

ORIGINAL ARTICLE

A fludarabine, thiotepa reduced toxicity conditioning regimen designed specifically for allogeneic second haematopoietic cell transplantation after failure of previous autologous or allogeneic transplantation

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We present a phase II study of fludarabine $5 \times 30 \text{ mg/m}^2$, thiotepa $3 \times 5 \text{ mg/kg}$ as preparative regimen specifically for allogeneic second haematopoietic stem cell transplantation (HCT) after failure of previous HCT. Forty-nine patients (median age 52 years, range 27–68) received an allogeneic second HCT after failed autologous ($n = 29$) or allogeneic ($n = 20$) HCT. Diagnoses were AML ($n = 18$), ALL ($n = 3$), multiple myeloma ($n = 11$), lymphoma ($n = 16$) and CML ($n = 1$). GVHD prophylaxis consisted of CYA and mainly low dose alemtuzumab (40 mg). The median follow-up for patients alive is 528 days (range 217–1344). In 43 of 49 (88%) evaluable patients response rates were CR = 19, PR = 14 and SD = 10 at one month. At one year, the probability (95% confidence interval) of relapse is 55.1 (38.2–72)% and the nonrelapse mortality (NRM) is 29 (14.2–44.4)%. Estimated survival at one year is 42.6 (28.7–56.6)% and event free survival is 38.1 (24.4–51.8)%. Survival was significantly better for patients experiencing relapse beyond one year, than for patients relapsing within one year from first transplantation (51.2 (33.5–68.9)% vs 27 (7–48.5)%; $P = 0.013$). We conclude that this regimen is feasible and well tolerated for allogeneic second HCT.

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Introduction

The use of dose intensive chemotherapy regimens followed by haematopoietic stem cell transplantation (HCT) is an

established curative treatment for patients with haematological malignancies refractory to or relapsing after conventional chemotherapy. However, patients experiencing relapse after HCT have a very poor prognosis. Currently, the best curative option for these patients is offered by an allogeneic second HCT.^{1–5}

However, since these patients already received one course of conventional high dose conditioning (CC) cumulative organ toxicity increases the risk of nonrelapse mortality (NRM). Especially, TBI or busulphan/cyclophosphamide based regimens are associated with substantial organ toxicity.⁶ Furthermore, extensive tissue damage increases the risk of severe acute GVHD.

The reduced toxicity conditioning transplantation concept is based on the notion that the GVL contributes significantly to the curative potential of allogeneic (allo)-HCT. Yet, the conditioning regimen provides for effective antileukaemic activity, without the toxicity of conventional conditioning.^{7–11}

We established a preparative regimen based on thiotepa and fludarabine for allogeneic second HCT aiming at low toxicity, high tolerability and good activity against lymphohaematopoietic disorders. Fludarabine has been widely used in different combinations for reduced toxicity conditioning.¹¹ It has potent lymphocytotoxic activity, hence, facilitating engraftment and furthermore, it enhances the cytotoxic activity of alkylating agents. Thiotepa, in turn, is an alkylating agent with potent activity against lymphohaematopoietic cells,¹² that is also used in several combinations for conditioning^{13,14} and has a very favourable toxicity profile. Thiotepa can be safely escalated up to 500 mg/m^2 until nonhaematological toxicities may be observed.^{15,16}

For GVHD prophylaxis we initially used CYA, MTX and for unrelated donor transplants antilymphocyte globulin (ATG). After 7 patients this protocol was replaced because of severe toxicity in 3 patients by CYA and low dose alemtuzumab (40 mg abs.) for all following 42 patients.

Here, we report the results of 49 consecutive patients with recurrent or persistent haematological malignancy after a previous CC regimen and autologous or allogeneic transplantation treated within a clinical phase II trial for allogeneic second HCT.

