



Allogeneic bone marrow transplantation with matched unrelated donors for patients with hematologic malignancies using a preparative regimen of high-dose cyclophosphamide and fractionated total body irradiation

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Summary:

Allogeneic bone marrow transplantation (BMT) from an HLA-identical sibling donor is effective therapy for patients with bone marrow failure states and those with hematologic malignancies. However, only a minority of them will have an HLA-identical sibling donor; unrelated donors, matched or partially mismatched, have been used successfully for patients lacking a related donor. Even though results with allogeneic transplants using unrelated donors are encouraging, the incidence of complications including graft-versus-host disease (GVHD) and graft rejection or late graft failure is increased compared to identical sibling transplants. The combination of cyclophosphamide and total body irradiation (TBI) has been used as an effective preparative regimen for allogeneic transplants, however, the total dosage and dosing schedule of both the cyclophosphamide and TBI has varied significantly among studies. To decrease the rate of graft rejection and late graft failure with volunteer donors, we evaluated a preparative regimen of high-dose cyclophosphamide (200 mg/kg over 4 consecutive days, days -8, -7, -6, -5) followed by fractionated TBI (1400 cGy administered in eight fractions over 4 days, days -4, -3, -2, -1). GVHD prophylaxis included FK506 and methotrexate. From July 1993 to January 1996, 43 adult patients, median age 38 years (range 18–58 years), were treated with this preparative regimen. Seventeen patients had low-risk disease and 26 had high-risk disease. Thirty-one donor/recipient pairs were matched for HLA-A, -B, and -DR by serology and molecular typing. Seven additional pairs were minor mismatched at the HLA-A or HLA-B loci. Four other donor/recipient pairs were HLA-A, -B, and -DR identical by serology but allele mismatched at either DRB1 or DQB. Forty patients were evaluable for myeloid engraftment. Engraftment occurred in all 40 patients at a median of 19 days. There

were no cases of graft rejection or late graft failure. Nephrotoxicity was the primary adverse event with 26 patients (60%) experiencing a doubling of their creatinine. Hepatic veno-occlusive disease occurred in seven patients, six of whom had high-risk disease. All patients who had relapsed or refractory disease prior to BMT achieved a complete remission following BMT. Six patients transplanted for high-risk disease relapsed a median of 377 days post-BMT. None of the patients with low-risk disease have relapsed following transplant; the Kaplan–Meier survival for those patients with low-risk disease is 62% and 37% for those patients transplanted with high-risk disease ($P = 0.0129$). The median Karnofsky performance status is 100% (range 70–100%). Therefore, a preparative regimen of high-dose cyclophosphamide and fractionated TBI is an acceptable regimen for patients receiving an allograft from unrelated donors.

Keywords: bone marrow transplant; preparative regimen; unrelated donors; hematologic malignancies; CY-TBI

Allogeneic bone marrow transplantation (BMT) from an HLA-identical sibling donor has been shown to be effective therapy for patients with bone marrow failure states and patients with acute and chronic leukemias as well as selected patients with multiple myeloma and lymphoma.^{1–6} Prolonged survival with recovery of normal hematopoiesis has been demonstrated in 60–90% of patients with severe aplastic anemia following allogeneic BMT;^{7,8} long-term disease-free survival of >50% has been demonstrated in patients undergoing BMT for acute and chronic leukemias.^{1–4} However, only a minority of patients will have an HLA-identical sibling donor and fewer than 5% of patients will have an HLA one locus mismatched family member who may serve as an appropriate donor. Unrelated donors, matched or partially mismatched, at the HLA loci have been used successfully in BMT therapy for severe combined immunodeficiency disease, aplastic anemia, acute leukemia, myelodysplastic syndrome (MDS) and chronic myelocytic leukemia (CML).^{9–11} McGlave *et al*¹² recently reported on 196 patients with CML, 133 of whom received unrelated

