Disease-Free Survival After Autologous Bone Marrow Transplantation in Patients With Acute Myelogenous Leukemia

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Autologous bone marrow transplantation (ABMT) makes it possible to escalate the dose of cytotoxic treatment to a lethal range. Disease-free survival (DFS) following myeloablative therapy and ABMT has been shown to be superior to conventional treatment in high risk patients with acute myelogenous leukemia (AML). It was the purpose of the present study to compare hematopoietic reconstitution, actuarial DFS, and relapse rate of patients transplanted in first complete remission (CR) of AML with those in second or subsequent CR, and to evaluate transplant related mortality. Fifty-two patients with AML, 22 in first CR (low risk) and 30 in second or subsequent CR (high risk), underwent total body irradiation (12.1 to 16.7 Gy) and cyclophosphamide (CY) treatment (200 mg/kg) followed by ABMT. The autograft was incubated with the active CY derivative Mafosfamide (ASTA Werke, Bielefeld, Federal Republic of Germany) to reduce the number of possibly contaminating clonogenic tumor cells. All patients showed three lineage engraftsments with platelet recovery observed as being the slowest. The transplant related death rate was low at 5.8%. There was no significant difference in the kinetics of polymorphonuclear (PMN) cell or platelet reconstitution between the low and high risk patient subgroups. The estimated probability of DFS (relapse) after ABMT in first CR was 61% (36%) compared with 34% (65%) in second or subsequent CR, the longest follow-up being 55 months and 57 months, respectively (median follow-up 31 months and 19 months, respectively). ABMT offers a stable long-term DFS when performed in first CR with no relapses occurring in over a year after transplantation. Six later relapses, however, were seen after ABMT in second or subsequent CR, although DFS was not statistically different from that of first remission patients (P = .72).

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MATERIALS AND METHODS

Patients. Fifty-two patients were included with a median age of 35 years (range, 17 to 53 years). Patients were eligible for this study if AML was diagnosed by examining bone marrow aspirates and histologic typing according to the FAB classification (Table 1). All transplants were performed either in CR1 or in subsequent CR following intensive conventional chemotherapy. The decision whether a given patient would receive a transplant in first or in subsequent CR was made mostly before referral to the University Hospital for ABMT and was not made by the transplant team. The patients we monitored from the beginning of their disease received the transplant in first remission except for two patients whose marrow was collected in CR1 and transplanted after 44 and 28 months of cryopreservation, respectively. Of 52 patients, the marrow of 50 was harvested in the same remission status as when the transplant was performed. The median time between the start of complete remission preceding ABMT and ABMT was 3 months (range, 1 to 8 months) for all patients. Of 52 patients, 51 did not have an HLA-matched sibling donor. One patient preferred ABMT over allogeneic bone marrow transplantation despite having an HLA identical sibling. Patient data are listed in detail in Table 1.

The study protocol was approved by the Joint Committee on Clinical Investigation of the Heidelberg University, and informed consent was obtained from patients before entering the study.

