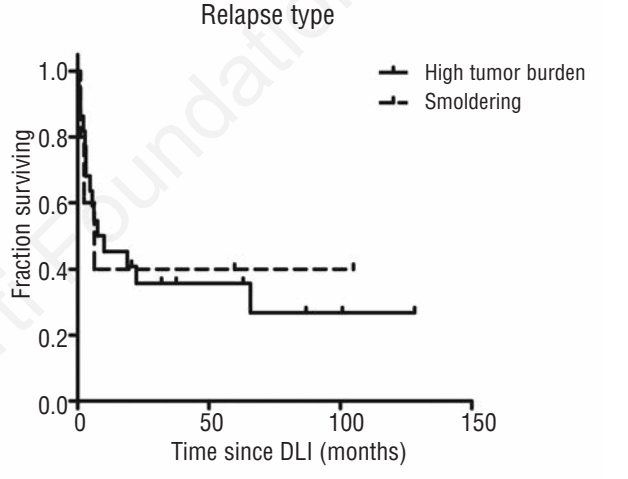

Figure 2. Overall survival. Kaplan-Meier curves showing overall survival after infusion of donor lymphocytes to 22 patients with high tumor burden relapse who received re-induction therapy followed by DLI (solid line) and five patients with smoldering relapse who received DLI without prior cytoreductive treatment (dashed line).

Table 2. Outcomes of five patients with smoldering relapse (patients are ordered according to grading of acute GvHD after DLI).

N. of patients	Sex	Age at transplant (years)	Disease prior to transplant	Conditioning regimen	Donor type	Prophylactic DLI	Acute GvHD prior to relapse	Time transplant to relapse (days)	% blasts BM at relapse	DLI dose (x10^6 CD3$^+$cells /kg)	IFN	Time DLI to acute GvHD (days)	Acute GvHD after DLI	Immuno-suppression for GvHD (days)	Follow-up after DLI (months)	Current status
1	male	63	MDS, IPSS 1.0	RIC	MUD	Day 206; 1.5x10^6 CD3$^+$cells/kg	grade 1	234	6	2.5	yes	-	No	0	6	Dead, primary disease
2	male	66	Poor-risk AML	RIC	MUD	Day 134; 0.15x10^6 CD3$^+$cells/kg	grade 1	176	8	1.5	no	39	grade 2	182	60	Alive, CR
3	female	50	Poor-risk AML	RIC	MMUD	No	no	65	5	2.5	no	52	grade 3	207	105	Alive, CR
4	male	66	Poor-risk AML	RIC	MMRD	Day 196; 2.5x10^6 CD3$^+$cells/kg	no	329	5	7.5	no	14	grade 3	20	1	Dead, GvHD-related
5	male	36	MK AML	MAC	MRD	No	grade 1	282	3 (2 PB)	50	yes	22	grade 4	51	2	Dead, GvHD-related

Relapse indicates first relapse after allogeneic stem cell transplantation; MK: monosomal karyotype; RIC: reduced-intensity conditioning; MAC: myeloablative conditioning; MRD: 10/10 matched related donor; MMRD: mismatched related donor; MUD: 10/10 matched unrelated donor; MMUD: mismatched unrelated donor; BM: bone marrow; PB: peripheral blood; IFN: interferon-α; CR: complete remission; '-': not available/not evaluated/not applicable.

95% CI: 0.52-4.97; *P*=0.41). Outcomes of patients with grade 1-2 as compared to grade 3-4 acute GvHD did not differ significantly (*data not shown*).

Finally, to determine the impact of remission status prior to transplant (first *versus* second complete remission), donor type (HLA-matched sibling *versus* other donor), type of conditioning regimen (reduced intensity *versus* myeloablative), and time between transplant and relapse (first relapse within 6 months after SCT *versus* first relapse more than 6 months after SCT) on overall survival after DLI, these factors were compared within the 27 patients who received DLI using a log-rank test. None of these variable differed significantly (*P*-values 0.73, 0.44, 0.13, and 0.89, respectively). Exploratory analysis of lymphocyte recovery after DLI and early chimerism data also did not indicate correlations with GvHD or outcome (*data not shown*).