marrow grafts, identical by serotyping and MLC analysis at the HLA-A, -B and -DR loci, and in 63 cases, where there was no identity at one HLA locus. Twenty-two patients failed to engraft and an additional 10 patients experienced late graft failure. The 2-year actuarial disease-free survival (DFS) for patients transplanted in first chronic phase within 1 year of diagnosis was 45%. Analysis revealed that transplantation with HLA-matched donor marrow ($P = 0.01$), transplantation at younger age ($P = 0.02$) and in first chronic phase ($P = 0.04$) had independent, beneficial effects on DFS. Kernan *et al*¹¹ reported on 462 patients who received marrow grafts from unrelated donors for leukemia ($n = 387$) and non-malignant disorders ($n = 72$). The actuarial probability of DFS at 1.5 years was 34% for patients with good-risk leukemia and 20% for patients with high-risk disease.

The development of the National Marrow Donor Program (NMDP) has facilitated the identification of unrelated donors and the procurement of unrelated donor marrow.¹¹ Current estimates suggest that HLA-A, -B and -DR matched unrelated donors can be found for approximately 35–50% of potential recipients with existing donor registry resources.¹³ Further expansion of NMDP in the United States as well as its close collaborations with large unrelated bone marrow donor registries located in other countries should improve the efficiency of donor searches. In addition, studies have also demonstrated the potential use of unrelated donors mismatched at one HLA antigen, making it possible to extend marrow transplantation to other patients, for whom a suitable matched, related donor is not available.¹¹

The combination of cyclophosphamide and total body irradiation (TBI) is an effective regimen for allogeneic transplants using either HLA compatible sibling donors¹⁴ or matched unrelated donors.¹¹ However, the total dose and schedule of both the cyclophosphamide and TBI has varied significantly among studies. To decrease the rate of graft rejection with volunteer donors, immunosuppressive preparative regimens are required to adequately prepare the patient for the unrelated allograft. With this in mind, an immunosuppressive preparative regimen incorporating maximum doses of cyclophosphamide over 4 days and fractionated TBI administered over 4 days was evaluated in patients with hematologic malignancies undergoing matched unrelated donor transplants. Wingard *et al*¹⁵ reported a similar regimen for patients with acute lymphoblastic leukemia (ALL) undergoing allografting with HLA compatible family donors; results were encouraging with no graft rejection and acceptable treatment-related toxicity.

Materials and methods

Patients

From July 1993 to January 1996, 43 adult patients were enrolled in this phase II study at Emory University Hospital. Patient characteristics are shown in Table 1. Patients with low-risk disease included (1) those with AML or ALL in first remission, (2) CML in chronic phase, (3) chronic

Table 1 Patient characteristics

Patient (<i>n</i>)	43
Age (years)	
median	36
range	18–58
Sex (M/F)	25/18
Disease categories	
AML	14 (35%)
CR1	0
CR2	2
>CR2, refractory, relapse	12
ALL	6 (14%)
CR1	3
CR2	0
>CR2, refractory, relapse	3
MDS	4 (9%)
RA, RARS	1
RAEB, RAEB-t	3
CML	15 (33%)
CP	11
AP, BP	4
CLL	2
Responsive disease	1
Refractory disease	1
Multiple myeloma (partial remission)	1
Myeloproliferative disease (stable)	1

