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Successful unrelated marrow transplantation for patients over the age of 40 with chronic myelogenous leukemia

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ABSTRACT

Some older patients (≥40 years) with chronic myelogenous leukemia (CML) who lack human leukocyte antigen (HLA)-identical sibling donors are not offered unrelated marrow transplantation because of concerns over excessive regimen-related toxicity, in particular due to graft-vs.-host disease (GVHD). The purpose of this study was to determine the efficacy and toxicity of unrelated marrow transplantation in older CML patients using a regimen designed to minimize the severity of GVHD. Thirty-one consecutive patients over the age of 40 with CML received unrelated marrow transplants between January 1988 and June 1997. Twenty-one patients were transplanted in chronic phase while ten were transplanted in the accelerated phase of their disease. Fifteen patients received transplants from phenotypically matched donors while 16 received marrow grafts from donors who were mismatched at one HLA locus. GVHD prophylaxis consisted of *ex vivo* T cell depletion of the donor marrow graft plus posttransplant cyclosporine administration. Durable engraftment was achieved in 29 of 31 patients (94%). The probability of developing grades II–IV or severe grades III–IV acute GVHD was 39.2 and 7.1%, respectively. There was no difference in the incidence of grades II–IV acute GVHD between patients transplanted with marrow grafts from phenotypically matched (38.1%) vs. those transplanted from mismatched unrelated donors (40%, $p = 0.99$). The 2-year probability of relapse for the entire population was 29.4%. Relapse was significantly higher for patients transplanted in accelerated phase (60%) than for those in chronic phase (13.8%, $p = 0.027$). The 2-year probability of overall survival and disease-free survival for the entire cohort was 56 and 45%, respectively. There was no significant difference in survival or disease-free survival for patients receiving phenotypically matched vs. mismatched marrow grafts. Immunological reconstitution for this cohort was compared with a younger (<40 years) patient population that had been similarly transplanted over the same time period. Immune function as assessed by total T cell, B cell, NK cell, and T cell subset reconstitution posttransplant was quantitatively equivalent in the two groups with most parameters normalizing within 18 months of transplant. We conclude that CML patients over the age of 40 who have either phenotypically matched or one antigen-mismatched unrelated donors can successfully undergo allogeneic marrow transplantation. T cell depletion of the marrow graft may be advantageous in these older patients by reducing GVHD severity, particularly in those patients transplanted with HLA-disparate marrow grafts.

KEY WORDS

Leukemia • Bone marrow transplantation • Unrelated donors

INTRODUCTION

Allogeneic bone marrow transplantation (BMT) is the only proven curative therapy for patients with chronic myelogenous leukemia (CML). Transplantation with marrow grafts from human leukocyte antigen (HLA)-identical sibling donors has resulted in long-term disease-free survival rates of 45–80% for patients in chronic phase and 10–50% for patients transplanted in the more advanced phases of the disease [1–4]. Most CML patients who are potential candidates for allogeneic BMT, however, lack an HLA-identical sibling.

Table 1. *Patient characteristics*

Sex (M/F)	**20/11**
Age (years)	
Median	**45**
Range	**40–53**
Disease status	
Chronic phase	**21**
Diagnosis to BMT <1 year	**7**
Diagnosis to BMT 1–2 years	**6**
Diagnosis to BMT ≥2 years	**8**
Accelerated phase	**10**
Donor/recipient histocompatibility	
Matched	**15**
Mismatched	**16**
A locus	**11**
B locus	**2**
DR locus	**1**
DQ locus	**2**
Recipient CMV serostatus	
Seropositive	**16**
Seronegative	**15**

For many of these patients, unrelated marrow transplantation has emerged as a viable therapeutic option, albeit at the expense of increased transplant-related mortality due to higher rates of graft rejection, more severe graft-vs.-host disease (GVHD), and an increased frequency of opportunistic infections [5–7]. These transplant-related complications have been particularly problematic for older patients (>40 years) undergoing unrelated marrow transplantation [5]. Because the incidence of CML increases with advancing age, the risk of these complications affect a substantial portion of potential candidates for unrelated BMT. Consequently, alternative approaches, such as interferon therapy or autologous BMT are often initially considered in these patients by some centers and unrelated marrow transplantation either not performed or deferred until patients enter the more advanced phases of CML [8,9]. This is particularly true for patients who lack phenotypically matched donors and have only mismatched unrelated donors from whom to receive marrow grafts. In an effort to make unrelated marrow transplantation more effective in this older patient population, we have designed a transplant regimen to reduce the regimen-related toxicity of unrelated marrow transplantation by using an intensive preparative regimen to facilitate durable engraftment along with T cell depletion of the donor graft to reduce the severity of GVHD. In this study, we present our results using this approach in 31 patients over the age of 40 with CML who underwent allogeneic marrow transplantation from both phenotypically matched and mismatched unrelated marrow donors.

