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# α-Interferon with Very-Low-Dose Donor Lymphocyte Infusion for Hematologic or Cytogenetic Relapse of Chronic Myeloid Leukemia Induces Rapid and Durable Complete Remissions and Is Associated with Acceptable Graft-versus-Host Disease

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## ABSTRACT

**Donor lymphocyte infusion (DLI) results in complete cytogenetic remission (CCR) of relapsed chronic-phase chronic myeloid leukemia (CML-CP) after allogeneic stem cell transplantation (SCT) in up to 80% of patients. The main complication of DLI is graft-versus-host disease (GVHD). Decreasing the dose of DLI is associated with less GVHD but also with a longer interval between treatment and CCR. We postulated that combining α-interferon (α-IFN) with DLI would enable us to decrease the dose of DLI, thereby limiting GVHD, and at the same time to decrease the interval between DLI and CCR for patients with either a hematologic or cytogenetic relapse. For molecular relapses, we hypothesized that because of a lower tumor load, very low doses of DLI without α-IFN could be an effective treatment. Two groups of CML-CP patients treated with DLI at a very low dose of 0.5 to 1.0 × $10^7$ mononuclear cells per kilogram, containing 2 to 6 × $10^6$ $CD3^+$ T cells per kilogram, were analyzed: 13 patients with a cytogenetic or a hematologic relapse after allogeneic SCT (group A) were treated with additional α-IFN therapy at a dose of 3 × $10^6$ U 5 d/wk, and 8 patients with a molecular relapse were treated without α-IFN (group B). Twelve patients from group A reached a CCR. The median interval between DLI and CCR was 7 weeks (range, 5-18 weeks) for group A. All patients with a CCR reached complete donor chimerism at a median of 10 weeks after DLI (range, 6-121 weeks). Eleven patients reached molecular remission at a median of 15 weeks after DLI (range, 8-34 weeks). In group B, all patients reached a molecular remission at a median of 14 weeks (range, 12-29 weeks). Five patients from group A developed acute GVHD grade II to IV and extensive chronic GVHD. In group B, 1 patient developed acute GVHD grade II to IV and subsequently developed extensive chronic GVHD. With a median follow-up of 62 months, 10 patients in group A are alive and in continuous CCR. One patient had a molecular relapse, for which she successfully received additional DLI; another patient reached molecular remission only after 5 doses of DLI. Two patients from group A died of a gram-negative sepsis, and 1 died of an acute myocardial infection. In group B, all patients are alive and in molecular remission with a median follow-up of 20 months. One patient's disease progressed but was successfully treated with DLI plus α-IFN. In conclusion, very-low-dose DLI in combination with α-IFN as treatment for cytogenetic or hematologic relapses of CML-CP after allogeneic SCT reduced the interval to obtain a CCR with acceptable GVHD when compared with the literature. Patients with a CCR also reached complete donor chimerism and complete molecular remissions. For patients with a molecular relapse, very-low-dose DLI alone is sufficient to induce molecular remissions in most patients and is associated with limited GVHD.**



## KEY WORDS

CML • DLI • α-Interferon • Transplantation • Relapse

## INTRODUCTION

Allogeneic stem cell transplantation (SCT) is the only curative treatment for patients with chronic myeloid leukemia (CML) [1]. Relapse rates after allogeneic SCT vary from 20% to 50%, depending on T-cell depletion of the graft [2]. Relapses of CML after allogeneic SCT can successfully be treated with donor lymphocyte infusions (DLI), resulting in complete remissions (CR) in up to 80% of patients [3-13]. The likelihood of achieving CR depends on the extent and type of relapse. Molecular or cytogenetic relapses of chronic phase (CP) CML respond more frequently than hematologic relapses [4-7,13], whereas patients with relapses in accelerated or blastic phase have a relatively low likelihood of responding to DLI [8,10]. The antileukemic response is often associated with the development of graft-versus-host disease (GVHD), especially when a high initial cell dose is given [14]. Low-dose DLI with up to $10^7$ $CD3^+$ T cells per kilogram body weight of the recipient is associated with a lower incidence of severe GVHD but also with a long interval between intervention and achievement of a complete cytogenetic remission (CCR). The median interval between DLI at a dose of $10^7$ $CD3^+$ T cells per kilogram for cytogenetic or hematologic relapse and CCR was reported to be 14 weeks (range, 6-19 weeks) by Mackinnon et al. [6] and to be up to 22 weeks by Dazzi et al. [7]. This long interval may put the patient at risk for disease progression [4-8,10,13]. Therefore, low-dose DLI is often followed by higher doses when an increase in tumor load is observed after the initial dose [6,7]. However, although higher doses of DLI ($>10^7$ $CD3^+$ T cells per kilogram) seem to decrease the interval to CCR, dose escalation may also be accompanied by more severe GVHD and a higher treatment-related mortality [14].