lymphocytic leukemia (CLL) with responsive disease, and (4) MDS (refractory anemia (RA) or refractory anemia with ringed sideroblasts (RARS)) with significant pancytopenia or chronic myeloproliferative disorder. Patients with either AML or ALL in first remission were considered for allografting with an unrelated donor based on disease characteristics, primarily poor-risk cytogenetics, age and donor availability. Patients were categorized with high-risk disease if they had either (1) AML or ALL in greater than first remission, refractory to induction therapy or in relapse at the time of BMT, (2) CML patients in accelerated phase, blast crisis, or second chronic phase, (3) advanced MDS (refractory anemia with excess blasts (RAEB) or RAEB in transformation (RAEB-t)) and (4) patients with other hematologic malignancies (multiple myeloma, lymphoma, etc) refractory to conventional therapies. The protocol and consent forms were approved by the Human Investigations Committee at Emory University. Written informed consent was obtained from all patients. Potential patients had morphologically documented disease. They were required to be at least 12 years of age, have a Karnofsky performance status of 80% or greater, an estimated creatinine clearance of 60 ml/min or greater, total bilirubin ≤ 1.4 times upper limit of normal (ULN), AST and ALT less than or equal to 1.5 times ULN, FVC and FEV1 greater than 70% predicted, a cardiac ejection fraction greater than 50%, no known hypersensitivity to cremophor or related products, and could not be carriers of the human immunodeficiency virus (HIV). A pre-study pregnancy test was required for all women to exclude those who were pregnant.

Donor–recipient matching

Serologic testing for HLA-A, -B and -C was performed by the standard NIH two-stage microlymphocytotoxicity assay

using commercial typing trays. Molecular testing for DRB1, DRB3, DRB4, DRB5 and DQB1 was performed with sequence-specific PCR primers. For approximately one-half of the donor/patient pairs, additional molecular testing for DRB1 and/or DQB1 was performed by automated, direct sequencing of their HLA genes (Mauer *et al*, unpublished). Every effort was made to resolve ambiguous HLA types by testing available family members of the patient. If an HLA-A, -B, -C, -DRB1, -DQB1 identical donor could not be identified, additional potential donors with mismatches at the C locus and/or DQB1 locus were considered. When no donors could be identified by these criteria, the search was extended to potential donors with one minor mismatch at the HLA-A, -B or -DRB1 locus as identified by the NMDP. In addition, when appropriate, donor selection was based on donor/recipient sex matching and cytomegalovirus (CMV) status of the donor and recipient.

Treatment regimen/supportive care

All 43 patients received an identical preparative regimen consisting of cyclophosphamide (total of 200 mg/kg over 4 consecutive days, days -8, -7, -6, -5) followed by fractionated TBI (1400 cGy administered in eight fractions over 4 days, days -4, -3, -2, -1). Cyclophosphamide was given intravenously over 1 h at a dose of 50 mg/kg/day; dose was based on the lesser of actual or ideal weight in non-obese patients and on adjusted (average of ideal and actual weight) for obese patients (30% over ideal weight). To prevent hemorrhagic cystitis, aggressive hydration (150–200 cc/h) and brisk diuresis was maintained during cyclophosphamide therapy. Mesna was not routinely used. Fractionated TBI consisted of two fractions per day of 175 cGy per fraction administered through opposed anterior/posterior fields separated by at least 6 h over 4 consecutive days for a total of eight fractions. The lungs were shielded with each treatment to restrict their dose to 800–850 cGy. Because of the lung shielding an electron boost to the chest wall corresponding to the lung blocks was given in two fractions to boost the dose to the intervening tissue, between the ribs and skin, to approximately 1100 cGy. For patients with a history of prior CNS disease, methotrexate (MTX) 12 mg intrathecally was administered for five doses beginning approximately 60 days after transplant as peripheral blood counts permitted, once or twice weekly. Folinic acid 5 mg was given orally q 6 h for six doses starting 24 h after each metotrexate dose.

All donor marrows were obtained from the NMDP, Canadian and German registries and reinfused on day 0. No bone marrows were *in vitro* T cell depleted; marrow manipulation consisted only of red cell removal by buffy coat for ABO incompatible grafts.