Discussion

Our study with a median follow-up of more than 5 years illustrates that patients with relapsed AML after allogeneic SCT can be cured when salvage re-induction therapy leading to a low tumor burden is combined with a rapid and profound, but controllable donor T-cell-mediated immune response resulting in limited GvHD. In the absence of GvHD after DLI, no long-term disease control was observed. In contrast, most patients developing GvHD after DLI did not experience a second relapse. These findings illustrate the prominent role for alloreactive donor lymphocytes in inducing a persistent GvL response in relapsed AML after allogeneic SCT, which was not obvious from other studies.[17] However, the benefit of this GvL response was virtually obliterated in patients who developed very severe GvHD, resulting in death due to GvHD-related complications including multiple-organ failure and infections.

Previous studies had already illustrated that reduction of the tumor burden was necessary to gain significant time to allow the immune response to develop.[14,19,26] We hypothesized that tumor reduction was also necessary to limit the inhibitory effect of the malignant cells on the induction of an immune response, to induce immunological danger signals to promote development of a primary immune response, and to induce leukopenia to promote homeostatic proliferation of the antigen-experienced T cells further favoring a GvL response.[20-22,24] Based on these prerequisites, we preferentially used gemtuzumab-ozogamycin as targeted chemotherapy for first-line treatment for patients with relapsed AML. Gemtuzumab-ozogamycin is a CD33 antibody linked to the cytotoxic drug calicheamycin. Gemtuzumab-ozogamycin-induced apoptosis of both CD33-positive and CD33-negative leukemic cells in the bone marrow may lead to presentation of antigens from the leukemic cell debris by local antigen-presenting cells.[27,28]

Table 3A. Outcomes of 22 patients with relapsed AML receiving re-induction therapy and DLI (patients are ordered according to grading of acute GvHD after DLI, see Table 3B).

N.	Sex	Age at transplant (years)	Disease prior to transplant	Conditioning regimen	Donor type	Prophylactic DLI	Acute GvHD prior to relapse	Time transplant to relapse (days)	% blasts in BM at relapse
1	M	55	MK AML	MAC	MRD	no	grade 1	148	<1 *
2	F	51	int-risk AML	MAC	MMRD	no	grade 2	360	50
3	F	18	poor-risk AML	MAC	MUD	day 121: 0.15x10^6 CD3$^+$cells/kg	no	211	75
4	F	29	poor-risk AML	MAC	MRD	no	no	194	- (94 PB)
5	F	59	poor-risk AML	MAC	MRD	no	grade 1	284	50
6	M	35	int-risk AML	MAC	MRD	no	no	183	70
7	M	32	poor-risk AML	MAC	MUD	no	no	270	40
8	F	27	poor-risk AML	RIC	MRD	no	grade 1	128	40
9	M	64	poor-risk AML	RIC	MUD	day 134: 0.15x10^6 CD3$^+$cells/kg	grade 1	183	90
10	M	37	poor-risk AML	MAC	MRD	no	grade 1	253	90
11	M	54	poor-risk AML	MAC	MRD	day 88: 1.0x10^6 CD4$^+$cells/kg	no	130	45
12	M	53	MDS, IPSS 2.0	RIC	MRD	no	grade 2	370	40
13	M	47	poor-risk AML	RIC	MUD	no	no	514	60
14	M	65	poor-risk AML	RIC	MRD	no	grade 2	213	15
15	M	63	poor-risk AML	RIC	MUD	day 220: 1.5x10^6 CD3$^+$cells/kg	grade 1	259	50
16	F	60	poor-risk AML	RIC	MRD	no	grade 1	158	18
17	M	36	MK AML	MAC	MRD	no	grade 2	216	<1 §
18	F	67	poor-risk AML	RIC	MUD	no	no	108	33
19	M	45	MK AML	MAC	MRD	no	grade 1	328	- (12 PB)
20	F	55	int-risk AML	RIC	MRD	no	grade 1	187	68
21	F	33	poor-risk AML	MAC	MMUD	day 120: 0.52x10^6 CD3$^+$cells/kg	grade 1	426	12
22	F	61	poor-risk AML	RIC	MRD	no	no	88	70