In earlier studies, α-interferon (α-IFN) was coadministered with DLI because of a possible enhancement of the immune response against the leukemic cells [3]. Multivariate analysis of larger groups of patients with relapsed CML treated with DLI did not show significant differences in response rate with or without additional α-IFN therapy. However, the cell dose infused in most of the patients described in these studies was high (median, 3-5 $\times$ $10^8$ mononuclear cells [MNC] per kilogram) [4,5]. Previously, we have shown that α-IFN increased the efficacy of generating leukemia-reactive CTL in vitro [15]. Others described an enhanced immune response in vivo by α-IFN [16]. A limited number of patients with a relapse of CML after allogeneic SCT entered remission after treatment with α-IFN alone [17]. Moreover, α-IFN has direct antiproliferative effects on CML cells in vitro [18]. More recently, we demonstrated that α-IFN increased the sensitivity of leukemic cells to Fas-induced apoptosis, mediated by recruitment of leukemic cells from the resting $G_0$ compartment to the $G_1$ phase [19]. On the basis of these findings, we analyzed in this study whether the addition of α-IFN to very-low-dose DLI (0.5-1.0 $\times$ $10^7$ MNC per kilogram) containing approximately 2 to 6 $\times$ $10^6$ $CD3^+$ T cells per kilogram can potentiate and accelerate the immune response against CML cells with acceptable GVHD in patients with a hematologic or cytogenetic relapse after allogeneic SCT. Patients with a molecular relapse of CML were treated with similar very-low-dose DLI without α-IFN, because we hypothesized that DLI alone might eradicate the relatively low tumor load in these patients. In case of progression of the disease in the latter patients, α-IFN was added to the DLI.

In this study, we demonstrate that for patients with a hematologic or cytogenetic relapse of CML-CP, the combination of very-low-dose DLI (0.5-1.0 $\times$ $10^7$ MNC per kilogram) with α-IFN at a dose of 3 $\times$ $10^6$ IU 5 d/wk resulted in a short interval between DLI and CCR and an acceptable incidence of GVHD. For patients with a molecular relapse, we confirmed that very-low-dose DLI alone is sufficient to induce molecular remission in most patients, with a low incidence of GVHD.

## MATERIALS AND METHODS

### Patients

Between January 1994 and January 2003, 21 sequential patients with a relapse after allogeneic SCT for CML-CP were treated with DLI. Two groups of patients were treated: between January 1994 and January 2000, 13 patients with a cytogenetic or a hematologic relapse (group A) and between January 2000 and January 2003, 8 patients with a molecular relapse (group B) received DLI. Patient characteristics are shown in Table 1. The definitions of relapses are described below.

### Transplantation and Follow-up

As a conditioning regimen, patients with HLA-identical sibling donors received cyclophosphamide 60 mg/kg intravenously on days −6 and −5 and 9 Gy of total body irradiation on day −1. Total body irradiation was 2 $\times$ 6 Gy on days −1 and 0 for patients with matched unrelated donors (URD) or with HLA-mismatched sibling donors. The stem cell graft was T-cell depleted by adding 10 mg of Campath-1G (Therapeutic Antibody Centre, Oxford, UK) or 20 mg of Campath-1H to the graft [20]. Recipients of matched URD grafts also received Campath-1G or -1H 5 mg/d in vivo on days −8 to −4 and cyclosporine 3 mg/kg intravenously from day −1.

