Autologous Bone Marrow Transplantation for Acute Myeloid Leukemia Using Busulfan Plus Etoposide as a Preparative Regimen

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We have studied the use of a new preparative regimen for the treatment of patients in remission of acute myeloid leukemia (AML) with autologous bone marrow transplantation. Chemotherapy consisted of busulfan 1 mg/kg every 6 hours for 4 days (total dose, 16 mg/kg) on days −7 through −4 followed by an intravenous infusion over 6 to 10 hours of etoposide 60 mg/kg on day −3. Autologous bone marrow, treated in vitro with 100 µg/mL of 4-hydroperoxycyclophosphamide, was infused on day 0. We have treated 58 patients up to the age of 60 years, 32 in first remission, 21 in second or third remission, and 5 with primary refractory AML unresponsive to high-dose Ara-C, but achieving remission with aggressive salvage regimens. Of the first remission patients, there has been 1 treatment related death and 5 relapses. With median follow-up of 22 months, the actuarial relapse rate is 22% ± 9% and disease-free survival is 78% ± 9% at 3 years. Patients with favorable French-American-British (FAB) subtypes (M3 or M4 EO) did especially well, with no relapses seen in 15 patients observed for a median of 30 months. Actuarial relapse rate at 3 years was 48% for first remission patients with less favorable FAB subtypes. Of patients in second or third remission, there were 5 treatment related deaths and 4 relapses. With median follow-up of 22 months, the actuarial relapse rate is 25% ± 11% and disease-free survival is 56% ± 11% at 3 years. Four of five primary refractory patients died during treatment and 1 remains in remission with short follow-up. These preliminary data are very encouraging and, if confirmed, support the use of autologous purged bone marrow transplantation using aggressive preparative regimens as one approach to improve the outcome of adults with AML.

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The goal of treatment of acute myeloid leukemia (AML) in young adults is cure. Remission induction therapy has become increasingly successful, and approximately 70% of adults under 60 years of age with de novo AML achieve initial complete remission. However, despite intense postremission chemotherapy, the majority of patients relapse, and relapse has become the limiting factor in the effort to improve long-term disease-free survival (DFS). Intensification of postremission chemotherapy, including the use of high-dose Ara-C, has led to some improvement in long-term disease control. However, recent results suggest that the limits of this approach have been reached. At best, only 30% to 40% of patients treated in remission remain in remission continuously, and less than one-quarter of patients under 60 years of age diagnosed with de novo AML are currently being cured.1,6

Experience with allogeneic bone marrow transplantation (BMT) demonstrates that ablative therapy has greater antileukemic efficacy than conventional chemotherapy and can substantially lower the relapse rate.7-12 For patients undergoing allogeneic BMT in first remission, relapse rates are approximately 20%, and it is possible that improved preparative regimens will lower this even further. However, the role of allogeneic BMT in the treatment of AML is destined to be circumscribed. Many patients are ineligible because of age or because of lack of a matched sibling donor. These constraints limit the use of allogeneic BMT to approximately 20% of adults under age 60. Furthermore, the substantial treatment-related mortality associated with allogeneic BMT reduces the overall DFS to approximately 50% and raises the question of whether, despite its increased antileukemic effectiveness, a better strategy would be to use this therapy as salvage for patients relapsing after chemotherapy.

Autologous BMT offers a way to use the antileukemic efficacy of ablative therapy without the morbidity and mortality of graft-versus-host disease (GVHD) that complicates allogeneic BMT. In addition to improved safety, autologous BMT would be broadly applicable, allowing the treatment of all adults up to age 60 who achieve initial remission. The reinfusion of autologous BM raises the concern that viable clonogenic leukemia cells would be infused, thereby producing an unacceptable risk of relapse. We have chosen to purge harvested marrow in vitro with 4-hydroperoxycyclophosphamide (4-HC) to reduce this risk. An additional concern about autologous BMT is that, in the absence of a graft-versus-leukemia effect, disease control would not approach that seen with allogeneic transplantation. Indeed, with the use of “standard” total body irradiation plus cyclophosphamide, relapse rates of 40% to 60% are seen in syngeneic transplants for first remission AML.13 One would expect that autologous BMT using equivalently effect preparative regimens would have a relapse rate of at least 50%.

