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Acute myeloid leukaemia

Long-term outcome in acute myelogenous leukemia autografted with mafosfamide-purged marrow in a single institution: adverse events and incidence of secondary myelodysplasia

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Summary:

We have analyzed the long-term outcome and toxicities in 98 patients with high-risk acute myelogenous leukemia (AML) who were treated with autologous bone marrow transplantation (ABMT) and monitored for a median observation period of 11.67 years. Between 1983 and 1994, 98 patients in our institution in first or second and higher complete remission (CR) underwent total body irradiation and high-dose cyclophosphamide prior to ABMT purged with mafosfamide. Twenty-seven out of the 90 evaluable patients (30%) were alive and in continuous CR for a median of 11.67 years (range, 6.39–15.53) after ABMT and could be considered as 'cured'. Among the 90 patients, 39 were transplanted at first CR and had a significantly higher survival rate than those transplanted at ⩾2 CR. Younger patients (<40 years) had a better prognosis and patients with FAB M1–4 had a more favorable outcome than those with M5. Long-term complications included four patients with cardiac complications, two with renal insufficiency. Five developed HCV infections, four myelodysplastic syndrome. The incidence of cataract among the long-term survivors was 44.4%. Therefore, a significant number of adult patients with AML in first CR derived long-term benefit from ABMT, despite the risks of a few long-term complications and of MDS (4.4%).
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Although the rate of complete remission (CR) for adult patients with acute myelogenous leukemia (AML) treated with conventional chemotherapy ranges between 65 and 80%, the disease-free survival at 5 years remains disappointingly low, and ranges between 10 and 15% in patients >55 years and 20–40% in younger patients.[1–7]

Allogeneic bone marrow transplantation has been proven to induce long-term cure. For those without a suitable donor, autologous bone marrow transplantation (ABMT) has been increasingly used as a treatment strategy, either as part of post-remission therapy for patients with intermediate and high risks, or after successful re-induction therapy for relapse.[8–14]

One of the major limitations of ABMT is the presence of residual leukemic cells in the autologous graft. One approach to this problem is the *ex vivo* treatment of the autograft to eradicate these leukemic cells yet spare the normal and primitive hematopoietic stem cells.[8,15–19]

Whereas most reports on ABMT, with or without purged marrow, focus on the encouraging results in terms of survival and disease free survival, very few data are available on the long-term adverse events associated with ABMT and the outcome after a follow-up of more than 10 years.

Patients and methods

Patient eligibility

Patients had to have a diagnosis of AML based on the morphologic examination of bone marrow aspirate or biopsy, as well as peripheral blood smear. The subtypes were classified according to the criteria defined in the French–American–British (FAB) system of classification of acute leukemia.[20]

Between July 1983 and November 1994, 98 patients with AML in first or subsequent CR received ABMT at our institution. They were mostly referred from other institutions and were primarily treated according to the German Multicenter Acute Myeloid Leukemia Trial.[21] Patients in first CR were referred for ABMT mostly because of the presence of poor risk factors at the time of diagnosis (high WBC, elevated LDH). Patients in relapse were treated either with NOVE[22] or with other equivalent salvage regimens. Indications for ABMT were made by the corresponding centers. The patients were analyzed for outcome, toxicities and prognostic factors (age, gender, FAB classification, status

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at transplant, time from diagnosis to transplantation, marrow dose of TNC/kg). None of these patients received hematopoietic growth factors (G-CSF/GM-CSF/SCF) or peripheral blood stem cell transplantation.

In the majority of patients, the marrow was harvested after the last course of consolidation chemotherapy in the same remission status as when the transplant was performed.

The database was checked, the survivors were interviewed, and corrections made when necessary from April to September 2000. The study protocol was approved by the Joint Committee on Clinical Investigation of Heidelberg University, and informed consent was obtained from patients before entering the study.

Patient characteristics

Of the total of 98 patients, eight were lost to follow-up and sufficient information could not be retrieved. Of the 90 patients that could be evaluated (Table 1), 50 were male and 40 were female. Median age at the time of transplantation was 37 years (range, 16–53). Distribution within the FAB classification was as follows: M1, 20%; M2, 19%; M3, 14.4%; M4, 31.1%; M5, 13.3%; and undetermined or missing 2.2%. Status at the time of transplantation was as follows: CR1, 43.3%; CR2, 45.5%; CR3, 10%; CR4, 1.1%. The median interval between time of diagnosis and time of transplantation was 15.6 months (range, 3.7 – 68.1).