After transplantation, peripheral blood, bone marrow, or both were collected at fixed time points for

**Table 1.** *Clinical Characteristics of Patients with CML Relapsing after Allogeneic SCT (AlloSCT)*

| Patient No. | Sex (Patient/Donor) | Source of Stem Cells | Donor Type | Age at SCT (y) | Months from AlloSCT to Relapse | Type of Relapse |
|---|---|---|---|---|---|---|
| 1 | F/F | BM | Sibling | 29 | 23 | Cytogenetic |
| 2 | M/M | BM | Sibling | 38 | 8 | Cytogenetic |
| 3 | M/M | BM | Sibling | 43 | 17 | Cytogenetic |
| 4 | M/M | BM | Sibling | 40 | 9 | Cytogenetic |
| 5 | F/M | PB | Sibling | 36 | 19 | Cytogenetic |
| 6 | M/F | PB | Sibling | 38 | 18 | Cytogenetic |
| 7 | M/M | PB | URD* | 33 | 12 | Cytogenetic |
| 8 | M/M | BM | URD | 28 | 27 | Hematologic |
| 9 | M/M | BM | Sibling | 27 | 11 | Hematologic |
| 10 | F/F | BM | Niece† | 45 | 21 | Hematologic |
| 11 | M/F | BM | URD | 29 | 7 | Hematologic |
| 12 | F/F | PB | Sibling | 48 | 9 | Hematologic |
| 13 | F/M | PB | Sibling | 37 | 66 | Hematologic |
| 14 | M/F | PB | Sibling | 21 | 12 | Molecular |
| 15 | F/M | PB | Sibling | 45 | 12 | Molecular |
| 16 | M/M | PB | Sibling | 26 | 13 | Molecular |
| 17 | F/F | BM | URD | 40 | 12 | Molecular |
| 18 | F/F | PB | Sibling | 48 | 5 | Molecular |
| 19 | F/M | PB | Sibling | 45 | 11 | Molecular |
| 20 | M/M | PB | Sibling | 44 | 23 | Molecular |
| 21 | F/M | PB | URD | 19 | 17 | Molecular |

M indicates male; F, female; BM, bone marrow; PB, peripheral blood; URD, unrelated donor.
*HLA-matched unrelated donor.
†HLA phenotypically identical.

morphologic examination and cytogenetic analysis, and from January 1, 2000, they were also collected for isolation of messenger RNA and DNA to quantify BCR/ABL transcripts and to analyze donor chimerism, respectively (see "Response Evaluation"). This was performed at intervals of 3 months during the first 3 years and at longer intervals thereafter unless there was a clinical suspicion of relapse. For patients treated before January 1, 2000, molecular and chimerism analysis was performed retrospectively from frozen samples. A molecular relapse was defined as the reappearance of BCR/ABL transcripts in blood or bone marrow or a 1-log increase when patients remained BCR/ABL positive after transplantation on at least 2 consecutive occasions. A cytogenetic relapse was defined as the reappearance of the Philadelphia chromosome in the bone marrow assessed with standard cytogenetics or >1% positivity by using fluorescence in situ hybridization (FISH). A hematologic relapse was defined as the reappearance of the characteristic morphologic features of CML.

### Treatment Protocol

Patients were treated with a DLI protocol that was approved by the Leiden ethical committee for medical research. Treatment for group A started immediately after the diagnosis of a relapse was made and consisted of α-IFN subcutaneously (Roferon; Roche, Mijdrecht, the Netherlands) at a dose of $3 \times 10^6$ IU 5 d/wk followed 2 to 4 weeks later by DLI at a dose of $10^7$ MNC per kilogram containing 4 to $6 \times 10^6$ $CD3^+$ T cells per kilogram. Group B was treated by DLI alone at a dose of $10^7$ MNC per kilogram. Patients with URD received $5 \times 10^6$ MNC per kilogram. After informed consent, MNC were harvested from the stem cell donor by using a Cobe Spectra apheresis system (Cobe BCT, Inc., Lakewood, CO). MNC were counted, $10^7$ MNC per kilogram were administered directly, and residual cells were cryopreserved in liquid nitrogen to allow administration of $3 \times 10^7$ viable MNC per kilogram after thawing for a second DLI in case of no response to the first infusion.

### Response Evaluation

After DLI, peripheral blood and bone marrow collections were performed every 4 weeks during the first 3 months and at longer intervals thereafter to allow morphologic examination, cytogenetic analysis, and isolation of RNA and DNA for BCR/ABL quantification and analysis of chimerism, respectively.

### Cytogenetic Analysis

This was performed by conventional banding techniques and with FISH. FISH was performed as described previously, with minor modifications [21]. Briefly, fluorescent-labeled probes for the BCR and ABL genes (LSI-bcr/abl or LSI-bcr/abl ES; Vysis Inc.,

Downers Grove, IL) identified the BCR/ABL fusion gene in interphase or metaphase nuclei in CML cells when the signals were juxtaposed.