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Patients and methods

Patients

Patient characteristics are listed in Table 1. Forty-nine patients were treated between April 2002 and July 2005; the date of evaluation was 25 January 2006. The study was initiated after internal board of review and ethics committee approval. Eligible for this trial were patients with haematological malignancies pretreated with one or more previous high-dose chemotherapies for conditioning followed by either autologous ($n=29$) or allogeneic ($n=20$) transplantation, who had persistent or recurrent disease. Patients were enrolled following written informed consent according to the declaration of Helsinki. Of 49 patients, 37 (75%) received at least one cycle of chemotherapy

Table 1 Patient characteristics

| | |
|---|---------------|
| <i>Patients, no.</i> | 49 |
| Age (years), median (range) | 52 (27–68) |
| Karnofsky score at transplantation, median (range) | 90 (50–100) |
| Sex, no. (%) | |
| Male | 27 (55) |
| Female | 22 (45) |
| <i>Disease, no. (%)</i> | |
| AML | 18 (37) |
| ALL | 3 (6) |
| Lymphoma | 16 (33) |
| Myeloma | 11 (22) |
| CML | 1 (2) |
| <i>1st transplantation → 2nd transplantation, no. (%)</i> | |
| Autologous → related | 6 (12) |
| Autologous → unrelated | 23 (47) |
| Related → related | 1 (2) |
| Related → unrelated | 4 (8) |
| Unrelated → unrelated | 15 (31) |
| <i>Time from first to second HCT (days), median (range)</i> | |
| First → second | 547 (45–4129) |
| Autologous → allogeneic | 805 (45–4129) |
| Allogeneic → allogeneic | 391 (51–2374) |
| <i>Disease status, no. (%)</i> | |
| Untreated relapse/progression | 12 (24) |
| Chemo-sensitive | 16 (33) |
| Chemo-refractory | 21 (43) |
| <i>Donors, no. (%)</i> | |
| HLA identical siblings | 7 (17) |
| Unrelated donors ^a | 42 (83) |
| <i>Donor/recipient sex, no. (%)</i> | |
| Matched | 31 (63) |
| Mismatched | 18 (37) |
| <i>CMV serology, no. (%)</i> | |
| Patient positive | 32 (65) |
| Patient negative | 17 (35) |
| <i>GVHD prophylaxis, no. (%)</i> | |
| CYA/mMTX/ATG40 ^b | 7 (14) |
| CYA/Campath40 | 42 (86) |

^aHLA matched for HLA-A, -B, -DR, -DQ. Six transplants were performed with one mismatch, one with two mismatches.

^bFour patients received CYA/ATG/MTX. One patient received CYA/MTX, one patient CYA/ATG and one patient CYA/ATG/MMF.

pretransplant, 12 (25%) were transplanted in untreated relapse/progression. Of the 37 chemotherapy-treated patients, 21 (57%) were defined as chemoresistant relapse, when no tumour reduction of at least 50% was reached. Of the 20 previous allogeneic-transplant patients, 10 (50%) had received donor lymphocyte infusions (DLI), prior to second transplant. Diagnoses and disease status were AML ($n=18$): untreated relapse ($n=5$), chemosensitive relapse ($n=6$), chemoresistant relapse ($n=7$); ALL ($n=3$) all in sensitive relapse; multiple myeloma ($n=11$): untreated relapse ($n=5$), sensitive relapse ($n=2$), resistant relapse ($n=4$); lymphoma ($n=16$; 15 non-Hodgkin's lymphoma, 1 Hodgkin's disease): untreated relapse ($n=1$), sensitive relapse ($n=5$), resistant relapse ($n=10$) and CML ($n=1$) in untreated relapse (2CP). Previously used conditioning regimens were busulphan (BU)/cyclophosphamide (CY) ($n=14$), BU ($n=1$), BEAM ($n=13$), melphalan ($n=9$), fludarabine/BCNU/melphalan ($n=7$),¹⁷ TBI (12 Gy)/VP-16/CY ($n=3$) and TBI (10 Gy)/melphalan ($n=2$).