Table 1 Patient characteristics

Age	
median	37 years
(range)	(16–53)
⩽40	$n = 56$
>40	$n = 34$
Sex	
M:F	50:40
FAB classification	
M1	18
M2	17
M3	13
M4	28
M5	12
NA	2
Remission status	
CR1	39
CR2	41
CR3	9
CR4	1
Marrow TNC-dose/kg	
$<1.0 \times 10^8$	47
$>1.0 \times 10^8$	38
NA	5
median	0.9×10^8
(range)	$(0.24–2.85 \times 10^8)$
Interval diagnosis–transplantation (months)	
median	15.6
(range)	(3.73–68.1)

FAB = French–American–British; M = male; F = female; TNC = total nucleated cell; CR = complete remission; n = number; NA = not available.

Marrow processing

All bone marrow grafts were collected, treated with mafosfamide, washed, and cryopreserved. They were then thawed at 42°C in a water-bath and immediately infused into the patient's central line without further manipulation. The marrow cell suspension was depleted of RBCs and neutrophils using a buffy-coat and a Ficoll–Metrizoate (FM) gradient centrifugation method as described by Gilmore *et al*.[23] The final cell suspension was washed twice using minimal essential medium (S-MEM), supplemented with ABO compatible human plasma (50%), and adjusted to a concentration of 2×10^7 WBC/ml. Mafosfamide was added at a median dose of 70 μg/2×10^7 WBC (range, 60–80) according to the individual sensitivity of granulocyte–macrophage colony-forming units, as described previously.[24] The marrow suspension was incubated with agitation at 37°C for 30 min. To terminate the cytotoxic effect of mafosfamide, the cell suspension was washed once at 4°C, resuspended with ABO compatible human plasma to a final volume of 200 ml, and transferred to four freezing bags (DELMED, Canton, MA, USA), 50 ml per bag. Fifty milliliters of cold S-MEM supplemented with 20% DMSO was added to each bag. The cell suspension was then immediately frozen at a controlled rate of 1 to 2°C per min from 10°C to −70°C, and at 7°C per min to −100°C (BV-10 Biological Freezer; Cryoson, Krefeld, Germany).

Conditioning regimen

High-dose therapy consisted of cyclophosphamide 50 mg per kg body weight (BW) intravenously on 4 consecutive days and hyperfractionated total body irradiation (TBI), at a median dose of 14.4 Gy (range, 12.1–16.7). One patient was treated with cyclophosphamide at the above doses and busulphan orally 4 mg per kg BW on 4 consecutive days because of previous radiation therapy. All patients were treated with prophylactic acyclovir until day 100 post autografting, as described previously.[24]

Statistical analysis

A series of variables were studied, including age, gender, FAB classification, status at time of ABMT, time from diagnosis to transplantation and risk factors using the log rank test.[25]

Event-free survival (EFS) was defined as survival without evidence of leukemia and is measured from the time of ABMT. To evaluate the probability of relapse, patients dying either from direct toxicity of the procedure or from any other cause not related to leukemia were censored. All variables were analyzed for their effect on the probabilities of relapse, EFS and overall survival (OS) using the methods of Kaplan and Meier.[26]

Results

Hematologic recovery

The median number of total nucleated cells (TNC) transfused per kg was 0.9×10^8 (range, 0.24–2.85 $\times 10^8$), and that of mononuclear cells (MNC) transfused per kg was 0.42×10^8 (range, 0.11–1.50 $\times 10^8$). The number of cells that were reinfused was given as counted at the time of cryopreservation.

Patients whose marrow harvest yielded $<1.0 \times 10^8$ TNC/kg had a 100 day mortality of 16% while patients whose marrow harvest contained $>1.0 \times 10^8$ TNC/kg had a 100 day mortality of 8.6%. There were otherwise no significant differences between patients whose marrow harvest yielded $>1.0 \times 10^8$ TNC/kg or less as regards disease-free survival or overall survival ($P = 0.37$, $P = 0.49$, respectively).