Supportive care measures included the routine use of prophylactic and empiric antibiotics. Amphotericin B (0.5–1.0 mg/kg/day) infused over 2 h was initiated as per current infectious disease protocols for persistent fever despite broad-spectrum antibacterials or for documented fungal infection. Intravenous immunoglobulin was not routinely administered. All patients underwent weekly surveillance blood cultures for CMV using either the shell-vial method

or by PCR determination, and pre-emptive ganciclovir was begun for any patient with a positive blood result.¹⁶ CMV-negative blood products were administered for CMV-negative donor/recipient pairs.

The first six recipients received granulocyte-macrophage colony-stimulating factor (GM-CSF; Immunex, Seattle, WA, USA) subcutaneously daily beginning on day 0 and continuing until the absolute neutrophil count (ANC) was greater than 500/mm³ for 3 consecutive days. Thereafter, patients were not routinely administered myeloid growth factors.

GVHD prophylaxis

All 43 patients received the same GVHD prophylaxis regimen consisting of FK506 administered by continuous intravenous infusion at a dose of 0.03 mg/kg/day, based on the lesser of actual or ideal body weight, beginning on day -1.¹⁷ Oral FK506 was substituted at four times the intravenous dose when tolerated and continued until day +180 when it was stopped unless chronic GVHD had occurred. Dose adjustments of FK506 have previously been described and were based primarily on renal function.¹⁷ If FK506 was discontinued, methylprednisolone 1 mg/kg/day in two divided doses was given for GVHD prophylaxis. If at week 9 following BMT there was no evidence of chronic GVHD, FK506 doses were tapered to 66% of the week 8 dose, at week 17 tapered to 33% of week 8 dose, and at week 26 discontinued. MTX was administered intravenously at 5 mg/m² for four doses on days 1, 3, 6 and 11. MTX doses were reduced or withheld for grade 4 mucositis or grade 3 or 4 nephrotoxicity or hyperbilirubinemia, based on modified SWOG criteria.¹⁸ Folinic acid rescue, 5 mg orally or intravenously every 6 h for four doses, was initiated 24 h following the last three doses of MTX.

Engraftment criteria

Myeloid bone marrow engraftment was considered to have occurred on the first day the ANC was at least 500/mm³ for 2 consecutive days. Standard cytogenetic and heteromorphism studies, when appropriate, were performed routinely to confirm donor origin. Platelet engraftment was the first day the platelet count was greater than 20 000/mm³ for 7 consecutive days without transfusion support. Failure to engraft was defined as no evidence of hematopoietic recovery by day +28, confirmed by biopsy. Graft failure was defined as initial hematopoietic recovery by day +28, followed by an ANC less than 500/mm³ for more than 3 consecutive days, independent of any myelosuppressive drugs, severe GVHD, CMV or other infection. Graft rejection was defined by initial engraftment documented to be of donor origin followed by loss of graft (ANC less than 500/mm³) and return of recipient hematopoiesis.

Assessment and treatment of GVHD

Patients were evaluable for acute GVHD if they engrafted. Patients were evaluable for chronic GVHD if they were alive and disease-free at 100 days or greater. Acute and

chronic GVHD were staged and graded using previously described criteria.¹⁹

If acute GVHD occurred, first-line treatment included methylprednisolone at 2.0 mg/kg/day in 4 divided doses. If a response was obtained after four days, the dose of methylprednisolone was tapered by 0.5 mg/kg/day every 4 days until discontinuation. If there was no response after 4 days, treatment was continued for an additional 4 days before instituting second-line therapy which included investigational protocols active at the time. Patients developing extensive chronic GVHD were placed on an alternate day regimen consisting of oral FK506 (0.12 mg/kg every other day) and prednisone (1 mg/kg every other day).

Adverse event monitoring

Patients were evaluated clinically throughout the study including all adverse events for possible preparative regimen toxicity. In addition, laboratory studies, including a complete blood count and serum chemistries were obtained routinely. Renal dysfunction was defined as a doubling of serum creatinine over baseline. The criteria for hepatic veno-occlusive disease (VOD) has been previously defined.²⁰ Mucositis was graded based on modified SWOG criteria.¹⁸

Statistical analysis

Fisher's exact test was used to compare the proportions of patients with high-risk vs low-risk disease and the development of hepatic VOD and to compare rates of mucositis between patients with high-risk vs good-risk disease. Student's *t*-test was used to compare the mean peak creatinines between patients administered amphotericin B and those patients who never received amphotericin B.

