Outcomes after myeloablative unrelated donor stem cell transplantation using both *in vitro* and *in vivo* T-cell depletion with alemtuzumab

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HLA-matched unrelated donor (MUD) stem cell transplantation (MUD) is complicated by a high incidence of graft-versus-host-disease (GVHD) resulting in significant morbidity and mortality. To circumvent this problem we included alemtuzumab for *in vivo* and *in vitro* T-cell depletion in a myeloablative MUD-SCT regimen. After SCT, no severe acute GVHD was observed in the 30 transplanted patients. Donor lymphocyte infusion administered at a later time point resulted in sustained anti-tumor responses in most patients with chronic myeloid leukemia. After donor lymphocyte infusion three patients developed severe acute GVHD. Due to good responsiveness to immunosuppressive therapy only two patients developed persistent chronic GVHD. The main advantage of the transplantation regimen including alemtuzumab is that not only mortality due to GVHD is limited but also extensive chronic GVHD, which potentially leads to chronic morbidity and diminished quality of life, is hardly observed.

Key words: alemtuzumab, stem cell transplantation, myeloablative, graft versus host disease

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Hematopoietic stem cell transplantation (SCT) using matched unrelated donors (MUD) has emerged as an important therapeutic option, because it increases the probability of finding a suitable donor to almost 90% in Caucasian patients.[1] However, this type of transplantation is associated with a higher rate of transplant-related mortality (TRM) compared to HLA-identical sibling transplantations, which can be largely attributed to acute and chronic graft-versus-host-disease (GVHD).[2] T-cell depletion of the stem cell graft remains the most effective method to prevent GVHD after SCT from related or unrelated donors. Campath-1H (alemtuzumab) is a humanized rat monoclonal anti-CD52 antibody, which can be used intravenously for T-cell depletion in the patient or directly added to the stem cell graft *in vitro* for purging.[3] Alemtuzumab has mainly been used for T-cell depletion in HLA identical sibling myeloablative[4] and many different non-myeloablative SCT regimens.[5-11] Experience on the use of alemtuzumab in myeloablative MUD-SCT is sparse. Three different small single center studies have been published,[12-14] two of which predominantly reported on pediatric patients. In these studies the use of alemtuzumab dramatically reduced the incidence of acute and chronic GVHD resulting in a low GVHD-related mortality. In this report we describe a myeloablative transplantation regimen for unrelated donors consisting of cyclophosphamide, total body irradiation and alemtuzumab administered both *in vivo* to the patient and *in vitro* to the graft. We report the results of a long-term follow-up of all adult patients (n=30) transplanted according to this regimen between January 1997 and June 2002 in the Leiden University Medical Center.

Design and Methods

The institutional Ethics Committee approved the transplant protocol. Written informed consent was obtained from all study patients according to the Helsinski Declaration. The characteristics of the patients are shown in Table. 1. The conditioning regimen consisted of alemtuzumab 5 mg/day intravenously on days –8 to –4, cyclophosphamide 60 mg/kg/day intravenously on days –6 and –5 and total body irradiation 6 Gy/day on days –8 and –7. T-cell depletion of the stem cell product was performed by incubation with 20 mg alemtuzumab for 30 minutes at room temperature under continuous agitation (Campath in the bag).[4] Post-transplant GVHD prophylaxis consisted of cyclosporine A, 3 mg/kg/day intravenously from day –1, changed to an oral dose as soon as tolerated by the patient and tapered off 3 months after SCT. Additionally, methotrexate (10 mg/m^2/day on days +3, +6 and +11) was administered to

patients who had received a peripheral blood stem cell graft. Standard post-transplantation supportive care was provided, including cotrimoxazole prophylaxis and weekly cytomegalovirus (CMV) monitoring, as described earlier.[4] Donor lymphocyte infusion (DLI) was administered to patients who relapsed or showed disease progression. The dose of DLI depended on the underlying disease and varied from 0.25 to 2.5×10^7 CD3$^+$ T cells per kg. DLI was administered in combination with α-interferon (Roferon A, 3×10^6 U daily subcutaneously) in patients with chronic myeloid leukemia (CML) with a hematologic or cytogenetic relapse and in relapsed acute leukemia patients. CML patients with only molecular disease did not receive α-interferon.

Criteria used for defining engraftment and graft failure, GVHD score, response evaluation, treatment-related mortality and disease free survival have been described before.[4] Survival curves were estimated according to the Kaplan-Meier method.

Results and Discussion

Here we report our results with a MUD-SCT regimen containing alemtuzumab for *in vivo* host T-cell depletion to prevent rejection and to reduce GVHD. For optimal GVHD prevention, alemtuzumab was also added *in vitro* to the graft at a dose of 20 mg, as reported earlier.[15] Cyclosporine and methotrexate were included for additional GVHD prophylaxis.