The recovery of the total leukocyte count to $>1000/\mu$l occurred at a median of 27 days (range, 12–81). Neutrophil recovery to $>500/\mu$l occurred at a median of 28 days (range, 13–81). The median recovery of platelet count to $>20\,000/\mu$l without substitution was 48 days (range, 11–356). There was a significant correlation between the dose of TNC/kg and granulocyte engraftment $>0.5 \times 10^9$ ($P = 0.01$) but not platelet recovery.

Nine patients died before reaching hematologic recovery (neutrophils $>500/\mu$l); seven due to transplant-related causes and two due to early relapse. Three patients showed pancytopenia with graft failure and died within the first 100 days after transplantation. Seven patients showed delayed platelet recovery and an unmaintained platelet count of $>20\,000/\mu$l was reached ranging from 107 to 356 days.

Immediate toxicity and adverse events

All patients experienced mild to severe nausea and vomiting at the time they received high-dose cyclophosphamide. Within the first 100 days after transplantation, four patients developed hemorrhagic cystitis (none fatal), two developed veno-occlusive disease (VOD); one died and the other recovered. Twelve developed pulmonary complications (including two interstitial pneumonitis and three fungal pneumonias) and one patient died due to acute respiratory distress syndrome (ARDS). Five of the 12 with pulmonary complications died (three of fungal pneumonia and one each of interstitial pneumonitis and pneumonia). The diagnosis of interstitial pneumonitis was made by X-ray and no organism was identified. One patient developed congestive cardiac failure and died. Four patients died due to septicemia (one of four from candida sepsis). Overall, there were 12 (13.3%) cases of therapy-related mortality within the first 100 days.

Within the first year, there were 39 deaths; 11 patients (12.2%) died due to early relapse in the first 6 months after transplantation and 13 patients (14.4%) died due to relapse in the following 6 months. A very uncommon observation was a patient who developed acute graft-versus-host disease (GVHD) of the liver (grade IV) after transplantation with a maximum bilirubin level of 22.0 mg/dl. In addition, this patient suffered from acute GVHD manifestations of the skin (grade II–III) and died as a sequel of these complications 110 days after transplantation.

Long-term outcome

The probabilities of event-free survival (EFS) and overall survival (OS) are demonstrated in the Figure 1a and b. Most relapses (37/45) occurred within the first 30 months. As of 15 March 2000, 27 patients (30%) were alive and well in continuous complete remission (CCR) at the last follow-up 6.4–15.5 years (median 11.7) after ABMT (Table 2).

We analyzed the prognostic factors that are associated with favorable long-term outcome. Younger autografted patients <40 years (Figure 2) had a better outcome than those >40 years (EFS, $P = 0.023$; OS, $P = 0.013$). Patients with FAB M1–4 had a more favorable outcome than those with M5 (EFS, $P = 0.03$; OS, $P = 0.05$). There was no significant difference in gender or time from diagnosis to transplantation between long-term responders and those who died. Patients autografted in CR1 had a more favorable long-term outcome than those transplanted in second or later remissions. Figure 3 shows that the actuarial 5 year probability of event-free survival in patients in CR1 is 48.3% (EFS, $P = 0.018$; OS, $P = 0.013$).

Long-term toxicity

Long-term toxicity is defined as toxicity occurring 6 months or later after transplantation.

Partial engraftment failure: There were seven patients (7.7%) with persistent thrombocytopenia for more than 100 days after ABMT. Four patients with thrombocytopenia recovered on days 193, 356, 323 and 201, respectively, and were alive in CCR at the completion of this study. Two ultimately developed secondary MDS and died as a consequence of MDS at 32.8 and 115.0 months after transplantation. One died of relapse of AML 148 months after transplantation.

Cardiac toxicity: Four patients (4.4%) suffered from grade II–IV (according to WHO) cardiac toxicity. The pretransplant examination revealed no cardiac abnormalities for these patients. In two patients, the cardiac insufficiency (grade II and III) was transient and reversible, and they were alive and performing well at 127 and 141 months after transplantation. The third patient had cardiac insufficiency grade II (WHO) and died at 11 months after transplantation due to relapse, while the last patient developed cardiac insufficiency grade IV and died due to heart failure at 13 months after transplantation.