MATERIALS AND METHODS

Patient population

The study population consisted of 31 consecutive patients over the age of 40 with CML who received bone marrow transplants from unrelated donors between January 1, 1988, and June 5, 1997, at the Medical College of Wisconsin. Patient disease status and other demographic data are shown in Table 1. CML was classified as being in accelerated phase according to previously published criteria [10]. Informed consent was obtained from each patient (or their guardians) and all treatment was administered under protocols approved by the institutional review committees of the Medical College of Wisconsin.

Donor selection and histocompatibility testing

Each recipient and donor candidate underwent extended serotyping for HLA-A, -B, -DR, and -DQ by standard microcytotoxicity assays. Beginning in February 1989, all recipients and donors who were judged suitably matched, based on screening serologic assays, underwent prospective oligotyping of HLA-DRB1 [11]. Prospective oligotyping for HLA-DQB1 was begun in January 1990, as previously described [12]. Donor/recipient pairs from transplants performed before these dates were retrospectively analyzed so that the degree of HLA-DR and -DQ compatibility for the entire patient population was evaluable. Additional typing information was available on 15 donor/recipient pairs from the retrospective assessment of HLA-A and HLA-B by one-dimensional isoelectric focusing (IEF [13]) or from solid-phase DNA sequencing of class I (HLA-A and -B) or class II (HLA-DR and -DQ) antigens [14,15]. Specifically, eight patients had class I disparity assessed by IEF, while in two additional donor/recipient pairs DNA sequencing of HLA-A and B alleles was performed. Four patients had HLA disparity evaluated by DNA sequencing of both class I and II alleles in patient and donor, while the remaining patient had class I and II disparity assessed by IEF and DNA sequencing (class II only). Any donor/recipient disparity that was detectable by serology, molecular typing, IEF, or DNA sequencing was considered a mismatch in this study. Disparity was categorized based on the highest level of typing information known at the time of analysis. A greater percentage of accelerated-phase patients (7 of 10 [70%]) received mismatched marrow grafts than did chronic-phase patients (9 of 21 [43%]).

Preparative regimen, GVHD prophylaxis, and supportive care

All patients were treated in laminar air flow or HEPA-filtered rooms. Pretransplant conditioning consisted of a regimen of high-dose cytosine arabinoside (3 gm/m^2 × 6, days –7 to –4), cyclophosphamide (45 mg/kg × 2, days –6 and –5), methylprednisolone (1 gm/m^2 × 4, days –2 to 0) followed by fractionated total-body irradiation to a total dose of either 13.32 or 14 Gy (days –2 to 0) [10,16]. GVHD prophylaxis consisted of *ex vivo* T cell depletion with the αβ T cell receptor antibody $T_{10}B_9$ plus posttransplant cyclosporine [17]. All patients received prophylactic acyclovir and trimethoprim-sulfamethoxazole both before and after transplantation. Cytomegalovirus (CMV)-seronegative patients received blood components from CMV-seronegative donors. Twenty-four of the 31 patients received growth factor therapy with granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), or combined G-CSF and GM-CSF beginning on the day of transplant to accelerate myeloid engraftment.

Assessment of engraftment, GVHD, and relapse

The date of engraftment was defined as the first of 3 consecutive days in which the absolute neutrophil count (ANC) was $\geq 500/mm^3$. Trilineage engraftment was documented by bone marrow examination in the majority of patients 3–4 weeks after transplant. Follow-up marrow studies were done 100 days, 6 months, 1 year, and at least yearly after transplantation to evaluate engraftment and relapse. Durable engraftment was confirmed by cytogenetic analysis, restriction fragment length polymorphism studies, or analysis of a variable number of tandem repeats in blood or marrow samples to distinguish donor from recipient cells. Acute GVHD was graded as 0–IV according to criteria of Glucksberg *et al*. [18], whereas chronic GVHD was defined as none, limited, or extensive [19]. Patients who had evidence of engraftment were evaluable for acute GVHD, while patients who engrafted and also survived more than 100 days were evaluable for chronic GVHD. Relapse was defined by either morphologic evidence of leukemia in the peripheral blood, marrow, or extramedullary sites or by the recurrence and sustained presence of the Philadelphia chromosome (Ph^+). Patients whose sole evidence of disease was positivity for the *bcr/abl* RNA transcipt by the polymerase chain reaction (PCR) were not classified as having relapsed.