We have chosen to evaluate the use of a new preparative regimen that might have greater antileukemic effectiveness. It has already been demonstrated in the setting of allogeneic BMT that more intensive preparative regimens can significantly lower the relapse rate.13,14 However, these gains have been offset by increases in treatment-related mortality that have resulted in no net benefit in DFS. Autologous BMT, in contrast, offers a setting in which it may be more practical to intensify preparative regimens and in which gains in disease control may not be offset by increases in GVHD and treatment-related mortality. We have used the combination of high-dose busulfan and etoposide based on the hypothesis that etoposide might be a more effective antileukemic agent than cyclophosphamide, and that, in the autologous setting, the immunosuppressive properties of cyclophosphamide would not be needed.
Our approach was to attempt to treat all adults up to age 60 in first remission of de novo AML with autologous BMT. We used high-dose Ara-C based induction therapy in an effort to achieve a high rate of initial remission and to do so rapidly with one course of therapy. We also hoped that the greater initial cytocutaneous achieved with such therapy might result in a lower tumor burden at the time of BM harvesting. No consolidation therapy was used and patients were taken directly to autologous transplantation. Some patients were referred from outside institutions in first remission and received alternate induction therapy. We also treated patients in more advanced stages of AML with the identical treatment regimen. We report the preliminary results of this single institution pilot study in which we are encouraged by the low relapse rates and the high DFS.

MATERIALS AND METHODS

Patients

Patients were treated between October 1986 and March 1992. Patients in first remission of de novo AML were eligible. (Patients with prior myelodysplasia, myeloproliferative diseases, or treatment-associated leukemia were excluded). Patients were considered to be in remission if they had normal BM morphology, resolution of abnormal cytogenetics, neutrophils greater than 1,500/µL, and platelets greater than 140,000/µL. Remissions were required to last for a minimum of 30 days before proceeding to BM harvest and transplant. In addition, we required adequate organ function with a bilirubin less than 1.5 mg/dL and aspartate transaminase (AST) and alkaline phosphatase less than twice the upper limit of normal. All patients gave written informed consent using forms approved by the Committee on Human Research of the University of California, San Francisco, or Alta Bates Hospital in accord with the Helsinki Declaration. For patients with more advanced disease, eligibility criteria were similar. We required that neutrophils be greater than 1,000/µL and platelets greater than 100,000/µL and remissions were not required to last for 30 days before harvest. The AST and alkaline phosphatase could be up to three times the upper limit of normal. Patients were designated as "primary refractory" if they had failed to achieve complete remission with high-dose Ara-C consisting of at least 12 doses over 6 days of 2 g/m² of Ara-C. These patients were eligible for transplant if they achieved remission with salvage therapy.

BM Collection and Processing

BM was harvested 1 to 30 days before initiation of preparative chemotherapy. All patients transplanted in second or third remission had BM harvested during that same remission. A minimum of 2 × 10⁸ nucleated cells/kg was collected (in one case requiring a second harvest). The nucleated cell/buffy coat was separated by centrifugation and prepared at a cell concentration of 2 × 10⁸/mL in M199 media (GIBCO Lab, Grand Island, NY) and 20% autologous plasma. The hematocrit was adjusted to between 5% and 10%. Freshly prepared 4-HC (Nova Corporation, Baltimore, MD) in M199 media was added to the cell suspension to obtain a final concentration of 100 µg/mL. Cells were incubated with 4-HC at 37°C for 30 minutes, with mixing every 5 minutes. The cell suspension was rapidly cooled to 4°C and centrifuged at 4°C at 3,000 rpm for 10 minutes. Cells were resuspended for freezing in M199 media to achieve a final cell concentration of 4 × 10⁹/mL in 5% autologous plasma and 10% dimethyl sulfoxide (DMSO). Eighty milliliter aliquots of final cell suspension were placed in polyolefin bags. These were frozen in a controlled rate freezer at -1°C/min to a temperature of -90°C. Cells were then transferred to the liquid phase of a liquid nitrogen freezer. At the time of autologous reinfusion, each bag was thawed in a 37°C water bath and immediately infused intravenously (IV).