Donors and grafts

HLA class I antigens were typed by serology or intermediate resolution DNA techniques (two digits) and HLA class II by high resolution DNA techniques (four digits).¹⁸ Donors were HLA-matched siblings in 7 transplants and HLA-matched (A,B,DRB1,DQB1) volunteers in 42 transplants. Six of 42 unrelated transplants were performed with one mismatch and one was performed with two mismatches. The median age of the donors was 36 years (range 18–62). All patients were transplanted with G-CSF mobilized unmanipulated PBSC except for one who received bone marrow. Thirty-one patients received a graft from a same sex donor, 18 patient–donor pairs were from different sexes. Thirty-nine patients were at risk for CMV reactivations (CMV positive donor or recipient).

Transplant characteristics

Of 49 patients included in this study, 28 (57%) had previously received one autologous transplantation, 1 (2%) had received two previous autografts and 20 (41%) had received one previous allograft. Altogether, 42 patients underwent unrelated (PBSCT 41, BMT 1) and 7 sibling transplantation (all PBSCT). Of the 29 previously autografted patients, 6 received a related allograft and 23 received an unrelated allograft. Of the 20 previously allografted patients, 5 had been previously transplanted from a related donor and 15 from an unrelated donor. Except for one patient, who was retransplanted from a different related donor all other previously allografted patients underwent unrelated transplantation from a different donor ($n=18$) or the same donor as the first transplantation ($n=1$; Table 1).

Conditioning regimen and GVHD prophylaxis

All patients received fludarabine 30 mg/m² on days –8, –7, –6, –5, –4 (total dose 150 mg/m²) as 1 hr infusion, and thiotepa 5 mg/kg on days –7, –6, –5 (total dose 15 mg/kg) 2 h after the end of fludarabine as a 2 h infusion. The graft was infused on day 0. For GVHD prophylaxis all patients received CYA 2.5 mg/kg every 12 h starting at day –3.

Four patients transplanted from unrelated donors received rabbit ATG (Fresenius, Graefelfing, Germany) 20 mg/kg on days -2, -1 (total dose 40 mg/kg) and MTX 5 mg/m² on days +1, +3, +6. One patient received CYA and ATG, one patient received CYA and MTX, and one patient received CYA, ATG and mycophenolate mofetil (MMF) 2 g/d starting at day +1. Forty-two patients received CYA and alemtuzumab (MabCampath, Schering, Berlin, Germany) 20 mg/d absolute on days -2, -1 (total dose 40 mg). Supportive care was performed as published previously.¹⁸

Statistical analysis

Overall survival, NRM and probability of relapse were plotted using the method of Kaplan and Meier using the Graph Pad Prism software. Statistical analysis was performed by two-way log rank test. Overall survival was calculated from date of transplant to date of death from any cause. NRM was measured from the date of transplant until death without evidence of progression. Subjects experiencing progression were censored at the date of documented progression. The probability of relapse was calculated from date of transplant until the date of documented relapse/progression.

Results

Engraftment

Engraftment was evaluable in 46 patients. Three patients died prior to engraftment. For evaluable patients the median to reach an absolute count of 0.5×10^9 per litre neutrophils (ANC) was 14 days (range 10–37), for 20×10^9 per litre platelets it was 10 days (range 7–38). At day 30+, 38 of 43 (88%) evaluable patients achieved full donor chimerism, three patients had mixed chimerism and in two patients chimerism analysis was not evaluable for technical reasons. No secondary graft failure occurred.

GVHD

Acute and chronic GVHD were graded as described.¹⁹ Acute GVHD (aGVHD) was evaluable in 46 of 49 patients (94%). We observed grade II aGVHD in 11 patients (24%) and grade III–IV aGVHD in 4 patients (9%; Table 2). All of the patients with severe GVHD had skin and gut involvement. One patient died because of GVHD grade IV of the gut in persistent disease. Thirty-nine patients were evaluable for chronic GVHD (cGVHD), in 22 (56%) no cGVHD was observed, 10 (26%) had limited cGVHD and 7 (18%) extensive.