### Molecular Analysis of BCR/ABL Transcripts

This was performed by quantitative real-time polymerase chain reaction (PCR) analysis as described previously [22]. Briefly, total RNA was isolated from bone marrow and peripheral blood aspirates by using Trizol (Invitrogen, Carlsbad, CA), according to the manufacturer's instructions. Random primed complementary DNA (cDNA) was synthesized from 1 μg of total RNA by using the first-strand cDNA synthesis kit for reverse transcriptase-PCR (AMV; Roche, Indianapolis, IN). Quantitative real-time PCR for BCR/ABL was performed on an ABI/Prism 7700 Sequence Detector System (Applied Biosystems, Foster City, CA). Expression of BCR/ABL transcripts was compared with a serial dilution of CML cells in MNC obtained from a healthy individual. The amount of porfobilinogendehydrogenase (PBGD) [22] expression in the samples was determined to correct for differences between samples and runs. To correct for the total amount of amplified cDNA, the ratio between absolute numbers of BCR/ABL transcripts and PBGD transcripts was calculated. The limit of detection for this assay was a BCR/ABL-PBGD ratio of $1.10^{-5}$.

### Chimerism Analysis

Donor chimerism was quantified in peripheral blood leukocytes, bone marrow cells, or both, as described recently [23]. For sex-mismatched transplantations, chimerism was analyzed by FISH with X- and Y-chromosome–specific probes. The probes used to identify the X- and Y-chromosome were a centromere–X-specific DNA probe (pBamX5) and a DNA probe specific for the heterochromatic region of the Y-chromosome (pY3.4). For sex-matched transplantations, genomic DNA was isolated with a Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN). Donor and patient were screened for informative polymorphic genomic markers by using the AmpFLSTR Profiler Plus ID Amplification Kit (Applied Biosystems). Posttransplantation samples were analyzed with 2 polymorphic markers informative for the number of hematopoietic cells from patient origin. Amplification was performed on a 96-well GeneAmp PCR System 9700 thermocycler (Applied Biosystems) by using AmpliTaq Gold DNA Polymerase (Applied Biosystems). PCR products were analyzed on an ABI Prism 310 Genetic Analyzer with GeneScan Analysis software version 2.1 (Applied Biosystems). The sensitivity for most markers was 1%.

### Study End Points and Statistical Analysis

Data were analyzed as of May 1, 2003. The median follow-up was 51 months (range, 14.5-102 months) for group A and 21 months (range, 9-35 months) for group B. Overall survival was estimated, by using the Kaplan-Meier method, from the date of first infusion of donor cells until death from any cause or last follow-up evaluation. Patients still alive at the time of analysis were censored at the last follow-up evaluation date. Complete cytogenetic response was defined by the absence of the Philadelphia chromosome by standard cytogenetics or <1% positivity with FISH. Whenever cytogenetic analysis was not performed, CCR was defined by the absence of BCR/ABL transcripts with real-time quantitative PCR analysis. Complete donor chimerism was defined as >99% quantified donor markers and <1% recipient markers. Complete molecular remission was defined by the absence of BCR/ABL transcripts as measured by PCR. Toxicity was graded according to the World Health Organization toxicity scale. Acute GVHD was scored according to the Glucksberg criteria [24], and chronic GVHD was scored according to the Shulman criteria [25].

## RESULTS

### Patients

Thirteen patients with a cytogenetic or hematologic relapse (group A; patients 1-13) and 8 patients with a molecular relapse (group B; patients 14-21) entered the study. Patient characteristics are shown in Table 1. The patients ranged in age at the time of SCT from 27 to 48 years (median, 38 years) in group A and from 19 to 48 years (median, 42 years) in group B. The time interval from allogeneic SCT to relapse ranged from 7 to 66 months (median, 17 months) for group A and from 5 to 23 months (median, 12 months) for group B. In group A, 3 patients received URD transplants, and in group B 2 patients had an URD. In 2 patients (patients 5 and 13), α-IFN was initially reduced in dose but was finally stopped after 50 and 54 weeks, respectively, because of severe flulike symptoms.