Renal toxicity: Three patients developed long-term renal complications. Two patients (2.2%) had chronic renal insufficiency, of whom one required hemodialysis. He developed renal failure 86 months after ABMT, was treated with dialysis for 26 months and died 112 months after transplantation due to renal complications. The cause of the renal failure was not known but was most probably unrelated to transplantation. The other patient required no specific treatment but died due to relapse of AML 148 months after

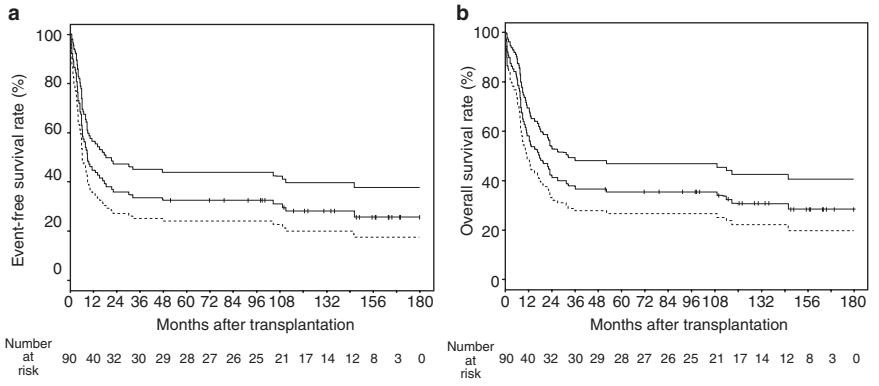

Figure 1 (**a**) Disease-free survival and (**b**) overall survival (±95% confidence level) for 90 AML patients autografted with mafosfamide-purged marrow.

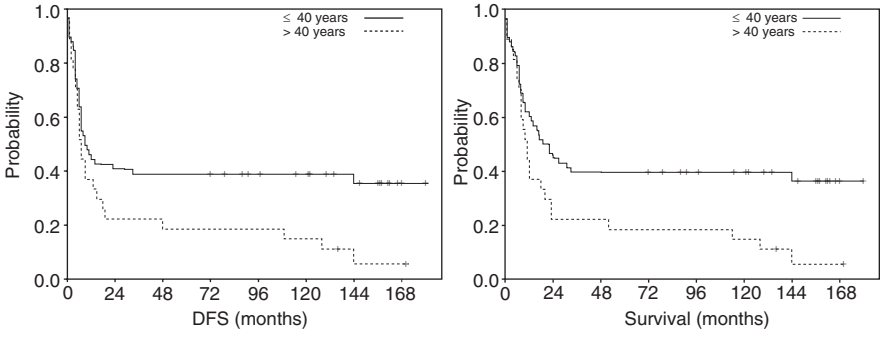

Figure 2 Correlation between age and DFS and OS in 90 patients autografted for AML.

transplantation. The third patient developed hemorrhagic cystitis after ABMT, then chronic interstitial nephritis with acute renal tubular necrosis, probably related to high-dose cyclophosphamide. He died (in CR) of multi-organ failure 8 months after transplantation.

Hepatic toxicity: Five patients developed antibodies against hepatitis C virus between days 100 and day 360 after transplantation. Three of them are alive and in CCR 102, 153 and 166 months after transplantation. Of the other two, one developed liver cirrhosis and died in CR 17 months after transplantation due to liver failure, and the other developed secondary hemochromatosis of the liver and died due to relapse 118 months after transplantation. In addition, another patient developed hepatic siderosis with evidence of bone marrow siderosis 7 months after transplantation and died 1 month later. The cause of hepatic siderosis was not clear.

Pulmonary toxicity: One patient developed bronchial asthma 22 months after transplantation, probably unrelated to ABMT. This patient underwent bronchodilator treatment and was doing well in CCR at 120 months after transplantation.

Dermatological toxicity: One patient had chronic GVHD of skin grade II and died 8 months after transplantation due to renal complications as mentioned above. One patient developed neurodermatitis after transplantation and was alive in CCR 78 months after transplantation. Four patients suffered from reactivation of herpes zoster infection 10, 86, 112 and 140 months after transplantation.

Neurotoxicity: Two patients developed generalized seizures of unknown etiology 7 and 12 months after transplantation; both died due to relapse of leukemia at 9 and 16.7 months, respectively, after transplantation. Another patient had peripheral neuropathy and was alive in CCR 141 month after transplantation. One patient developed restless leg syndrome after transplantation with muscle twitches in both legs, mostly at night. Investigations revealed no abnormalities in serum electrolytes, magnesium, iron, hormones or any neuro-muscular disorder. This patient underwent different kinds of treatment without success. She was in CCR and alive for 135 months after transplantation.