In order to decrease the risk of viral infections due to delayed lymphocyte recovery, we administered only a total dose of 25 mg of alemtuzumab *in vivo* to the patient, which is much lower than the dose of 100 mg used in earlier myeloablative and non-myeloablative transplantation regimens.[3,4,13,14,15] We made this dose reduction because in a large study comparing T-cell depletion with no T-cell depletion, transplant-related mortality was increased in T-cell depleted patients due to increased graft failure and infections, despite a lower incidence of GVHD.[16]

With this regimen we observed engraftment in all patients after administration of a median of 2.5×10^6 per kg CD34-positive cells (range 0.8-19.0). The median time to neutrophil engraftment was 17 days, the median time to platelet engraftment 26 days (Table 1). In one of the patients with myelodysplastic syndrome, the graft was rejected 2 months after transplantation; all other patients had stable engraftment. Chimerism was analyzed 3 months after SCT in 24 of 26 evaluable patients. Full donor chimerism was observed in 75% of patients, while mixed chimerism with a high percentage of donor cells (91-99%) was found in 21% of patients (Table. 1). In one patient the percentage of donor cells at 3 months was only 20% due to relapsed acute lymphoblastic leukemia. During later follow up, persistent full donor chimerism was observed in all patients not developing disease relapse. Of the five patients with mixed chimerism with a donor cell percentage over 90%, three converted to full donor chimerism spontaneously and two after DLI.

No severe acute GVHD (grade III-IV) was observed after SCT (Table 2). Only mild grade I-II GVHD of the skin developed in 43% of patients, which quickly resolved with topical corticosteroid therapy in all patients. Chronic GVHD developed in only three patients after SCT (12% of evaluable patients), resolving after treatment in two patients. Only one patient developed extensive chronic GVHD, that did not resolve

Table 1. Characteristics of the patients and transplantation.

No. of patients	*30*
Median age (years)	34 (18-48)
Male:female	20:10
Diagnosis	
CML	
chronic phase	8
accelerated phase	2
blast crisis	2
AML	
first CR	1
second CR	6
ALL	
first CR	1
second CR	5
partial remission	1
Myelodysplastic syndrome	2
Non-Hodgkin's lymphoma	1
Fanconi's anemia	1
Median time from diagnosis to SCT (years)	0.9 (0.4-19.9)
Disease status at transplantation	
Complete remission	13
Partial remission	2
Chronic phase/stable disease	15
Risk status	
High	16
Low*	14
Source of stem cells	
Bone marrow	19
Mobilized peripheral blood	11
Stem cell dose (×10^6 per kg CD34$^+$ cells)	2.5 (0.8-19.0)
HLA compatibility+	
Fully matched	24
One locus mismatch	6
Median time to engraftment (days)	
Neutrophil$^{\&}$	17 (9-29)
Platelet§	26 (13-73)
Chimerism at 3 months	
Full donor	18
Mixed donor-patient	6
Not evaluated	6

*CR: complete remission; CML: chronic myeloid leukemia; AML: acute myeloid leukemia; ALL: acute lyphocytic leukemia; *first CR or first chronic phase (CML); +Determined for ten HLA loci (HLA A, B, C, DRB1 and DQB1) by molecular methods at high resolution level; $^{\&}$Defined as the first of two consecutive days with an absolute neutrophil count above 0.1×10^9/L; §Defined as the first of seven consecutive days with a platelet count above 20×10^9/L without transfusion.*

Table 2. Graft-versus-host disease after SCT and after DLI.

GVHD after SCT		
Acute GVHD grade	*Number (n=30)*	*Development into chronic GVHD*
0	16	–
I	10	2
II	2	0
III-IV	0	–
Not evaluable	2	–
Chronic GVHD		*Number not resolving*
No	23	–
Limited	2	0
Extensive	1	1
Not evaluable	4	–
GVHD after DLI		
Acute GVHD grade	*Number (n=10)*	*Development into chronic GVHD*
0	3	–
I	4	2
II	0	–
III-IV	3	2
Not evaluable	0	–
Chronic GVHD		*Number not resolving*
No	4	–
Limited	3	0
Extensive	2	1
Not evaluable	1	–

with immunosuppressive therapy. Ten patients received DLI at a median of 17.2 months (range 4.2-31.6) after SCT. Nine of these patients received low dose DLI for progression of CML, one patient was given a high dose of DLI for relapsed acute lymphocytic leukemia early after SCT. DLI administration induced graft-versus-tumor effects in eight of the nine CML patients resulting in sustained complete molecular remissions. In one CML patient molecular disease persisted in spite of increasing doses of DLI. The patient with relapsed acute lymphocytic leukemia died 7 weeks after DLI administration due to progressive disease. After DLI, three patients developed severe acute GVHD and two patients chronic extensive GVHD (Table 2). GVHD secondary to DLI administration was responsive to standard immunosuppressive therapy in most patients. Chronic extensive GVHD persisted in only one patient.