Marrow collection, processing, "purging," cryopreservation, and retransfusion. Marrow was collected by multiple aspiration from
cells were washed once at 4°C, resuspended with ABO compatible
serum/2 x 10^9 WBC in the first 14 cases. The dose was then escalated to
dissolved in 10 mL Medium 199 was added at a concentration of 60
and adjusted to a concentration of 2 x 10^9 WBC/mL. Mafosfamide,
Ct
described by Gilmore
gradient centrifugation using the IBM Blood Cell Processor 2991 as
depleted of RBC and most of the polymorphonuclear (PMN) cells
the posterior iliac crest and the sternum. The cell suspension was
performed rapidly in a 42°C waterbath without the DMSO being
removed. The cell suspension was then aspirated into 50-mL syringes
for 30 minutes. To terminate the cytotoxic effect of Mafosfamide,
cells were washed once at 4°C, resuspended with ABO compatible
human plasma to a final volume of 200 mL, and transferred to four
human plasma supplemented with 20% DMSO was added to each
bag. The cell suspension was then immediately frozen at a controlled
cold 5-MEM supplemented with ABO compatible human plasma
(70 zg in the remaining cases except for eight cases in which 80 g
was used. The cell suspension was incubated with agitation at 37°C
70°C in the remaining cases except for eight cases in which 80 µg/2 x 10^9 WBC in the first 14 cases. The dose was then escalated to
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was used. The cell suspension was incubated with agitation at 37°C
for 30 minutes. To terminate the cytotoxic effect of Mafosfamide,
cells were 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), 50 mL per bag. 50 mL 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 minute from +10°C to -70°C, and at 7°C per
minute up to -100°C (BV-10 Biological Freezer, Cryoson GmbH,
Federal Republic of Germany).
The frozen bags were stored in liquid nitrogen. Thawing was
performed rapidly in a 42°C waterbath without the DMSO being
removed. The cell suspension was then aspirated into 50-mL syringes
and immediately injected into the patient’s central line (Hickman
catheter) at a rate of 10 mL/min.

Pretransplant regimen. The hyperfractionated total body irra-
diation (TBI) was slightly modified according to Shank et al.7 The
total body dose at body center was calculated to be between 12.1 and
13.7 Gy in the first 11 cases, then escalated to a dose range between
13.8 and 14.9 Gy in 39 cases, and between 15.0 and 16.7 Gy in the
remaining 12 cases; 120 cGy per fraction were given 3 times on days
1, 2, and 3, and twice on day 4, 4 hours apart for 11 fractions (total
12.1 to 13.7 Gy) or 12 fractions (total 13.8 to 14.9 Gy), three
fractions per day. The dose range between 15.0 and 16.7 Gy included
12 fractions, 130 cGy each. A 23 MeV linear accelerator source
(Saturne) (32 cases) or, alternatively, a Co60 gamma source (5 cases)
was used with a midline dose rate of 7 to 18 cGy/min. The lung dose
was limited to 9 Gy. Additional boosts with electrons of maximal 15
MeV (depending on the chest wall thickness) were given to the
previously shielded areas, particularly the ribs, to reach the total
maximum dose.

After TBI (days -9, -8, -7, -6) CY was given IV on each of 4
consecutive days (-5, -4, -3, -2) at a daily dose of 50 mg/kg. After
1 day of rest transplantation was performed on day 0.

Intensive care posttransplantation. The patients were kept in
reverse isolation from the beginning of high dose CY until a peripheral
PMN concentration of 500/µL was reached. Prophylactically
they all received acyclovir, ketoconazole, and CMV-hyperimmun-
globulin together with partial antibiotic decontamination of the gut6
up to 100 days posttransplantation. When fever exceeded
38.5°C, broad spectrum antibiotics were administered; when fever
persisted more than 4 days despite appropriate antibacterial treat-
ment, amphotericin B was added systemically. All platelet support
was HLA-A/B-matched and CMV-negative if the patient was
negative as well. All blood products were irradiated with 20 Gy to
avoid the risk of graft-versus-host disease (GVHD) induction.

Statistical evaluation. Clinical and laboratory data were
retrieved from the bone marrow transplant data base and analyzed
by standard statistical methods using the SAS software program.
Kaplan-Meier product limit estimates were used to evaluate actuarial
DFS, actuarial relapse, and reconstitution of blood cells. Based on
those calculations, we found the median DFS and relapse follow-up,
and the median hematopoietic reconstitution times.

To compare DFS, relapse rate, and hematopoietic reconstitution
data of patients who were actually disease-free with that of those
who had relapsed, we used the generalized Wilcoxon comparative
test statistics (Breslow). The equivalent Wilcoxon rank-sum test was
used for the evaluation of censored data, such as the duration of
remission preceding ABMT and following ABMT, as well as the
time between start of remission and ABMT in those two patient
groups. All analyses were understood as explorative. Formal signifi-
cance levels of P = .05 were used without adjustment for multiple
testing. The probability of DFS or relapse was calculated from the
day of marrow transplantation (day 0) and was analyzed as of June
1, 1989.