### Cytogenetic Response

Twelve of 13 patients from group A entered a CCR. The median interval between DLI and CCR was 7 weeks (range, 5-18 weeks; Table 2). Patient 5 developed a second (molecular) relapse after discontinuation of α-IFN because of side effects. She received additional doses of DLI, and this was followed by a second molecular remission. Patient 13 did not reach cytogenetic remission until after a fifth dose of DLI. This patient did not receive α-IFN continuously

**Table 2.** *Results of DLI Treatment in Patients with Relapsing CML after Allogeneic SCT*

| Patient No. | Chemotherapy Before DLI | MNC/kg | DLI-CCR (wk) | DLI-Molecular Remission (wk) | DLI-Complete Donor Chimerism (wk) | Grade Acute GVHD | Grade Chronic GVHD | Alive |
|---|---|---|---|---|---|---|---|---|
| 1 | No | $1 \times 10^7$ | 6 | 14 | 10 | 0 | 0 | Yes |
| 2 | No | $1 \times 10^7$ | 6 | 8 | 6 | II | 0 | Yes |
| 3 | No | $1 \times 10^7$ | 6 | 18 | 6 | 0 | 0 | Yes |
| 4 | No | $1 \times 10^7$ | 6 | 34 | 7 | II | Limited | No |
| 5 | No | $1 \times 10^7$* | 15 | 15† | 15 | 0 | 0 | Yes |
| 6 | No | $1 \times 10^7$ | 5 | 9 | 9 | 0 | 0 | Yes |
| 7 | No | $0.5 \times 10^7$ | 10 | 10 | 10 | III | Extensive | No |
| 8 | No | $1 \times 10^7$ | 16 | 20 | 16 | 0 | Limited | Yes |
| 9 | No | $1 \times 10^7$ | 18 | 26 | 10 | IV | Extensive | Yes |
| 10 | Hydrea | $0.6 \times 10^7$ | NR | NR | NR | I | Extensive | No |
| 11 | Hydrea | $1 \times 10^7$ | 7 | 20 | 7 | III | 0 | Yes |
| 12 | No | $1 \times 10^7$ | 9 | 9 | 9 | I | 0 | Yes |
| 13 | Hydrea | $1 \times 10^7$* | NA | NR | 121† | 0 | 0 | Yes |
| Median | | | 7 (5-18) | 15 (8-34) | 10 (6-121) | | | |
| 14 | No | $1 \times 10^7$ | | 29 | | 0 | 0 | Yes |
| | | $3 \times 10^7$ | | 16 | | | | |
| 15 | No | $1 \times 10^7$ | | 13 | | 0 | 0 | Yes |
| 16 | No | $1 \times 10^7$ | | 13 | | 0 | 0 | Yes |
| 17 | No | $5 \times 10^6$ | | 12 | | I | Limited | Yes |
| 18 | No | $1 \times 10^7$ | | 14 | | III | Extensive | Yes |
| 19 | No | $1 \times 10^7$ | | 17 | | 0 | 0 | Yes |
| 20 | No | $1 \times 10^7$ | | 26 | | 0 | 0 | Yes |
| 21 | No | $5 \times 10^6$ | | 12 | | I | 0 | Yes |
| Median | | | | 14 (12-29) | | | | |

MNC indicates mononuclear cells; DLI-CCR, interval between first donor leukocyte infusion and complete cytogenetic response; NR, no remission; NA, not assessed.

*Needed additional doses of DLI.

†Measured from the date of first DLI. Patient 14 progressed to a hematologic relapse after the first DLI; subsequent treatment with DLI + α-IFN induced complete molecular remission.

because of side effects, and eventually α-IFN was stopped.

## BCR/ABL and Chimerism Response

Twelve of 13 patients from group A reached complete donor chimerism at a median of 10 weeks (range, 6-121 weeks), and 11 of them reached a complete molecular remission at a median of 15 weeks (range, 8-34 weeks; Table 2; Figure 1). Seven of 8 patients from group B reached a molecular remission at a median of 14 weeks (range, 12-29 weeks; Table 2). Patient 14 was initially treated with DLI alone ($10^7$ MNC per kilogram) without α-IFN, according to protocol, but because of rapid progression from a molecular relapse to a hematologic relapse, the patient was treated with $3 \times 10^7$ MNC per kilogram and α-IFN 13 weeks after the first DLI, resulting in complete molecular remission. Donor chimerism was always mixed at relapse for patients from group A. Ten patients from group A converted to complete donor chimerism after 1 dose of DLI; 2 patients needed additional doses. Patient 5 received 2 doses, and patient 13 needed 5 doses of DLI (total cell dose, $7.4 \times 10^8$ MNC per kilogram) to a obtain complete donor chimerism (Figure 1). Complete donor chimerism was present at the time of molecular relapse in 6 patients from group B. The other 2 patients (patients 18 and 20) converted to complete donor chimerism after DLI at 5 and 12 weeks, respectively.