Susceptibility for infections: Among the 27 long-term survivors, 13 patients (48%) reported increased susceptibility to viral infections, mostly the common cold, influenza and herpes zoster virus. Among these, five patients (three male, two female) suffered from recurrent urinary tract infections and four patients from recurrent pneumonia.

Ocular toxicity: Among the long-term survivors, 12 of 27 patients (44.4%) developed cataracts in both eyes after a median of 76 months (range, 48–140) after TBI. One patient developed photosensitivity 60 months after transplantation without cataract formation. She was alive and doing well in CCR 176 months after transplantation.

Table 2 Characteristics of 27 AML long-term survivors

Patient ID number	*Age*	*Sex*	*FAB*	*Remission status at Tx*	*Marrow TNC-dose/kg* $\times 10^8$	*Interval Dx–Tx (months)*	*Date of Tx*	*EFS (years)*
01	27	M	M3	CR3	1.33	68.1	06.08.87	12.8
02	36	F	M1	CR1	0.62	7.2	03.12.86	13.5
07	16	F	M2	CR1	0.65	7.0	13.09.90	9.6
10	33	F	M4	CR1	0.35	12.5	10.07.86	13.9
14	17	F	M4	CR1	0.67	4.2	25.11.93	6.4
16	34	M	M4	CR1	0.72	10.1	16.10.86	13.6
17	48	F	M4	CR2	0.74	18.9	21.07.88	11.8
22	42	F	M4	CR2	0.61	33.4	29.06.89	10.9
24	42	F	M2	CR3	1.03	42.4	26.09.85	14.7
26	18	M	M2	CR1	0.58	27.1	08.04.93	7.0
27	26	F	M4	CR2	2.07	22.4	04.10.91	8.6
28	30	F	M4	CR1	NA	3.8	22.11.84	15.5
40	22	M	M2	CR1	1.36	11.0	10.04.86	14.6
44	23	M	M4	CR2	2.01	65.5	21.05.86	14.0
50	21	M	M4	CR1	1.22	9.3	21.10.87	12.6
58	40	F	M4	CR1	0.86	9.3	09.10.86	13.6
59	29	M	M1	CR2	0.63	20.3	02.04.92	5.8
63	37	M	M1	CR1	1.23	6.9	26.10.89	10.5
71	28	F	M1	CR1	0.78	6.6	18.04.90	10.1
73	46	F	M1	CR2	NA	29.4	31.10.91	8.5
81	39	M	NA	CR1	0.50	6.3	11.07.91	8.8
83	38	F	M1	CR1	1.76	6.6	14.09.89	10.7
89	22	M	M1	CR2	1.50	9.2	16.01.86	14.4
92	22	F	M1	CR1	1.84	5.0	30.01.86	14.3
94	40	M	M3	CR1	0.48	18.8	15.09.88	11.7
95	39	F	M3	CR1	2.85	10.3	05.01.89	11.4
96	49	F	M1	CR1	1.35	6.8	17.08.89	11.2

FAB = French–American–British; M = male; F = female; Tx = transplantation; TNC = total nucleated cell; Dx = diagnosis; EFS = event-free survival; CR = complete remission; NA = not available.

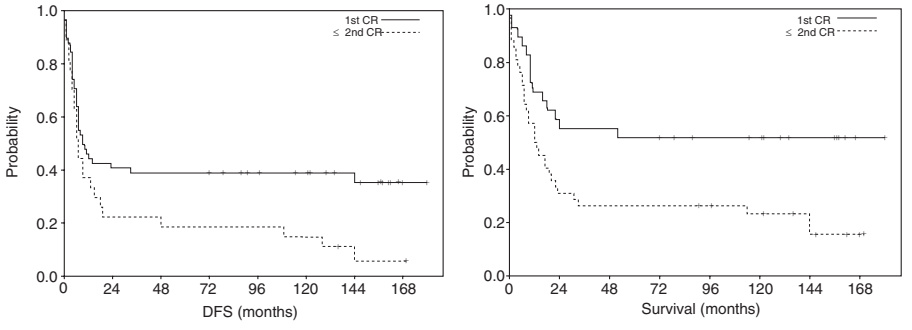

Figure 3 Correlation between remission status and DFS and OS in 90 patients autografted for AML.