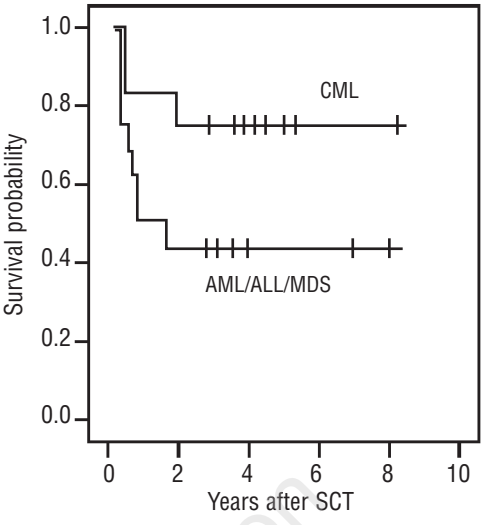

Figure 1. Overall survival: chronic myeloid leukemia (CML) versus acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL) and myelodysplastic syndrome (MDS).

Due to good responsiveness to immunosuppressive therapy and a low incidence of persisting chronic extensive GVHD, mortality associated with GVHD was only 7%. CMV reactivation occurred in 71% of the patients at risk for CMV disease. Most patients with CMV reactivation were successfully treated with ganciclovir and only one patient developed CMV disease. No Epstein Barr virus-related disease was observed. Transplant-related mortality was 17%. Four patients died from infectious causes (CMV disease, bacterial sepsis, candida sepsis and aspergillosis), one patient from rejection, two from GVHD and one from severe autoimmune hemolytic anemia. In total, tansplant-related mortality due to infectious causes was 13%. Seventeen patients were alive at follow-up. The median follow-up time was 50 months (range 34-99). Disease-free survival was 53%. Overall survival was 57% with a mean survival of 4.9 years. The percentage of CML patients surviving was higher (75%) than the percentages of patients with acute leukemia or myelodysplastic syndrome (43%) (Figure 1). The cumulative incidence of relapse was 33 % in the group of patients with acute leukemia or myelodysplastic syndrome. Relapse occurred too early for safe DLI administration with acceptable GVHD (median 84 days, range 54-138). All these patients died from their relapse. At last follow-up, all CML patients were in complete molecular remission with negative bcr-abl levels, except for one CML patient with persisting molecular disease despite escalating doses of DLI. All other patients were in complete remission.

We conclude that a MUD-SCT regimen in which alemtuzumab is administered *in vivo* and *in vitro* for the prevention of rejection and GVHD prophylaxis and DLI is administered for progressive disease at a later time

after SCT is successful in CML patients. We observed excellent engraftment and limited mortality due to infectious disease with this regimen. Although acute and chronic GVHD was observed, especially after DLI administration, GVHD responded well to immunosuppressive therapy. The main advantage of this regimen is that not only is mortality due to GVHD low, but also that hardly any persisting chronic GVHD is observed. Persisting chronic extensive GVHD results in significant morbidity and is regarded to be an important negative predictor of quality of life after SCT.[17,18] Persisting chronic GVHD has been shown to occur in 34% of patients in non T-cell depleted MUD-SCT resulting in a decreased performance status and quality of life compared to patients treated with antithymocyte globulin for T-cell depletion.[19]

The alemtuzumab-containing regimen that we describe here may prove successful in other slowly progressive diseases (for example low grade lymphoma and chronic lymphocytic leukemia) in which DLI administration can be delayed until a safe time after SCT. This regimen is less ideal for acute leukemia patients due to early relapses after SCT. However, the overall survival of 43% we observed in the group of patients with acute leukemias or myelodysplastic syndrome patients (Figure 1) is relatively high, considering that 88% of these patients were high-risk (transplantation in second complete remission or prognostically bad cytogenetics). These data are consistent with relapse rates in two other myeloablative MUD-SCT studies in acute myeloid leukemia[13] and acute lymphoblastic leukemia[14] using alemtuzumab for T-cell depletion. Larger studies will be needed to determine the efficacy of this transplantation regimen in acute leukemias and myelodysplastic syndrome.

PB, FB and IS performed the data analysis. PB, FF, RW and RB performed further interpretation of the data and wrote the manuscript. WF, MO and GH contributed to reviewing the manuscript.

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