*Relapse indicates first relapse after allogeneic stem cell transplantation; N.: patient number; M: male; F: female; MK: monosomal karyotype; int-risk: intermediate risk; MAC: myeloablative conditioning; RIC: reduced-intensity conditioning; MRD: 10/10 matched related donor; MMRD: mismatched related donor; MUD: 10/10 matched unrelated donor; MMUD: mismatched unrelated donor; BM: bone marrow; PB: peripheral blood; '-': not available/not evaluated/not applicable. *Cytogenetic relapse with clonal evolution. §Molecular relapse BCR-ABL p190.*

Table 3B. Outcomes of 22 patients with relapsed AML receiving re-induction therapy and DLI, continued (patients are ordered according to grading of acute GvHD after DLI).

N.	Time relapse to treatment (days)	Re-induction therapy	Time start treatment to DLI (days)	DLI dose ($x10^6$ $CD3^+$cells/kg)	IFNα	Time DLI to acute GvHD (days)	Acute GvHD after DLI	Immuno-suppression for GvHD (days)	Follow-up after DLI (months)	Current status
1	14	GO	13	5.0	no	-	no	no	3	dead, primary disease
2	7	MITO/ETOP	31	1.5	no	-	no	no	3	dead, primary disease
3	6	GO/MITO/ETOP	22	3.9	no	-	no	no	1	dead, infection
4	2	DAU/ARAC	28	100	yes	-	no	no	5	dead, primary disease
5	4	GO/DAU/ARAC	31	5.0	yes	-	no	no	66	dead, primary disease
6	6	2xGO/CLOF	91	1.5	yes	-	no	no	8	dead, primary disease
7	13	DAU/ARAC	26	1.5	yes	-	no	no	22	dead, primary disease
8	14	ARAC/AMSA	20	5.0	no	-	no	no	6	dead, primary disease
9	12	GO	13	2.5	no	-	no	no	1	dead, infection
10	7	DAU/ARAC	29	15	yes	7	grade 1	no	6	dead, primary disease
11	19	DAU/ARAC	21	5.0	yes	30	grade 1	yes, 219[¥]	38	alive, CR
12	8	DAU/ARAC	21	5.0	yes	108	grade 1	yes, 165[¶]	21	alive, CR
13	29	ARAC/AMSA	26	5.0	no	75	grade 1	yes, 223	10	dead, GvHD-related
14	4[§]	GO[§]	0 §	10	yes	41	grade 2	yes, >365	128	alive, CR
15	14	DAU/ARAC	24	7.5	yes	38	grade 3	yes, >365	32	alive, CR
16	9	GO/IDA/ARAC	28	5.0	no	27	grade 3	yes, 66	101	alive, CR
17	0*	Imatinib *	0*	3.0	yes	92	grade 3	yes, 154	87	alive, CR
18	32	ARAC/AMSA	21	2.5	no	21	grade 4	yes, 5	1	dead, GvHD-related
19	11	DAU/ARAC	26	100	yes	26	grade 4	yes, 51	3	dead, GvHD-related
20	9	ARAC/AMSA	22	5.0	yes	14	grade 4	yes, >365 + MSC	20	dead, GvHD-related
21	14	GO	15	7.8	yes	60	grade 4	yes, 78 + MSC	63	alive, CR
22	3	ARAC/AMSA+itMTX/ARAC	20	1.0	no	35	grade 4	yes, 28	2	dead, GvHD-related