Incidence of early osteoporosis after ABMT: Among the long-term survivors, two female patients (7.4%) experienced early osteoporosis without a previous family history or any other risk factors for osteoporosis. The symptoms of osteoporosis began 3 years after transplantation and became worse over time. They were transplanted at an age of 30 and 40 years and they were alive in CCR at 186.4 and 163.5 months after transplantation consecutively. The first patient had severe osteoporosis with multiple spontaneous rib fractures. She is 46 years old now and receiving therapy with fluoride, vitamin D_3, calcium and estrogen.

Reintegration into previous employment: Among the 27 long-term survivors, 13 patients (48%) resumed their previous occupation. Four patients (14.8%) took up part-time employment because of fatigue. Three patients (11%) were not able to enter into employment again after transplantation, while four patients (14.8%) preferred to receive early pension because of loss of stamina and of exhaustion after moderate work. Three female patients could not be evaluated as they were home-makers before transplantation. They were, however, able to resume their previous daily activities after transplantation.

Myelodysplasia: We observed four patients who developed myelodysplasia (MDS) 32, 46, 112 and 144 months after transplantation. The last patient, in addition to MDS, developed secondary AML with >50% blasts 146 months after transplantation. The details of these four patients are summarized in Table 3. Cytogenetic analysis in all these

Table 3 Features of patients who developed myelodysplasia (MDS)

Patient	*Age at Tx*	*Sex*	*AML type at diagnosis*	*Interval from Tx to MDS (months)*	*MDS type*	*Cause of death*[a]	*Interval from MDS to death (days)*
WB	39	M	M2	144	RAEBt	AML	72
FG	50	M	M4	46	RAEB	Septicemia	41
HG	53	M	M5	112	RA	Septicemia	28
AM	21	M	M4	32	RA	Bleeding	14

[a]Immediate causes subsequent to relapse.

cases had not been undertaken initially, and it remains debatable whether the relapse was of the original disease. For the purposes of statistical analysis, we consider these patients to have died from relapse. No other secondary malignancies were observed. We considered that the risk of MDS among all 90 patients studied was 4.4%. However, if we considered the incidence of MDS only among the patients who survived more than 30 months, this would have been four cases of MDS out of 34 patients (11.8%).

Discussion

Many studies have suggested that ABMT might be beneficial for AML patients with high-risk factors or with recurrent disease, especially if a compatible, related donor cannot be identified.[1–12,27] However, conclusive statements on the long-term benefits and adverse effects have been limited by the short follow-up period in most studies. In this report, we have summarized the long-term toxicities associated with the procedure as well as the outcome after a median follow-up of 11.7 years.

Among the 90 patients who were evaluable, 27 (30%) were alive and well between 6.4 and 15.5 years after ABMT. Many other authors have reported comparable rates of EFS and survival among patients with AML. It has been reported that ABMT in the second and third CR has achieved results similar to those obtained with allogeneic bone marrow transplantation.[10,12,15,28] The results of the Eastern Cooperative Oncology Group (ECOG) study and of the EORTC indicated that the outcome of ABMT in first CR rivalled that of allogeneic BMT.[10,17] Long-term disease-free survival after ABMT in AML might be better than conventional-dose post-remission therapy.[10,12] Present evidence suggests that maintenance chemotherapy after early consolidation without ABMT does not prolong survival in AML patients.[29]

In the report of the European Group for Bone Marrow Transplantation (EBMT), the probability of long-term disease-free survival (DFS) was about 40% for patients autografted for acute leukemia in first complete remission (CR1) and 30%, if treated in CR2.[8,9,11]

Our study confirms that the probability of long-term DFS for patients autografted in first CR is significantly better than that of patients transplanted in second or higher CR (EFS, $P = 0.018$; OS, $P = 0.013$), with 46.1% overall survival. In our hands, ABMT has therefore achieved a CCR rate that is more favorable than the rates accomplished with conventional chemotherapy. The very long-term outcome after transplantation was similar to that reported in the literature.[10,12,30,31]

Criticism could be raised that this was not a randomized trial and that we could not exclude selection of patients. The uncontrolled design of this retrospective study may have favored the selection of low-risk young patients. Furthermore, the significantly better prognosis of younger patients <40 years (DFS, $P = 0.023$; OS, $P = 0.013$) is an obvious bias. However, the influence of age should not be overestimated, as indicated by other authors.[32,33] In the study period between 1983 and 1989, cytogenetic analysis was not generally available. Hence it was not possible to identify any relationship between chromosomal aberration with the long-term outcome after more than 10 years.