Preparative Chemotherapy

Busulfan (1 mg/kg) was administered orally (PO) every 6 hours for 4 days (total dose, 16 mg/kg) on days -7 to -4. Etoposide (60 mg/kg) was administered by IV infusion over 6 to 10 hours on day -3. Etoposide was mixed at a concentration of 0.6 mg/mL in normal saline and infused at a rate of 1 L/h, resulting in an infusion rate of 1 hour for every 10 kg of weight. The first 31 patients (16 in first remission and 15 in subsequent remission) were dosed based on actual body weight. Twenty-seven subsequent patients (16 in first remission and 11 in subsequent remission) were dosed based on the mean of ideal and actual body weight. BM was thawed and infused on day 0. The median cell dose was 2.4 × 10⁹ nucleated cells/kg (range, 1.3 to 5.7).

Supportive Care

Patients were hospitalized in private rooms with high efficiency particle (HEPA) air filtration. Acyclovir (5 mg/kg) administered IV every 8 hours was begun on day -2 and continued until hospital discharge, at which point patients received 200 mg PO three times a day (TID) until 1 year after transplant. Trimethoprim/sulfamethoxyl (1 double-strength tablet PO twice a day [BID]) was administered on weekends throughout hospitalization and continued until at least 3 months posttransplant or until the CD4 lymphocyte count exceeded 200/µL. Gammaglobulin (500 mg/kg) was administered IV every 3 weeks for 4 doses beginning on day -1. Amphotericin (0.3 mg/kg) was begun on day +1. Amphotericin doses were then escalated for antibiotic-refractory fever. Broad spectrum antibiotics, usually consisting of cefazadime and vancomycin, were begun when neutrophil levels decreased to less than 500/µL. Other antibiotics were used as needed. Platelets were transfused to maintain a platelet count greater than 20,000/µL or higher if bleeding persisted.

Statistical Evaluation

Remission, DFS, and survival were all calculated from the date of BMT. Actuarial DFS and relapse were calculated using Kaplan-Meier analysis on a MacIntosh II personal computer (Apple Computers, Cupertino, CA). Relapse rates were compared using Mantel-Cox test. Comparisons between groups were performed by contingency table x² analysis using Statview Software (Abacus Concepts, Inc, Calabasas, CA). In calculating actuarial relapse, treatment-related deaths were censored. In calculating actuarial DFS, all adverse events were counted. Data was analyzed as of June 15, 1992.

RESULTS

Patients

The clinical features of the 32 patients treated in first remission are listed in Table 1. Most patients were treated at the University of California, San Francisco according to a uniform plan in which remission was induced with high-dose Ara-C combined with either daunorubicin (22 patients) or mitoxantrone (5 patients). None of these patients received consolidation chemotherapy before BMT. Five other patients were referred for treatment after receiving standard-dose induction chemotherapy, and two of these patients received a single course of consolidation therapy with standard-dose Ara-C-based treatment before BMT. All patients received transplants within 6 months of achieving initial remission. Cy-
togenetic evaluation was routinely performed on all patients initially diagnosed at the University of California, San Francisco. All eight patients with French-American-British (FAB) subtype M3 who were tested had t(15, 17) and two of these also had trisomy 8. All four patients with FAB type M4 EO that were tested had inversions of 16q. One of two patients with FAB M2 had t(8, 21).

The clinical features of patients in second or subsequent remission are listed in Table 1. Nineteen patients were in second remission and two in third remission. Five patients who had failed to achieve remission with initial high-dose Ara-C and achieved remission only after aggressive salvage therapy (primary refractory) are included in the "advanced disease" group. Prior therapy was variable, but 19 of 26 patients (73%) had relapsed after prior exposure to high-dose Ara-C, including 5 primary refractory patients and 14 of 21 (67%) second and third remission patients. Median length of first remission for these 21 patients was 11 months (range, 1 to 40 months) and only 1 patient had an initial remission greater than 2 years. Few patients had favorable cytogenetics. One patient (primary refractory) had t(15, 17), one patient (initial remission 13 months) had inversion of 16q, and two patients (initial remissions of 4 and 11 months) had t(8, 21).