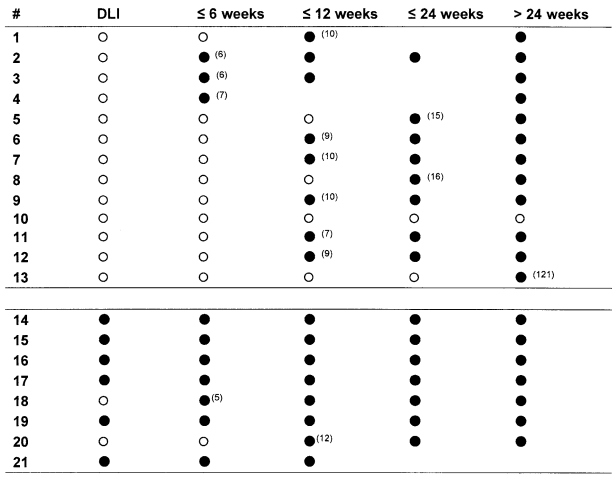


**Figure 1.** Kinetics of donor chimerism in patients with a relapse of CML after allogeneic SCT treated with DLI. Kinetics of donor chimerism for group A (cytogenetic or hematologic relapse; patients 1-13) and group B (molecular relapse; patients 14-21); between brackets is the first week of complete donor chimerism. ○ indicates mixed donor chimerism; ●, complete donor chimerism.

### GVHD and Other Toxicity

Seven of 13 patients from group A developed acute GVHD: 2 patients had grade I, 2 patients had grade II, 2 patients had grade III, and 1 patient had grade IV. Two patients developed limited chronic GVHD, and 3 developed extensive chronic GVHD (Table 2). GVHD occurred both before and after CCR was reached at a median of 8 weeks (range, 3-28 weeks) after DLI (data not shown). In group B, 3 patients developed acute GVHD: 2 patients developed grade I, and 2 patients developed grade III aGVHD. The latter patients also developed extensive chronic GVHD. All 6 patients with acute GVHD grade II to IV after DLI (5 patients in group A and 1 patient in group B) had a relatively short interval between SCT and relapse (median, 9 months; range, 5-12 months) when compared with patients with no relevant GVHD (median, 17 months; range, 9-66 months). Patient 12 developed transient aplasia of the bone marrow. Patients 4 and 6 in group A and patient 17 in group B developed an idiopathic thrombocytopenic purpura (ITP) diagnosed by thrombocytopenia in peripheral blood and sufficient megakaryopoiesis in the bone marrow. None of these 3 patients had detectable antiplatelet antibodies. Two patients responded well to treatment with prednisone, and 1 patient underwent splenectomy, after which the platelets normalized. Patient 6 also developed hypothyroidism. Most patients treated with α-IFN developed mild flulike symptoms. In 2 patients (patients 5 and 13), severe flulike symptoms resolved after cessation of the IFN.

### Survival

Ten of 13 patients from group A are alive with a median follow-up of 62 months (range, 37-102 months) after DLI. The 1-year overall survival is 100%, 3-year survival is 85%, and 5-year survival is 76% in group A (Figure 2). Two patients from group A died as a result of a gram-negative sepsis while they were receiving immunosuppressive treatment for GVHD, and 1 patient died as a result of acute myocardial infarction. In group B, all patients are alive with a median follow-up of 20 months (range, 9-35 months) after DLI.

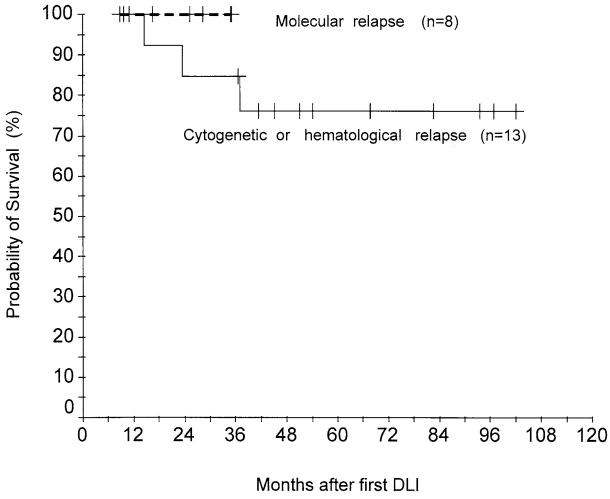


**Figure 2.** Survival probability of patients with a relapse of CML after allogeneic SCT treated with DLI: Kaplan-Meier curve of overall survival for group A (cytogenetic or hematologic relapse) and group B (molecular relapse) measured in months from first DLI.