RESULTS

Hematopoietic reconstitution. The ex vivo Mafosfamide
incubation reduced the number of CFU-GM in the marrow
graft to a median of 17% (range, 2% to 100%). Despite such
an inhibition of committed progenitor cells, all evaluable
patients showed engraftment of three cell lineages as con-
virmed by examination of bone marrow smears. The median
number of MNC transfused per kg was 4.6 x 10^9 (range, 0.6
to 21.0); CR1, 4.65 x 10^9 (range, 0.6 to 13.0); CR2+, 4.6 x
The median number of CFU-GM per kg given back to the patient following Mafosfamide treatment was $0.105 \times 10^4$ (range, 0.001 to 6.7); CR1, $0.12 \times 10^6$ (range, 0.006 to 2.7); CR2+, $0.10 \times 10^6$ (range, 0.001 to 6.7). The following analyses exclude two patients who died too early to be evaluated. The estimated time to reach 500 PMN/µL was 29 days posttransplant for 50% of patients (CR1, 30 days; CR2+, 29 days; $P = .43$), or 48 days to reach a stable 20,000/µL platelet count (CR1, 71 days; CR2+, 38 days; $P = .07$). Thirteen of 50 evaluable patients (CR1, 8 of 21; CR2+, 5 of 29) showed persistent severe thrombocytopenia with less than 20,000 platelets/µL for more than 100 days posttransplant and therefore needed prolonged platelet support.

One AML patient who already reached 2,400 leukocytes/µL and 44,000 platelets/µL on day 34 posttransplant almost lost her graft due to a common cold viral infection. She spontaneously reconstituted but needed platelet support for more than 1.5 years, the longest support in our series. One patient showed progressive pancytopenia about 1 year after ABMT due to a chronic herpes virus infection; 20 months posttransplant he developed a myelodysplastic syndrome of all three cell lines and eventually died of gastrointestinal bleeding. Chromosomal analysis did not show any aberrations.

The statistical evaluation of the time to reach a certain blood cell concentration after ABMT was not significantly different in patients who were actually disease-free or in patients who had relapsed (PMN, $P = .60$; platelets, $P = .84$).

**Early transplant-related morbidity and mortality.** In almost all patients we observed a transient supraventricular tachycardia from the beginning of high dose CY until about 3 weeks post-ABMT. The basic pulse rate went up to 110 to 140/min with profound orthostatic dysregulation. As shown by echocardiography, the left ventricular contractility was not significantly impaired.

Three patients (5.8%) died during the first 100 days posttransplant of transplant-related complications, such as sepsis, cardiac failure, or interstitial pneumonitis (one patient transplanted in CR1, one in CR2, and one in CR3). At autopsy one patient showed cardiac single fiber necrosis and intramyocardial hemorrhage. The echocardiography values prior to ABMT revealed a normal left ventricular contractility. In the case of interstitial pneumonitis, no CMV or related viral infection could be documented. All pulmonary function tests at the beginning of the pretransplant therapy were normal.

**Actuarial disease-free survival and relapse.** For statistical comparison, both patient groups, relapsed or disease-free, were not significantly different as far as the duration of remission preceding ABMT ($P = .80$) or the time between start of remission and ABMT ($P = .13$) is concerned. Of 52 patients, 24 have relapsed or died too early to be evaluated (2 patients: 8 of the 22 patients autografted in CR1, 12 of the 23 patients transplanted in CR2, 3 of the 5 patients transplanted in CR3, and 1 of the 2 patients transplanted in CR4. The actuarial DFS was 61% for patients autografted in CR1 at a longest follow-up of 55 months (median disease-free follow-up 31 months), compared with 34% in patients transplanted in second or subsequent CR at a longest follow-up of 57 months (median disease-free follow-up 19 months) (Fig 1). Actuarial DFS in both patient groups, low and high risk, was not statistically different ($P = .72$).