Treatment Results

Of 32 patients in first remission, there was 1 treatment-related death, 5 relapses (at 2, 3, 7, and 9 months), and 1 death at 20 months of causes unrelated to treatment or leukemia. Twenty-five patients remain in continuous remission. Actuarial relapse rate at 3 years is 22% ± 9% and DFS is 76% ± 9% (median follow-up, 22 months) (Fig 1). For 15 patients with FAB subtypes M3 or M4 EO observed for a median of 30 months, there have been no adverse events and DFS is 100%. For 17 patients with other FAB subtypes (with median follow-up of 19 months), actuarial relapse rate at 3 years is 48% ± 18%, significantly higher than that in patients with FAB M3/M4 EO (P = .009) (Fig 2). There was no correlation between colony-forming unit granulocyte-macrophage (CFU-GM) content and outcome.

Of 26 patients with more advanced disease, there were 9 treatment-related deaths and 4 relapses (at 2, 3, 7, and 9 months). Thirteen patients continue in remission from 3 to 56 months. Actuarial relapse rate at 3 years is 24% ± 10% and DFS is 48% ± 10% (median follow-up, 22 months). The five patients with primary refractory disease did poorly. Four of five patients died during treatment and one remains in remission at 7 months. Of the 21 patients with initially chemotherapy-responsive disease, actuarial relapse is 25% ± 11% and DFS is 56% ± 11% at 3 years (Fig 3). Neither age, length of first remission, nor interval between remission and transplant were prognostically significant for relapse rate or DFS.

Toxicity

Hematologic recovery was slow. Neutrophils recovered to 100, 500, and 1,000/μL at median times of 22, 32, and 41 days, respectively. Platelet transfusion support was required for a median of 52 days, and six patients (2 of whom relapsed early) required platelet transfusion support for more than 100 days. Platelets reached levels of 50,000 and 100,000/μL at median times of 79 and 170 days, respectively. Red blood cell (RBC) transfusion support was required for a median of 56 days, and seven patients (2 of
Patients were hospitalized for a median of 48 days (mean, 56.5) after BM infusion. Mucosal, skin, and gut toxicity were similar to those seen in first remission patients. Necrotizing enterocolitis was required for a median of 17 days and parenteral nutrition for a median of 33 days. Two patients had life-threatening gastrointestinal bleeding and recovered. Hepatotoxicity was more significant than in the first remission group. Although the median peak bilirubin was only 1.9 mg/dL, five patients had peak bilirubins greater than 6 mg/dL and three patients died of hepatic failure due to veno-occlusive disease.

There were nine treatment-related deaths (35%). Three were due to veno-occlusive disease and four to infection (cytomegalovirus pneumonia, resistant herpes simplex pneumonia, aspergillus pneumonia, and disseminated fusarium). Two patients died of graft failure (both primary refractory patients). Treatment-related deaths were significantly more common in patients with primary refractory disease, occurring in 4 of 5 patients as opposed to 0 of 21 patients in second or third remission ($P = .018$). However, even after excluding the primary refractory patients, treatment-related mortality was higher in second or third remission patients than in first remission patients ($P = .02$).

**DISCUSSION**

We initiated this study to evaluate the role of autologous BMT using BM purging and a new aggressive regimen in treatment of patients in first remission of AML. We treated all eligible patients up to age 60 without intentional patient selection. We attempted to recruit a high-risk group of patients by using an aggressive remission induction regimen consisting of high-dose Ara-C plus either daunorubicin or mitoxantrone and by taking patients directly to BMT without intervening postremission chemotherapy. The use of high-dose Ara-C–based induction chemotherapy may have recruited into our patient group patients with relatively resistant forms of leukemia that may not have entered remission with conventional dose therapy. Our preliminary results suggest that our treatment approach using autologous BMT is remarkably effective, with low treatment-related mortality and a low relapse rate combining to produce a 3-year DFS of 76% ± 9%.