Nonhaematological toxicities

Nonhaematological toxicity was scored by the common toxicity criteria (CTC) according to the NCI definitions. Multiple toxicities could be accumulated in single patients. Mucositis occurred in 21 patients but only 5 required narcotics (Table 2). In nine patients 14 severe nonhaematological toxicity events (grade 4–5) occurred, with fatal outcome in two of these patients. There were seven severe renal toxicities, one with fatal outcome; one severe cardiac

Table 2 Nonhaematological toxicities, incidence of acute GVHD and cause of death

| (a) Nonhaematologic toxicities (CTC) | 3 | 4 | 5 |
|--------------------------------------|------------------------|-----|----|
| Mucositis (events, <i>n</i>) | 5 | 0 | 0 |
| Cardiac (events, <i>n</i>) | 0 | 0 | 1 |
| Pulmonary (events, <i>n</i>) | 0 | 5 | 0 |
| Renal (events, <i>n</i>) | 1 | 6 | 1 |
| Neurologic (events, <i>n</i>) | 0 | 1 | 0 |
| <hr/> | | | |
| (b) Acute GVHD (grade) | II | III | IV |
| Patients, <i>n</i> | 11 | 1 | 3 |
| <hr/> | | | |
| (c) Cause of death | Patients, <i>n</i> (%) | | |
| Progression/relapse | 22 (67) | | |
| Infectious | 8 (24) | | |
| aGVHD | 1 (3) | | |
| Organ toxicity: renal | 1 (3) | | |
| Organ toxicity: cardiac | 1 (3) | | |

Nonhaematological toxicities were scored by the NCI common toxicity criteria (CTC). Grade 3–5 toxicities are shown, numbers indicate events. Severe acute GVHD grade II–IV is shown, numbers indicate patients.

toxicity with fatal outcome; five severe pulmonary toxicities and one severe neurological toxicity. In three out of the initial seven patients, who received GVHD prophylaxis according to the protocol without alemtuzumab, severe (grade 4–5) toxicity events occurred.

Infectious complications and CMV reactivations

While all patients experienced neutropenic fever that required the use of i.v. antibiotics, 5 (10%) patients experienced bacterial sepsis and 11 (22%) experienced pneumonia (bacterial: *n* = 8; CMV: *n* = 1; HSV: *n* = 1 and fungal: *n* = 1). Of 39 patients at risk, 23 (59%) experienced CMV reactivations and 1 developed CMV pneumonia with fatal outcome.

Cause of death

Overall there were 33 (67%) deaths, 22 (45%) were primarily caused by progression or relapse of the malignancy, 11 (22%) deaths were caused by nonrelapse causes. Reasons were infection (*n* = 8), cardiac failure (*n* = 1), renal failure caused by haemolytic uraemic syndrome (HUS; *n* = 1) and aGVHD grade IV (*n* = 1). The estimated NRM at 1 year is 29% (95% CI 14.2–44.4%; Figure 1).

Response

The best response rates for all patients following second transplant were CR (*n* = 19), PR (*n* = 14) and SD (*n* = 10) patients. Six patients were not applicable for response evaluation because of early death, four of which were leukaemia related. Of 43 evaluable patients 20 (47%) relapsed or progressed post-transplantation, 3 with AML never reached remission and subsequently died from leukaemia, while 6 with low grade lymphoma and myeloma who progressed, are alive under salvage therapy. Of all 49 patients 16 are alive and 9 are in complete remission. Of note, 8 of 9 (89%) of the relapse free survivors had cGVHD

(limited: $n = 7$, extensive: $n = 1$) compared to 9 out of 30 (30%) of the remaining patients. The estimated probability of relapse/progression at 1 year is 55.1% (95% CI 38.2–72%; Figure 1).