*Relapse indicates first relapse after allogeneic stem cell transplantation; N.: patient number; IFNα: interferon-α; GO: gemtuzumab-ozogamycin; MITO: mitoxantrone; ETOP: etoposide; DAU: daunorubicin; ARAC: cytarabine; CLOF: clofarabin; AMSA: amsacrine; IDA: idarubicin; MTX: methotrexate; MSC: mesenchymal stem cell infusion; CR: complete remission; '-': not available/not evaluated/not applicable. [§]Patient relapsed and received DLI 32 days before GO (date of DLI = start treatment). *Patient received DLI and IFN followed by imatinib for 8 months (date of DLI = start treatment). [¥]Patient developed a pericarditis, which was treated with immunosuppression. [¶]Patient developed chronic GvHD, which was treated with immunosuppression.*

After gemtuzumab-ozogamycin was withdrawn from the market, subsequent patients received myeloablative therapy with cytarabine-based re-induction regimens, which also led to control of leukemia in several patients, but at the cost of more systemic toxicity. Unfortunately, even with high-dose chemotherapy, failure to obtain sufficient initial disease control was observed in approximately 28% of patients with high tumor burden relapse, advocating treatment with immunological interventions in circumstances of minimal residual disease.

Some patients displayed a relapse of AML with limited leukemic load and an indolent course, i.e. a smoldering relapse.[29,30] These patients could benefit from our DLI-based strategy without prior cytoreductive treatment. This approach resulted in a profound immune response in four out of five patients. Apparently, the immune response can develop with limited tumor burden, under circumstances similar to a molecular relapse of chronic phase chronic myeloid leukemia. In chronic phase chronic myeloid leukemia, GvL reactivity by DLI can develop without chemotherapy or lymphodepletion, although the interval between administration of the T cells and the clinical response may last several months.[18,31] Previously, we demonstrated that treatment of patients with low-dose interferon-α could shorten the interval between the DLI and onset of the immune response.[23] Therefore, interferon-α was implemented in our strategy to boost the immune response by activating rapid and efficient antigen-presentation.[23,32-34] In contrast to treatment of chronic myeloid leukemia, we used relatively high doses of DLI to induce a rapid immune response with high amplitude, although we anticipated that this would occur at the cost of GvHD.

In the patients with sufficient initial disease control, development of acute GvHD was observed in approximately 56% of patients at 3 months after DLI. Importantly, interferon-α was withheld instantly when the first clinical signs of GvHD were observed. No clear difference in distribution pattern or severity of GvHD was observed between patients who developed GvHD within 3 weeks after DLI and patients who developed GvHD after boosting with interferon-α. Obviously, the adverse impact of severe GvHD on morbidity and mortality limits the window of opportunity for a favorable outcome of patients with relapsed AML. The necessity for systemic immunosuppression to taper the immune response increases susceptibility to opportunistic infections. Nevertheless, our results illustrate that relapse of AML after transplant can be successfully controlled by an alloimmune response.

Since all patients in our study received a T-cell-depleted graft, it may be questioned whether our strategy could be equally successful in patients with relapsed AML after T-cell-replete allogeneic SCT. Patients transplanted with a T-cell-

Table 4. Cox regression models with time-dependent covariates constructed to estimate the impact of development of grade 1-3 or grade 4 acute GvHD compared to absence of acute GvHD on the hazards for overall survival, relapse-free survival and relapse incidence (cause-specific hazard) from the starting point of DLI.

	Overall survival			Relapse-free survival			Relapse incidence		
Grading of acute GvHD	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value
No GvHD	1	-	0.03*	1	-	0.11	1	-	-
Grade 1-3	0.22	0.06-0.86	0.03	0.28	0.07-1.16	0.08	0.09	0.01-0.87	0.04
Grade 4	1.61	0.52-4.97	0.41	1.28	0.41-4.00	0.67	-**	-	-

** P values indicating overall significance of the variable. **No relapses were observed in patients developing grade 4 acute GvHD after DLI, therefore these patients were omitted from the model for relapse. Hazard ratios in the model indicate changes in risk for the time interval between onset of acute GvHD until end of follow-up with respect to a baseline patient who has not experienced acute GvHD at the same time. The grade represents the maximum grade of acute GvHD during follow-up. 95% CI indicates 95% confidence interval.*