Although BMT treatment-related mortality was low (3%) in first remission patients, the regimen has considerable toxicity and requires meticulous patient support to avoid a higher death rate. Patients experience 3 weeks of agranulocytosis and spend approximately 4 weeks with less than 500/µL neutrophils. Approximately 8 weeks of platelet transfusion support are required, often including 2 or more weeks in the outpatient setting. Nonhematologic toxicity in the skin and mucosa was significant and probably greater than that seen with more standard preparative regimens such as busulfan/cyclophosphamide or total body irradiation/cyclophosphamide. Our observation that treatment-related mortality increased significantly in more heavily pretreated patients in second or subsequent remission suggests that the current regimen is at the maximally tolerated dose.
This treatment program produced a low relapse rate of 22% ± 9% in first remission patients. This needs to be interpreted in the context of our patient group. Despite our goal of recruiting a high-risk group of patients, the FAB distribution of our patients was highly skewed, with 47% of patients having M3 or M4 EO. Ordinarily, one would expect 15% to 20% of young de novo AML patients to have these features at diagnosis. Because these patients have a high remission rate and a low drop-out rate before transplant, a more representative group of transplant patients would include 25% of patients with these favorable characteristics. Even so, our success in this patient group has been noteworthy, with 100% DFS in 15 patients observed for a median of 30 months. Patients with FAB M4 EO associated with inversions of chromosome 16 have been reported to have a favorable response to chemotherapy, with a DFS in the range of 50%, 17,18 Patients with FAB type M3 also fare relatively well with chemotherapy, with a DFS of 35% to 45%.19,20 However, even though considered “favorable,” relapse rates are still 50% or greater in these groups of patients treated with nonablative chemotherapy, considerably different the zero relapse rate seen with our regimen.

For our first remission patients with less favorable FAB subtypes, the relapse rate was 48% at 3 years. This compares favorably with results achieved with conventional postremission chemotherapy, especially if patients with favorable characteristics are excluded. If the relapse rates in our favorable and less favorable FAB groups hold true, a more representative group of patients (in which only 25% had favorable characteristics) would be expected to have DFS of 60%. The antileukemic efficacy of this BMT treatment approach is confirmed by the results in patients in second or subsequent remission and whom a relapse rate of 24% was observed. These patients are unselected and do not have low-risk features (median age of 38 years, short initial remissions, and few favorable FAB types). Additionally, two-thirds of these patients had relapsed after previous exposure to high-dose Ara-C and were unlikely to be cured with any nonablative approaches. Although median follow-up is still relatively short at 22 months, most relapses that occur in this setting occur early, and the relapse rate is unlikely to increase substantially.

We chose our preparative regimen, high-dose busulfan plus etoposide, based on the hypothesis that the substitution of etoposide for cyclophosphamide might produce a lower relapse rate than more traditional regimens. Busulfan has become widely accepted as an acceptable substitute for total body irradiation in preparative regimens. The combination of busulfan plus cyclophosphamide has produced relapsed rates less than 20% when used in allogeneic BMT for AML in first remission.9,10 A recent study suggests that total body irradiation may be a superior preparative regimen in this setting with less relapse, but this requires confirmation.12

The combination of busulfan plus etoposide was first used in the setting of second allogeneic BMT in five patients who had relapsed after an initial allogeneic transplant.21 Two of five patients were disease-free at the time of that report. Escalating doses of etoposide (30 to 40 mg/kg) were added to busulfan plus cyclophosphamide in 11 patients undergoing allogeneic or autologous transplant.22 The investigators concluded that the regimen could be administered with tolerable toxicity, but expressed concern over skin and hepatic toxicity. Higher doses of etoposide (60 mg/kg) were administered in combination with busulfan and cyclophosphamide to 24 patients with high-risk hematologic toxicity.23 Preliminary results were encouraging, with a relapse rate of 20% and DFS of 40%. More recently, 38 patients were treated with busulfan, cyclophosphamide, and 40 mg/kg of etoposide in the setting of either allogeneic or autologous BMT with encouraging results.24