Survival

Sixteen patients survive with a median follow up of 528 days (range 217–1344). The median follow up for all patients is 206 days (range 2–1344). At 1 year the estimated survival for all patients is 42.6% (95% CI 28.7–56.6%) and the estimated event free survival is 38.1% (95% CI 24.4–51.8%; Figure 2). There was no significant difference in survival for patients having received a previous autograft or previous allograft (48.3%, 95% CI 30.1–66.5% vs 33.7%, 95% CI 12.5–54.9% at 1 year, $P = 0.47$). Significant differences ($P = 0.013$) in survival could be observed between the subgroups having experienced a relapse within one year after first transplantation (27% survival at 1 year, 95% CI 7–48.5%) and those having experienced relapse beyond one year after first transplantation (51.2% survival at 1 year, 95% CI 33.5–68.9%; Figure 3). In the subgroup beyond one year, 14 (45%) of 31 patients are alive, while in

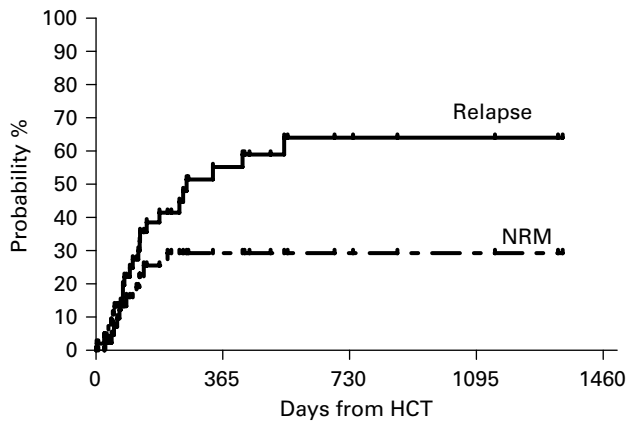


Figure 1 Nonrelapse mortality (NRM) and probability of relapse/progression. The estimated NRM at 1 year is 29% (95% CI 14.2–44.4%) and the estimated probability of relapse/progression at 1 year is 55.1% (95% CI 38.2–72%).

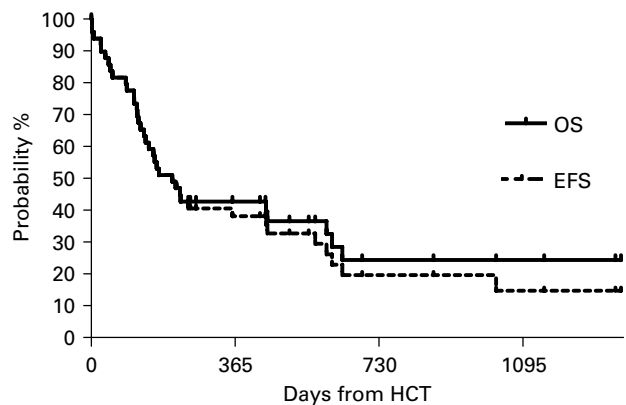


Figure 2 Overall survival (OS) and event free survival (EFS). At 1 year the estimated survival for all patients is 42.6% (95% CI 28.7–56.6%) and the estimated event free survival is 38.1% (95% CI 24.4–51.8%).

the subgroup within one year only 2 (11%) of 18 patients are alive.

AML patients

The largest subgroup is of patients with AML ($n = 18$). Disease status at transplant was untreated relapse ($n = 5$), chemosensitive relapse ($n = 6$) and chemoresistant relapse ($n = 7$). Of all 14 evaluable AML patients 10 (71%) achieved CR and 5 of the 14 (37%) are alive and in CR with a median follow up of 442 days (range 217–1149). The estimated survival for the total of 18 patients at 1 year is 25.9% (95% CI 5–46.8%; Figure 4).