Kaplan–Meier curves were computed for overall survival.²¹ Few patients relapsed and those who did died shortly thereafter so that disease-free and overall survival were largely equivalent. The log-rank test for the difference in survival curves was performed to evaluate differences between those patients with high-risk and low-risk disease.

Results

Patient selection

Forty-three patients underwent allografting using an unrelated donor with this preparative regimen. Seventeen patients had low-risk disease and 26 had high-risk disease. Five patients with high-risk disease had previously undergone an autologous transplant for AML and had relapsed following the transplant; they underwent an unrelated allograft either in complete remission (two patients), early relapse (one patient), or in relapse (two patients).

Donor selection

All donors were obtained from the NMDP, Canadian and German registries. Donor characteristics are shown in Table

2. The median donor age was 37 years (range 22–54 years). Twenty-three (47%) donors were male and 20 (53%) were female. Thirty-five donor/recipient pairs were sex matched and eight were mismatched.

Thirty-one donor/recipient pairs were matched for HLA-A, -B and -DR by serology and molecular typing. Seven additional pairs were minor mismatched at the HLA-A locus or HLA-B locus. There was only one patient with refractory AML who received a class I major mismatched allograft. Four other donor/recipient pairs were HLA-A, -B and -DR identical by serology but allele mismatched at either DRB1 or DQB. The median interval from the initiation of donor search to transplant was 4 months (range 1–11 months).

Engraftment

The median infused marrow nucleated cell dose was 2.80×10^8 /kg (range 0.9–4.70 $\times 10^8$ /kg). Forty patients were evaluable for myeloid engraftment; one patient died of CNS hemorrhage on day 16 post-transplant and two patients died of infectious complications on days 15 and 21 following transplant. These three patients had high-risk disease and, in addition, one received a major HLA-A mismatched bone marrow. Myeloid engraftment occurred in the other 40 patients at a median of 19 days (range 11–32 days). None of these 40 patients experienced either graft rejection or late graft failure. There was no association between nucleated cell dose and time to myeloid engraftment ($P = NS$). There was no significant difference in time to myeloid engraftment between patients receiving GM-CSF and those not receiving myeloid growth factors. Of these 40 patients, cytogenetic and/or heteromorphism studies were available for 29 patients which confirmed 100% donor origin, generally by day 90, in all of these evaluable patients.

Table 2 Donor and typing characteristics

Donor age (years)	
median	37
range	22–54
Recipient–donor matching	
HLA-matched (A, B, DRB1)	31 (72%)
HLA-A minor mismatched	5
HLA-A major mismatched	1
HLA-B minor mismatched	2
HLA-B major mismatched	0
HLA-DRB1 minor mismatched	4
HLA-DRB1 major mismatched	0
Recipient–donor CMV status (recipient/donor)	
positive/positive	10 (23%)
positive/negative	15 (35%)
negative/positive	1
negative/negative	17 (40%)
Recipient–donor sex and parity (recipient/donor)	
male/male	18 (42%)
male/female	3 (7%)
male/non-parous female	4 (9%)
female/female	6 (14%)
female/male	1
female/non-parous female	11 (26%)

Twenty-nine patients reached a stable platelet count >20 000 at a median of 29 days (range 12–88 days) following BMT. Fourteen patients died prior to platelet transfusion independence.

Acute GVHD

Forty patients were evaluable for acute GVHD. Sixteen patients (40%) developed grade 2–4 acute GVHD; severe grade 3 or 4 acute GVHD developed in nine patients (22%). The median time to onset of acute GVHD was 25 days (range 11–71 days).