The use of high-dose etoposide as part of preparative regimen has been further explored in allogeneic transplantation in combination with fractionated total body irradiation. In the initial dose escalation study of this regimen treating patients with high-risk hematologic malignancy, the relapse rate was 32% and DFS was 43%.25 The investigators concluded that the maximally tolerated dose of etoposide in this combination was 60 mg/kg. Of seven patients treated with 70 mg/kg of etoposide, three died and four relapsed. Subsequent studies of this regimen have confirmed its high level of activity with low relapse rates in high-risk patients.26,27 Treatment using this regimen has produced DFS in four of eight adults with primary refractory acute leukemia and three of six adults with Philadelphia chromosome-positive acute lymphoblastic leukemia, settings in which disease control has been previously very difficult to achieve.28 More recently, this regimen has been used to treat adults in first remission of acute lymphoblastic leukemia with excellent preliminary results.30

We chose to purge the marrows of all patients in this study with 4-HC. The role of marrow purging in autologous BMT for AML remains controversial. Several pieces of evidence support the importance of marrow purging in this setting. Results of the European Bone Marrow Transplant Registry suggest that purging with mafosfamide reduces relapse and increases leukaemia-free survival.31 However, this could be shown convincingly only in a subset of patients who received their autografts within 6 months of achieving remission, and the conclusions are not compelling because of the retrospective nature of this registry data. In another study in which patients were nonrandomly assigned to receive either unpurged peripheral blood stem cells or mafosfamide-purged BM, the use of purged marrow resulted in a lower relapse rate and higher DFS.32 Indirect evidence for the usefulness of purging with 4-HC has come from correlation of in vitro studies with clinical outcome. In one report, “intensity” of purging, as judged by the surrogate marker of elimination of CFU-GM colonies, was associated with a significant decrease in relapse.33 In further studies, the sensitivity to 4-HC of clonogenic leukemia cells isolated from harvested marrow has been shown to have a striking correlation with disease control.34 However, the requirement for purging has been challenged by data in several studies in which autologous BMT using nonpurged marrow has produced reasonably low relapse rates.35-38 Furthermore, it is clear that 4-HC purging of BM from patients with AML results in a significant delay in engraftment.39
The role of autologous BMT in the treatment of AML remains uncertain at this time. Most reports demonstrate a low mortality rate from the procedure, with treatment-related death in 3% to 7% of patients in first remission of AML.\textsuperscript{31,35,36,40-45} Relapse rates have varied between 36% and 68% and DFS varied between 25% to 61%. Studies in which relapse rates exceed 50% have all used nonpurged BM and have not made an attempt to intensify the preparative regimen, factors that may be important in producing optimal results. That ablative chemotherapy with autologous BM rescue has greater antileukemic efficacy than conventional chemotherapy is clearly demonstrated by the substantial salvage rate of treating patients in second or subsequent remission.\textsuperscript{37,38,40,43,46-49} In several reports, relapse rates have been 43% to 65% and DFS has been 22% to 52%. These results exceed any reasonable expectation from conventional therapy.

New approaches are needed if we are to make further progress in the treatment of adult AML. Nonablative postremission chemotherapy has been unable to produce relapse rates of less than 50% or DFS greater than 50%. There are currently no new agents or schedule modifications that are likely to substantially change these results. Allogeneic BMT will not be a broad solution to the problem, both because of limitations based on age and donor availability and because of reluctance to accept treatment-related mortality of 20%. Autologous BMT has the potential to impact on this problem and appears to be applicable to all adults up to the age of 60, provided that they can achieve initial remission. Autologous BMT will be an attractive option if treatment-related mortality can be kept under 5%, a mortality not different from that seen with high-dose conventional chemotherapy. If our preliminary results are confirmed, autologous BMT would become the treatment of choice for patients in first remission of AML.

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