Discussion

In the present study we report the results of 49 consecutive patients treated with high dose chemotherapy and allogeneic second stem cell transplantation after previously failed high dose chemotherapy. For this group of patients no conventional therapeutic approach exists to date to induce long

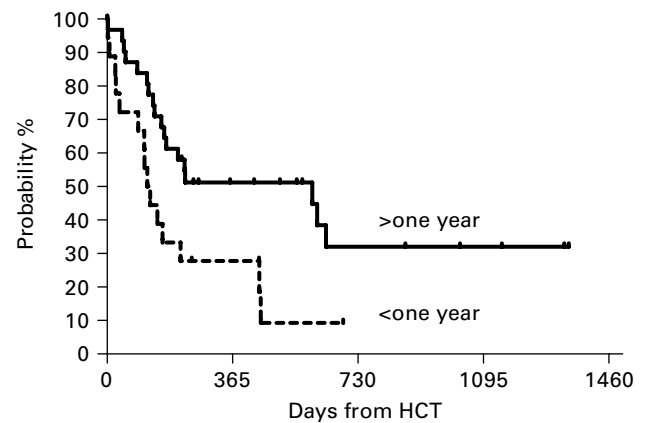


Figure 3 Overall survival according to early and late relapse. Estimated overall survival of patients transplanted within one year from first transplantation (27% survival at 1 year, 95% CI 7–48.5%; broken line) and beyond one year from first transplantation (51.2% survival at 1 year, 95% CI 33.5–68.9%; straight line; $P = 0.013$).

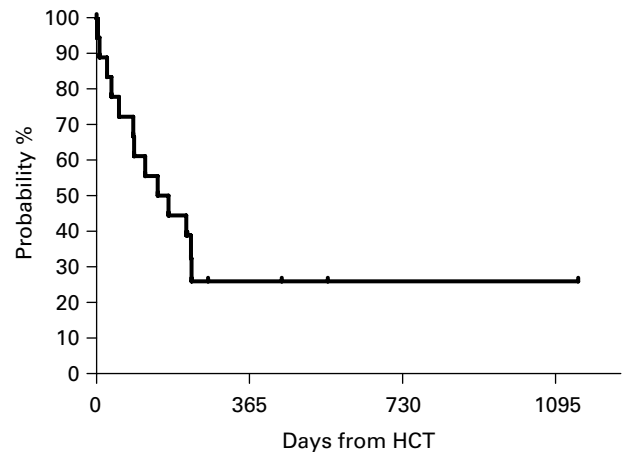


Figure 4 Overall survival of patients with AML. The estimated survival of AML patients at 1 year is 25.9% (95% CI 5–46.8%).

term remissions, the only potentially curative treatment option is a second transplant from an allogeneic sibling or unrelated donor.^{1,5}

However, this group of patients is at a particularly high risk of NRM because of accumulated organ toxicities, as well as opportunistic infections. These, in turn, increase the risk of severe GVHD. Based on these considerations, we established a study protocol with fludarabine and thiotepa providing for low organ toxicity, yet offering potent antileukaemic and antilymphoma activities. Fludarabine is widely used in different combinations for reduced-intensity conditioning (RIC) and has increasingly replaced CY in conditioning regimens.^{20,21} It has been shown to be as immunosuppressive as CY, yet with less toxicity. Additionally it enhances alkylator induced damage by inhibiting DNA repair, hence, potentiating the antileukaemic activity of thiotepa in targeting resting cells and malignant stem cells.²² Thiotepa, in turn, is tolerated with little organ toxicity and offers potent antileukaemic as well as stem cell toxic activity.^{3,13,16,23,24}

Our conditioning regimen was well tolerated with acceptable organ toxicity, considering all the patients had been heavily pretreated, all patients had aggressive disease and a high median age. Other investigators reported an NRM rate ranging between 50 and 80% for patients receiving a conventional high dose chemotherapy regimen for second transplantation after the failure of a previous auto- or allograft.^{4,6,25} We observed an estimated rate of 29% NRM at 1 year, that was mainly attributable to infectious complication. Our regimen facilitated engraftment in all evaluable 46 patients. Donor chimerism was evaluable in 43 of 49 patients, 38 of 43 (88%) achieved complete donor chimerism at one month and no graft failure occurred.