Chronic GVHD

Thirty patients were evaluable for the development of chronic GVHD. Sixteen patients (53%) developed limited or extensive chronic GVHD. Of these 16 patients, seven had acute GVHD which progressed to chronic GVHD and nine patients had *de novo* onset. The median time to the onset of histologically proven chronic GVHD was 182 days (range 34–340 days).

Adverse-related events

Nephrotoxicity was the primary adverse event noted in this patient population and was primarily attributable to FK506 and antibiotics/amphotericin B. Twenty-six patients (60%) had a doubling of their baseline serum creatinine during the study period, requiring adjustments in the FK506 dose. The median peak serum creatinine was 3.2 mg/dl (range 1.1–9.8 mg/dl). In general, renal function improved when the FK506 dose was reduced or temporarily held. The median peak creatinine was significantly higher in the 27 patients who received amphotericin B (2.7 mg/dl vs 1.8 mg/dl, $P = 0.046$). Hemodialysis (HD) was required in 11 patients (26%). Nine of the patients requiring HD were transplanted for high-risk disease and had been heavily treated prior to transplant with both chemotherapy and amphotericin B and two had low-risk disease, one of whom required HD for thrombotic thrombocytopenic purpura (TTP) (9/26 vs 2/17, $P = 0.117$). All patients who required HD have died.

Hepatic dysfunction unrelated to GVHD was in general mild and presumed to be due to the preparative regimen. Clinically apparent hepatic VOD occurred in seven patients (16%) and was a contributing cause of death in four. The median peak bilirubin was 16 mg/dl (range 3.1–66 mg/dl). Six of these seven patients had high-risk disease and had been heavily pretreated which was not statistically significant when compared to patients with low-risk disease who developed hepatic VOD (6/26 vs 1/17, $P = 0.215$).

Idiopathic interstitial pneumonitis and/or adult respiratory distress syndrome (ARDS) developed in six patients (14%) prior to day +30 and was a contributing cause of death in one. Four patients (9%) developed mild to moderate hemorrhagic cystitis in the early post-BMT period which responded completely to conservative measures including forced diuresis and increased platelet transfusion.

Grade 3 mucositis developed in all patients and required temporary narcotic analgesics. Eight patients developed

grade 4 mucositis, requiring withholding of the day +11 MTX dose. All eight patients had high-risk disease which was significant when compared to patients with low-risk disease (8/26 vs 0/17, $P = 0.0144$); in addition, four of these eight had accompanying nephrotoxicity at the time the day +11 MTX dose was held.

Opportunistic infection

Twenty-six of the 43 patients were at risk for development of CMV infection by virtue of patient and/or donor CMV seropositivity (Table 1). Fourteen of these patients (54%) developed CMV viremia at a median of 46 days (range 25–203 days) post-transplant. Three patients subsequently developed CMV interstitial pneumonitis and one patient developed intestinal CMV.

Documented invasive fungal infection occurred in seven patients (16%) (two *Candida krusei*, three *Aspergillus flavus*, one *Aspergillus fumigatus* and one *Fusarium*). Both *Candida krusei* infections developed before engraftment in patients with grade 3 mucositis who were receiving prophylactic fluconazole. One of these patients failed to engraft and later developed disseminated *Rhizopus*, which was the cause of death. The other *Candida krusei* infection cleared following engraftment while on high-dose amphotericin B. Two of the disseminated *Aspergillus* infections were detected at autopsy in patients who had been profoundly immunosuppressed following the development of grade 4 acute GVHD despite receiving high-dose empiric amphotericin B.

Despite the use of acyclovir prophylaxis, 14 patients had HSV cultured, usually from the oral cavity post-BMT after acyclovir prophylaxis was completed; two patients were found to be acyclovir resistant and one patient developed HSV esophagitis which responded to prolonged acyclovir therapy. Twelve patients developed varicella-zoster virus at a median of 305 days (range 33–715 days) following allografting. There were 11 episodes of Gram-negative infections and 33 episodes of Gram-positive infections in 27 patients resulting in the removal of nine Hickman catheters.