Meanwhile, a retrospective study from the registry data from the EBMT and the IBMTR showed a significant overall survival advantage of about 10% for the AML patients who were autografted with mafosfamide/4HC-purged marrow *vs* non-manipulated ABMT. This difference could be demonstrated 2 years after ABMT.[11,27] Our data are in alignment with these observations. Obviously, these were not prospective randomized trials and should be interpreted with caution.

The emphasis of this report has been on the long-term adverse events after more than 10 years. The most significant observation is the incidence of 4.4% of myelodysplasia, which evolved at a median of 79 months (range, 32–144) after ABMT. These four patients initially showed delayed platelet engraftment with a median of 161.5 days (range, 46–323). According to the standards at the time of transplantation, all four patients had an adequate amount of TNC (median 0.7×10^8/kg; range, 0.39–1.1×10^8/kg) in the autografts. One patient developed additionally secondary AML and died shortly thereafter. The other three patients all ultimately died of the consequences of MDS. In addition, seven patients had persistent thrombocytopenia more than 100 days after ABMT. Without the benefits of the initial cytogenetic finding or other genetic markers, the origin of the MDS cell clone remains speculative. Although there are extensive reports on the incidence of MDS after ABMT for non-Hodgkin's lymphoma, Hodgkin's disease and breast cancer[34–39] no data on the incidence of MDS in AML patients after ABMT are available.

Long-term cardiac toxicity occurred in four patients (4.4%), one of whom also died as a result of cardiac complications. Although the role of cyclophosphamide, of TBI, or of cumulation of both remains to be clarified, other

authors have reported similar rates of cardiac toxicity in patients treated with high-dose cyclophosphamide.[40,41]

The incidence of GVHD after autologous transplantation has been reported occasionally and is probably less than 1%.[42] We encountered two patients who had biopsy-proven GVHD. Whether this might be due to *ex vivo* purging of the marrow with mafosfamide is open to speculation.

As most of our patients were transplanted in the 1980s, the rate of the HCV infection after BMT was relatively high at 5.5% compared to 6.6% incidence of post-transfusion hepatitis C at that time.[43] Currently, the incidence of post-transfusion hepatitis C is much lower, almost negligible, due to the highly sensitive testing for HCV. The incidence of cataract formation after conditioning regimens containing TBI with ABMT in our study was 44%, compared to 50% in the reports of the European Group for Blood and Marrow Transplantation.[44]

It is of note that a lower incidence of fatal late events was observed among long-term survivors transplanted in CR1 (1/18) as opposed to those transplanted in CR2 or CR3 (3/9). This observation suggests that additional chemotherapy regimens prior to transplantation probably have a negative impact on long-term toxicity.

For patients transplanted with mafosfamide-purged marrow, no definite 'cut off' value for the minimal dose of total nucleated cells (TNC) has been reported. Demirer *et al*[45] determined a significant difference in engraftment as well as in outcome for patients with acute leukemia who were transplanted with unpurged marrow using a dose of TNC $>2.0 \times 10^8$/kg. Applying a multivariate analysis to our patient population, patients with a cell dose of $>1.0 \times 10^8$ TNC/kg experienced a significantly reduced 100-day mortality compared to those transplanted with a cell dose of $<1.0 \times 10^8$ TNC/kg after adjusting for disease status and patient age at transplant. There was, however, no statistically significant difference in long-term EFS or overall survival between the two groups ($P = 0.37$, $P = 0.49$, respectively). Although a correlation between the dose of TNC/kg and engraftment with a peripheral blood granulocyte count of $>0.5 \times 10^9$ was found ($P = 0.01$), no such relationship with platelet recovery could be established. Thus, in contrast to the report of Gorin *et al*[46] we could not find any relationship between cell dose and long-term outcome. However, it should be noted that Gorin analyzed the correlation between outcome and CFU-GM dose in mafosfamide-purged marrow and not the marrow dose of TNC.

In summary, our retrospective analysis shows that at a median follow-up of 11.7 years a third of adult patients with AML derived long-term benefit from high-dose chemotherapy and ABMT, albeit with significant long-term toxicities.

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