Of the 24 patients who relapsed, the actuarial time to relapse for 50% of patients was 137.5 days post-ABMT (range, 3 to 918): 109 days for patients autografted in CR1.
ABMT IN AML PATIENTS

Fig 2. Actuarial relapse rate for patients with acute myelogenous leukemia following autologous bone marrow transplantation in first or subsequent complete remission (CR) (= CR1+), in first CR (= CR1), or in second and subsequent CR (= CR2+).

The actuarial relapse rate was 36% for patients autografted in CR1 compared with 65% in patients transplanted in second or subsequent CR. There was no significant difference in the estimated relapse rate in both patient subgroups ($P = .62$).

Two patients had their marrow harvested in first CR and transplanted in second CR. One of them is disease-free 42 months post-ABMT, whereas the other patient relapsed 15 months posttransplant. In 11 of 30 patients the duration of remission after ABMT exceeded the remission duration prior to ABMT and last relapse (Table 2).

DISCUSSION

ABMT is based on dose escalation of consolidation treatment to a lethal range. The enhancement of antileukemic effect depends mainly on two conditions: (1) the tumor remaining in the patient must respond to lethal dose escalation of pretransplant therapy, and (2) the escalated dose therapy must be primarily myelotoxic with acceptable extramedullary toxicity.

The transplant-related death rate is of crucial importance for the acceptance of the autologous transplantation approach. If AMBT just means dose escalation with stem cell rescue, the treatment-related mortality should not significantly exceed that of conventional consolidation treatment as reported to be between 3% and 20%9-13 nor that of conventional induction treatment for adult AML patients being between 17% and 32%.14-16 In the present study the transplant-related death rate was 5.8% compared with 16% and 27% reported for transplants in high risk patients1,17 and between 0% and 15% in low risk patients.17-20

Ex vivo purging normal marrow cells of AML cells with the active CY derivative 4-HC was shown to be effective in the transplanted AML LBN rat model with 100% cure rate at a dose range between 18 and 24 $\mu$g/mL.21 Although purging the autograft of residual clonogenic tumor cells with CY derivatives seems to contribute to DFS in relapsed patients,1 there are still no data available from randomized prospective trials to substantiate this. As reported at the 1988 European Bone Marrow Transplantation Group meeting, the multicenter registration data for the first time gave evidence that ex vivo marrow purging using Mafosfamide is significantly superior to nonpurging in AML patients transplanted in CR1 after TBI. This was confirmed at the 1989 survey on 723 AML cases.22 The antitumor effect of the 4-HC incubation depends on the number of RBC and/or nucleated cells present in the marrow graft.23,24 Aldehyde dehydrogenase of RBC and nucleated cells seems to inhibit the 4-HC antitumor activity. Therefore, the target cell suspensions in the present study were highly depleted of RBC and PMN, leaving an almost pure mononuclear cell suspension for Mafosfamide incubation with a hematocrit of between 1% and 6%. Except for two patients in whom almost no CFU-GM reduction was seen after Mafosfamide treatment, the overall CFU-GM sensitivity to Mafosfamide showed little variation at a median CFU-GM inhibition of 83%. We therefore saw no need to adjust the Mafosfamide incubation dose to the individual CFU-GM sensitivity as proposed by Gorin et al.17 The 17% CFU-GM recovery in our series after Mafosfamide purging is higher than recently reported by Rowley et al,25 being less than 10%. The reason we did not further increase CFU-GM inhibition was the observation that platelet reconstitution in particular was affected by aggressive purging, leaving less than 10% CFU-GM in the autotransfusate. One could assume that the
mostly pure mononuclear cell suspension including early hematopoietic precursor cells was more sensitive to Mafosfamide or 4-HC at a given dose than the buffy coat preparation used by the Hopkins group producing more aldehyde dehydrogenase. As further demonstrated by Rowley et al\textsuperscript{25} we also looked at a possible correlation between CFU-GM inhibition after purging and DFS following ABMT. Opposed to their observation that CFU-GM recovery of less than 1% resulted in actuarial DFS of 36% compared with 16% for those with a recovery of more than 1%, we could not see such a correlation (CFU-GM recovery more than 17% vs less than 17%; \( P = .17 \)) in our study.