Relapse and survival

All patients who had relapsed or refractory disease prior to BMT achieved a complete remission following BMT. Six patients transplanted for high-risk disease (two refractory AML, one AML in CR2, two with RAEB-t and one refractory ALL) relapsed a median of 377 days post-BMT (range 139–480 days). None of these five patients had developed GVHD. Three of these patients received donor lymphocyte transfusions using their unrelated donor and one is currently alive 530 days following the BMT; the other two have died of recurrent disease. None of the patients with low-risk disease have relapsed following BMT.

Twenty-four patients have died, 15 prior to day +100. The primary causes of early (prior to day 100) and late (post day 100) death are shown in Table 3. The Kaplan–Meier estimate of survival for all patients is shown in Figure 1. A comparison of the Kaplan–Meier estimate of survival between low-risk patients and high-risk patients is

Table 3 Primary causes of death

	Prior to day 100	Post-day 100
GVHD	6	4
CMV	2	0
ARDS/Sepsis	2	0
Cerebral hemorrhage	2	0
VOD	1	0
Multi-organ failure	2	1
Recurrent leukemia	0	4
Total	15	9

GVHD = graft-versus-host disease; CMV = cytomegalovirus; ARDS = adult respiratory distress syndrome; VOD = veno-occlusive disease.

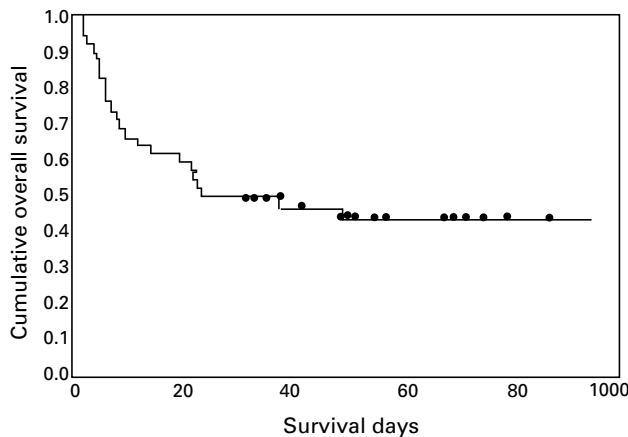


Figure 1 The Kaplan–Meier analysis for overall survival of 43 adult patients undergoing allogeneic bone marrow transplantation using unrelated donors following a preparative regimen of high-dose cyclophosphamide (200 mg/kg over 4 days) and fractionated total body irradiation (1400 cGy administered in eight fractions over 4 days).

shown in Figure 2 ($P = 0.0129$). The median follow-up of patients is 529 days (range 198–962 days). The median Karnofsky performance status of all survivors is 100% (range 70–100%).

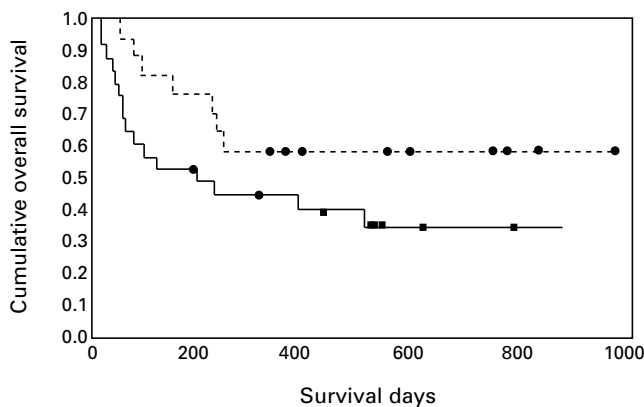


Figure 2 The Kaplan–Meier analysis comparing the overall survival between 26 patients with high-risk disease (—) and 17 patients with low-risk disease (---) ($P = 0.0129$). All patients received a preparative regimen of high-dose cyclophosphamide (200 mg/kg over 4 days) and fractionated total body irradiation (1400 cGy administered in eight fractions over 4 days).