## DISCUSSION

In this study, we pioneered the use of very-low-dose DLI in combination with α-IFN to induce CCR in patients with a cytogenetic or hematologic response after allogeneic SCT and the use of very-low-dose DLI alone in patients with a molecular relapse. We report the results of DLI treatment for relapsed CML patients in our hospital from 1994 to 2003. Up to 1999, relapse after allogeneic SCT was diagnosed by morphologic examination, cytogenetic analysis of blood and marrow cells, or both. In this cohort of patients with a cytogenetic or a hematologic relapse of CML-CP after allogeneic SCT (group A), we illustrated that the combination of α-IFN and very-low-dose DLI (0.2-0.6 $\times$ $10^7$ $CD3^+$ T cells per kilogram) induced a rapid and durable CCR in 92% of the patients. Two patients not continuously receiving α-IFN needed additional doses of DLI to achieve a CCR. CCR occurred at a median of 7 weeks after DLI for patients with a cytogenetic or a hematologic relapse (Table 2). Complete molecular response and complete donor chimerism occurred in all patients with a CCR (Table 2). Treatment according to this regimen resulted in an acceptable incidence of both acute GVHD grade II to IV in 5 of 13 patients and also extensive chronic GVHD in 3 of 13 patients (Table 2). From 2000 onward, we monitored CML patients after allogeneic SCT prospectively with quantitative BCR/ABL and chimerism measurements at fixed time points. As a consequence, relapses were detected at an (earlier) molecular level. Patients with a molecular relapse were treated with very-low-dose DLI without α-IFN, because we assumed that DLI alone could eradicate the lower tumor mass in molecular relapses compared with the larger tumor mass in advanced CML relapses. In case of progression, α-IFN was added to the treatment. Low-dose DLI without α-IFN induced a molecular response in 7 of 8 patients with a molecular relapse (group B) with acute GVHD grade III, and extensive chronic GVHD occurred in only 1 of 8 patients (Table 2). The nonresponding patient went into remission when α-IFN was added to a higher dose of DLI.

The CCR rate of 92% accomplished in our study for hematologic or cytogenetic relapses is similar to the CCR rates of 57% to 80% reported in the literature after DLI for patients with relapsed CML in CP after allogeneic SCT [3-14]. Mackinnon et al. [6] reported a mean time of 14 weeks between DLI and CCR for patients treated with $10^7$ donor $CD3^+$ T lymphocytes per kilogram. In this study, patients often needed extra infusions with higher doses of T lymphocytes and were sometimes also treated with α-IFN, resulting in long intervals between the initial treatment and remissions. The median time to cytogenetic remission in the study of Dazzi et al. [7] was 18 weeks for the group treated with a bulk dose and 22 weeks for the escalated-dose group. We postulated that adding α-IFN to the treatment with very-low-dose DLI would decrease the interval between DLI and CCR. In our study, we indeed observed a median time of 7 weeks between DLI and CCR, which is a clear difference as compared with the results of Mackinnon et al. and Dazzi et al. Two patients (patients 5 and 13) not continuously receiving α-IFN needed extra doses of DLI and reached their remissions relatively late. These observations support the synergistic effect of α-IFN with DLI. Moreover, the T-cell dose administered ($10^7$ MNC per kilogram contain approximately $0.5 \times 10^7$ $CD3^+$ cells per kilogram) in our study was approximately 50% lower than the effective doses in the 2 other studies mentioned [6,7]. Finally, the median time to molecular response in our study for patients with a molecular relapse who were treated by DLI alone (14 weeks) is comparable to the median time to molecular response of patients with a hematologic or cytogenetic relapse who were treated with additional α-IFN (15 weeks). A possible explanation is that although the tumor load to be eradicated in patients with a cytogenetic or hematologic relapse is higher than in molecular relapses, α-IFN accelerated this eradication.