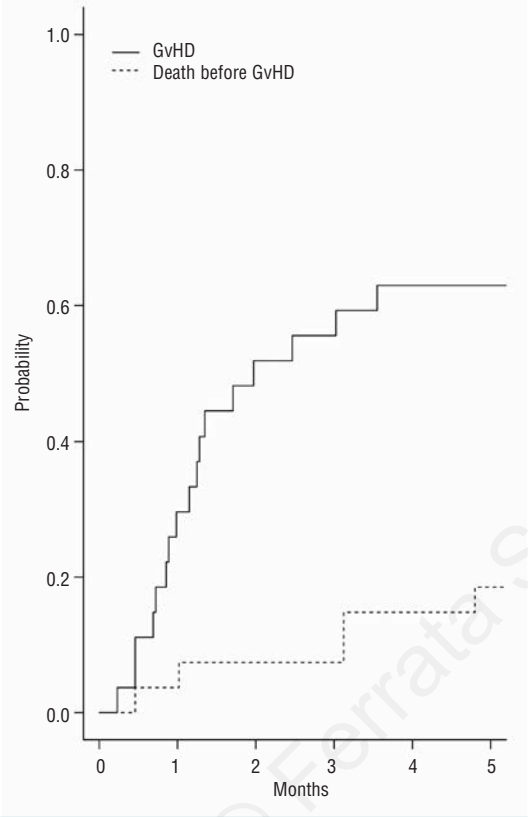

Figure 3. Cumulative incidence of acute GvHD after DLI.

replete allograft are more likely to have experienced an alloimmune response after transplantation, and patients who relapse may, therefore, be selected to be resistant to a cellular immune intervention. However, several patients in our cohort with long-term survival after relapse had already shown limited GvHD or had received prophylactic DLI prior to their relapse. In these patients, re-introduction of DLI-induced GvL with acute GvHD was seen. This observation supports the assumption that our strategy is able to boost a GvL response in patients who apparently have had an immune response prior to relapse with insufficient amplitude, which can be similar in patients without severe GvHD after T-cell-replete allogeneic SCT. On the other hand, we assumed that patients with severe GvHD at the time of relapse were indeed resistant to alloreactivity by donor T cells.

To limit potential selection bias all patients with relapsed AML after allogeneic SCT who were treated at our institution during a 10-year time period were included in our retrospective analysis. Although cure was apparently achieved in a substantial fraction of patients with relapsed AML, overall survival at 2 years after relapse of the entire cohort was only 23%. This outcome was negatively influenced by the seven patients who were not eligible for our treatment strategy and received palliative treatment only. In patients eligible for cytoreductive treatment and DLI, the 2-year overall survival improved to approximately 36%. On top of this, the likelihood of a favorable outcome was correlated with low tumor burden and development of controllable GvHD after DLI. Prevention of high tumor burden relapses might further improve the outcome of relapsed AML after allogeneic SCT. Early detection of an impending relapse with highly sensitive markers of minimal residual disease, including *WT1* gene expression, may be useful to guide immunological interventions.[35] In addition, the use of new cellular therapies which circumvent the negative impact of GvHD may be attractive, such as infusion of hematopoiesis-restricted or leukemia-specific T cells.[36] Due to the small sample size of the dataset, no additional multivariate analyses could be performed to investigate the impact of conditioning regimen, degree of HLA-matching, or disease stage at transplant.[25] Also at present, no registered targeted therapies are available, but perhaps less toxic re-induction therapy, compared to high-dose chemotherapy, might lead to reduced GvHD after DLI.

In summary, a regimen combining efficient salvage re-induction therapy, followed by infusion of donor T cells in the neutropenic phase after cytoreduction and subsequent interferon-α administration if needed to induce donor-derived anti-tumor immunity may result in cure of patients with relapsed myeloid malignancies after allogeneic SCT. In contrast, the prognosis in the case of absent or excessive acute GvHD remains dismal due to refractory disease or GvHD-related mortality. We conclude that achievement of limited tumor burden in combination with relatively high-intensity immune responses, as reflected by controllable acute GvHD, are essential for the cure of relapsed AML after allogeneic SCT.

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Authorship and Disclosures

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