Discussion

For successful allografting in patients with leukemia, a preparative regimen must be sufficiently immunosuppressive, myeloablative and anti-leukemic. In addition, the regimen must have relative ease of administration with acceptable toxicity to the patient. For allogeneic BMT incorporating mismatched related donors or matched unrelated donors, attempts have been made to increase immunosuppression by the preparative regimen, usually by increasing the total dose of TBI or by the addition of other immunosuppressive drugs such as high-dose steroids or ATG.^{22,23} Such approaches have generally increased the toxicity of the transplant. Bearman *et al*²⁴ compared 52 patients who received HLA-compatible marrow transplants from unrelated volunteer donors to 104 patients transplanted from HLA-identical siblings. The actuarial probability of severe regimen-related toxicity was 31% after the unrelated BMT and 21% after sibling transplants ($P = 0.1041$). In this comparison, patients who received cells from unrelated donors were usually treated on more intensive immunosuppressive protocols. Regimens employing TBI greater than 1200 cGy have been shown to be associated with significantly more severe regimen-related toxicities including renal and hepatic toxicity and multiorgan failure than less intensive preparative regimens.^{24,25} Miralbell *et al*²⁵ found that in their analysis of 84 patients with malignant hematologic diseases receiving allogeneic BMT, TBI dose and the presence of GVHD were significantly correlated with renal dysfunction following transplant.

In this study we attempted to increase immunosuppression by increasing the total dose of cyclophosphamide over 4 days while maintaining a total TBI dose of 1400 cGy. Major treatment-related toxicity was primarily renal with 26 (61%) patients experiencing a doubling of their baseline serum creatinine during the study period, requiring a FK506 dose adjustment. Once the FK506 dose was reduced or held, renal function generally improved. It is extremely difficult to assess toxicity related to only the preparative regimen, especially renal toxicity, since patients receive multiple nephrotoxic drugs during their post-transplant period. In this study, the median peak creatinine was also found to be significantly higher in those patients who also received amphotericin B. Hepatic VOD was seen in 16% of patients and severe mucositis, which required withholding the day +11 MTX dose, in 17% of the patients. It is important to note that renal toxicity, the occurrence of hepatic VOD, and the development of severe mucositis were more frequently present in those patients with high-risk disease who have generally been exposed to more aggressive treatment approaches prior to transplant. The 17 patients with low-risk disease tolerated this preparative regimen with acceptable levels of toxicity. In the future, patients with high-risk disease or those patients who have been heavily pretreated should receive a less intensive regimen, perhaps by reducing the total dose of fractionated TBI to 1200 cGy.

Myeloid and platelet recovery was reasonable and donor engraftment was complete by 90 days in all patients who could be evaluated. Evaluation of the marrow or peripheral blood of the 29 patients who had cytogenetic and/or heteromorphism studies performed, demonstrated 100% donor

origin. The anti-leukemic response of this regimen was also reasonable; all patients with disease prior to BMT entered a complete remission, and there have been no relapses in patients with low-risk disease following BMT.

In summary, a preparative regimen of cyclophosphamide, total dose 200 mg/kg over 4 days, and fractionated TBI, total dose of 1400 cGy over 4 days, is an acceptable preparative regimen in patients receiving an allograft from unrelated HLA-compatible volunteer donors. The increased immunosuppression was well tolerated in patients with low-risk disease allowing for complete engraftment and successful anti-leukemic benefit. In patients with high-risk disease, even though engraftment was acceptable and patients entered complete remission and/or responded to the therapy, toxicity appeared to be increased, probably related to the extensive treatment prior to the transplant.

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