Since three of the first seven patients who received CYA/ATG/MTX for GVHD prophylaxis experienced severe toxicities, which were attributable to MTX, the protocol was amended and ATG and MTX were replaced by low dose alemtuzumab. This was previously shown to be very effective for acute GVHD prevention with low toxicity profile.²⁶ However, commonly used dosages consisting of 100 mg total alemtuzumab induce intensive immunosuppression, which in turn increases the risk of infection and may also increase the risk of relapse by preventing development of mild to moderate GVHD. Hence, we chose 40 mg total dose of alemtuzumab.

The overall incidence of severe acute GVHD was low with only 3 of 42 (7%) patients in the alemtuzumab group with grade 3–4 GVHD, compared to 1 of 7 patients in the MTX group (16%). Chronic GVHD developed in 17 of 39 evaluable patients; 7 were scored as extensive. Such low aGVHD rates have also been reported for alemtuzumab at a dose of 100 mg.²⁷

We found very good early disease control, especially in the AML group: 10 of 14 evaluable patients achieved CR at one month. Of note, 12 were transplanted in uncontrolled disease. In this poor prognosis group, we observed an estimated survival of 25.9% at 1 year with 5 of 14 patients (37%) alive and in CR. This compares to data obtained for first transplantation in AML with refractory disease.²⁸ Of the 9 long term disease free survivors, 8 (89%) experienced

cGVHD, compared to 9 of 30 (30%) of the remaining patients evaluable for cGVHD. This compares to other reports that show a superior outcome for patients with cGVHD.^{29,30} The estimated survival for all patients at 1 year is 42% and the estimated probability of relapse is 55%. We further found the outcome to be significantly worse in patients with relapse or progress within a year from the first transplantation compared to latter relapses with an actuarial survival of 27 vs 51% at 1 year ($P=0.013$), respectively. In addition to reflecting the more aggressive course of disease and less chemosensitivity as it is commonly observed in early relapses of haematological disease,^{12,31} this may also reflect the shorter recovery time for organ toxicity after first transplantation. However, with our regimen we did not observe increased toxicity in this early relapse group, which has been reported for other conditioning regimens.¹ No significant differences in survival could be observed between previously autotransplanted and allotransplanted patients (48.3 vs 33.7% overall survival at 1 year, $P=0.47$).

Our data represent the first consistent study with one conditioning regimen designed especially for second transplantation, while other reports are mainly retrospective and have heterogeneous regimens. Also, our group of patients includes 20 patients who previously had an allogeneic transplantation and relapsed, in contrast to other studies which mainly included previous autotransplants.^{2,3} Finally, our cohort included predominantly unrelated transplants (42 of 49), while other studies mainly report on sibling transplants.^{1,3} It may be postulated that a peripheral graft from an unrelated donor confers more GVL activity.^{17,32} Although not specifically tested, we favour a change of donor for the second transplant after a first allogeneic transplant.

We conclude that this regimen is feasible in offering a second transplant after failure of previous high dose chemotherapy and an autologous or allogeneic stem cell transplant. Conditioning and GVHD prophylaxis according to our protocol allows for acceptable toxicity and GVHD rates, while engraftment is not hampered.

Meanwhile, further amendments of our protocol included the reduction of the total alemtuzumab dose to 10 mg for sibling and unrelated donors as well as the reduction of fludarabine to 90 mg/m² to enhance GVL activity and reduce infectious complications.

Although the number of patients is still small and the follow up is short, response and survival rates indicate that an allogeneic second transplant offers a potentially curative option for this patient cohort.

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