We also analyzed the effect of α-IFN treatment in combination with very-low-dose DLI on the incidence of GVHD. The incidences of acute GVHD grade II to IV in 5 and extensive chronic GVHD in 3 of 13 patients of group A in our study were similar to those reported in other studies [3-5,7-14], with the exception of the study by Mackinnon et al. [6]. Incidences of 33% to 90% of GVHD grade II to IV have been reported and were correlated with the number of donor lymphocytes infused [3-5,8-13]. In a recent large retrospective study comprising 344 patients with relapsed CML treated with DLI, a low initial cell dose of $<2 \times 10^7$ MNC per kilogram was associated with 26% GVHD [14]. The median dose of MNC administered to patients in these studies [3-5,7-14], which ranged from a median of $1.7 \times 10^8$ to $6 \times 10^8$, was higher than the MNC dose of $0.1 \times 10^8$/kg that our patients received. These data illustrate that the addition of α-IFN to very-low-dose DLI seemed to reduce the necessity of giving escalating doses of DLI, with an acceptable incidence of GVHD in patients with a hematologic or cytogenetic relapse. In other studies, escalated doses were often necessary after a low initial cell dose without α-IFN [6,7,14]. Finally, we observed that a short interval between allogeneic SCT and relapse was associated with an increased incidence of significant acute GVHD after DLI, especially for cytogenetic and hematologic relapses. Five of 11 patients who received DLI within 12 months after allogeneic SCT developed acute GVHD grade II to IV, compared with none of 10 patients who received DLI more than 12 months after allogeneic SCT. A possible explanation is that antigen-presenting cells, which are still of recipient origin in the first months after allogeneic SCT, are gradually replaced by antigen-presenting cells of donor origin during the first year after allogeneic SCT. However, this association was not found in 2 recently published analyses of prognostic factors for acute GVHD after DLI [26,27]. Differences between those studies and our study population were T-cell depletion of the graft and the number of T cells administered with DLI. All our stem cell grafts were T-cell depleted, and, therefore, conclusions can be drawn only for these types of transplants, because conflicting reports have been presented on the response rate and incidence of GVHD after DLI. In a recent study comprising 593 DLIs, T-cell depletion at transplantation was not a significant predictor either for response or for acute GVHD grade II to IV in multivariate analysis [27]. In contrast, in another multivariate logistic analysis of prognostic factors for acute GVHD after DLI for relapsed CML, T-cell depletion at transplantation was associated with an increased risk of GVHD after DLI [26].

Because our study was a single-arm prospective study and other studies were retrospective analyses, only a comparative trial may give a definite answer as to whether the addition of α-IFN to very-low-dose DLI is advantageous for treating CML relapses after allogeneic SCT. The introduction of imatinib, a new and highly effective agent against CML, has changed treatment algorithms for CML already [28]. In a recent study, imatinib seemed to be effective in patients with CML who relapsed after allogeneic SCT even after failing DLI, without a risk of GVHD [29]. It would be of interest to determine in a prospective comparative trial not only whether the addition of α-IFN to very-low-dose DLI enforces the immune response against CML, but also whether imatinib given after SCT might increase the interval between transplantation and relapse and thus also the SCT-DLI interval. α-IFN might improve the response rate, whereas imatinib might prolong the interval between transplantation and DLI, which is associated with less GVHD, as shown in this study.

Our observation of the occurrence of ITP in 3 patients, 2 of whom were treated with α-IFN, has not been reported by others as a complication associated with DLI therapy for relapse CML. α-IFN treatment by itself has been associated with autoimmune phenomena [30], but because in our study 1 patient who was not receiving α-IFN also developed ITP, a correlation with this drug is not a likely explanation. Others reported aplasia of the bone marrow, with thrombocytopenia as a consequence [31]. However, in our patients, megakaryopoiesis was intact, and thrombocytes increased after standard treatment for ITP.

In conclusion, treatment of a cytogenetic or hematologic relapse of CML after allogeneic SCT with a combination of very-low-dose DLI ($10^7$ MNC per kilogram, containing approximately $5 \times 10^6$ $CD3^+$ T cells per kilogram) and α-IFN resulted in a relatively short interval to obtain a durable CCR and molecular CR in >90% of our patients. Furthermore, this regimen induced a clinically acceptable rate of acute GVHD, especially when DLI was given more than 12 months after allogeneic SCT. Patients with a molecular relapse could be effectively and safely treated with a very low dose of DLI containing only approximately $5 \times 10^6$ $CD3^+$ T cells per kilogram. When patients do not respond to DLI alone, the addition of α-IFN to a second dose of DLI could induce a remission.

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