According to data from syngeneic bone marrow transplantation in AML,\textsuperscript{26,27} the relapse rate for ABMT is expected to be between 50% and 60% due, at least in part, to the lack of graft vs leukemia effect. Yeager et al\textsuperscript{1} reported an actuarial relapse rate of 46% for high risk AML patients at a median follow-up of 13 months compared with 65% in our study at a similar median follow-up of 19 months. Six relapses occurred more than 1 year after ABMT at 30, 20, 19, 18, 13, and 12 months in patients transplanted in second or subsequent CR, whereas no late relapse was seen in patients autografted in first CR at a median follow-up of 31 months, suggesting a stable disease-free plateau for low risk AML patients following ABMT. In our study the actuarial DFS in low risk AML patients was 61% compared with 50% reported by Burnett et al\textsuperscript{18} and 22% reported by Stewart et al\textsuperscript{19} without marrow purging or 69% by Gorin et al\textsuperscript{17} with Mafosfamide purging. From our series it is noteworthy that three of seven patients transplanted in third or fourth CR remain disease-free for 22, 40, and 44 months post-ABMT without any maintenance therapy.

It is believed that studies of bone marrow transplants in AML in CR1 are difficult to evaluate critically because they involve selected patients, some of whom have been in remission for several months or have not come to transplant at all due to early relapse, comorbid disease, refusal, insufficient graft, poor hematologic regeneration after induction or consolidation treatment, or other causes (42.5\textsuperscript{28} and 41%\textsuperscript{29} of all patients eligible for bone marrow transplantation). Although it appears that allogeneic bone marrow transplantation and intensive chemotherapy are roughly equivalent for curing adult patients with acute leukemia in the first remission,\textsuperscript{30} controlled trials are needed in which patients with AML in CR1 are randomized into groups receiving postremission chemotherapy or ABMT. Until these data are available, autologous bone marrow transplants, particularly in CR1 AML, should be regarded as investigational. When considering the time between onset of CR and ABMT as a patient selection criterion, Preisler et al\textsuperscript{4} published similar data with CR1 patients treated with conventional chemotherapy for the first 8 months after onset of complete...
remission; 58% of those patients randomized to stop chemotherapy at 8 months postinduction therapy remained alive for more than 3 years. These survival data are similar to those provided by our study for low risk AML patients.31

Transplants in CR2 or greater remission are less difficult to critically evaluate because it is generally believed that chemotherapy produces no long-term survival in patients who relapse while receiving treatment. Bone marrow transplantation appears to be the only therapeutic option with a curative potential in adults with acute leukemia in the first relapse or CR2.30 However, when comparing conventional chemotherapy plus maintenance therapy with ABMT, a proper control group of conventionally treated patients probably has a survival greater than 5%, as shown by Preisler and Raza,31 placing our ABMT data into perspective.

It is well-known that platelet reconstitution after ABMT occurs slowly probably due to the cryopreservation procedure, a defective stem cell transfused into the patient, or an autoimmune phenomenon. Purging might contribute to a delay in platelet recovery, which has also been observed with nonpurged autografts.18,19

The most important step forward in ABMT is to improve the therapeutic efficacy of the pretransplant regimen. With either treatment, radiotherapy and/or chemotherapy, the limit of toxicity is reached. One way to further increase antitumor effect could possibly be to add immunomodulatory treatment, such as the infusion of lymphokine activated killer cells or, as shown in the allogeneic transplant situation, the induction of an autologous GvHD-like syndrome.32 More effective ex vivo purging is also an important consideration in improving DFS with ABMT for AML, although the validity of purging particularly in CR1 AML has to be evaluated by randomized trials.

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