PCR assay for detection of minimal residual disease

Total cellular RNA was prepared from peripheral blood, peripheral blood buffy coats, or peripheral blood mononuclear cells isolated by Ficoll-Hypaque (Pel-Freez, Mequon, WI), bone marrow, or cultured B cells and K562 (Ph^+) cells. Based on previous data demonstrating that both are equally sensitive for detecting residual disease in CML patients [20], peripheral blood and marrow cells were used interchangeably. Cells were either viably frozen or directly added to 4 M guanidinium isothiocyanate. RNA was prepared from 3 to 5 million cells. The conditions used for extraction of RNA and PCR amplification have been previously detailed [21,22]. All assays were performed in duplicate, beginning with independent RNA isolations. Mock RNA preparations were used as negative controls in every assay. The criteria used for defining positive assay results have been previously published [22]. Assay sensitivity was monitored through the addition and detection of 0.05 ng RNA from the Ph^+ cell line K562, mixed within a replicate assay for each unknown sample. This quantity of RNA represents nucleic acid isolated from approximately five K562 cells, which requires two rounds of nested primer PCR for detection.

Immune reconstitution studies

Samples were obtained for immune phenotyping and proliferative response to T cell and B cell mitogens at 8 targeted posttransplant time points. Sampling periods included days 30 and 100; 6, 12, and 18 months; and 2, 3, and 4 years. If a patient was sampled multiple times during an interval, only the data from the sample nearest to the target time point was used for analysis. Data were available from 25 of the 31 patients in this study. These data were compared with those from 36 patients <40 years of age with CML who were similarly transplanted and tested over the same time period.

Lymphocyte subsets were measured by two-color direct immunophenotyping of ethylene diamine tetraacetic acid anti-coagulated whole blood samples. Antibodies that were directly conjugated to phycoerythrin or fluoroscein isothiocyanate were obtained from Coulter Immunology (Hialeah, FL) or Becton Dickinson (Mountain View, CA). The panel included antibodies to CD45, CD14, CD3, CD4, CD8, CD56, and CD20, with the appropriate isotype controls. CD8-positive T cells were defined based on co-expression of CD3 or T cell receptor-$\alpha\beta$. Either Q-Prep (Coulter Immunology) or FACS lysis buffer (Becton Dickinson) was used to lyse red blood cells after antibody staining. Within 24 hours of staining, cells were analyzed using an Epics Profile II (Coulter Immunology) or a FACSCalibur flow cytometer (Becton Dickinson). A debris-free lymphocyte gate that was based on forward- and side-angle light scatter characteristics was established, and the proportion of stained cells within the gate for each subset measured. Lymphocyte purity was determined using CD45 and CD14. Subset percentages were corrected for the percent of $CD45^+$cells in the lymphocyte gate. Data were expressed as the absolute number of the indicated subset calculated from a white blood cell count (WBC) and differential performed on the day of sampling as follows:

$$\text{Absolute cells per mm}^3 = \text{\% of subset in lymphocyte-gated cells} \times \text{WBC} \times \text{\% of lymphocytes}$$

Proliferation of mononuclear cells that were isolated from heparinized peripheral blood to multiple dilutions of the mitogens phytohemagglutinin-purified (PHA-P; Difco, Detroit, MI), concanavalin A (ConA; Aldrich Chemicals, Milwaukee, WI), and pokeweed mitogen (PWM; Gibco, Grand Island, NY) was determined. Briefly, lymphocytes were suspended in RPMI 1640 with 25 μM Hepes (Gibco) and further supplemented with penicillin/streptomycin and L-glutamine (Gibco) and 10% heat-inactivated, human AB serum (locally produced from heparinized plasma). Mitogen-stimulated cells were plated in triplicate at 10^5 cells per well in flat-bottomed 96-well plates, incubated for 72 hours in a well-humidified atmosphere of 5% CO_2, and pulsed for the final 5 hours of culture with 1 μCi ^{3}H-thymidine (New England Nuclear, Boston, MA) per well. The cells were harvested onto glass fiber filters and their radioactivity measured in a liquid scintillation counter (Packard Instruments, Meriden, CT). Data were expressed as the average counts per minute (cpm) of triplicate cultures minus the average cpm of cultures incubated with medium alone. Mitogen concentrations yielding the maximal proliferative response were reported. Two normal control samples were tested with each mitogen assay.

Statistical analysis

Endpoints were calculated at the date of last contact, with a latest follow-up date of July 5, 1997. The median duration of follow-up for the patient population was 51 months (range, 1–87 months). Cumulative actuarial probabilities of relapse, disease-free survival, overall survival, and acute GVHD were calculated using the Kaplan-Meier method [23]. Patients were censored at the time of death in the analysis of acute GVHD. One patient who had graft rejection was censored at the time of second transplant. A second patient who rejected the marrow graft and had autologous reconstitution with return of CML was also classified

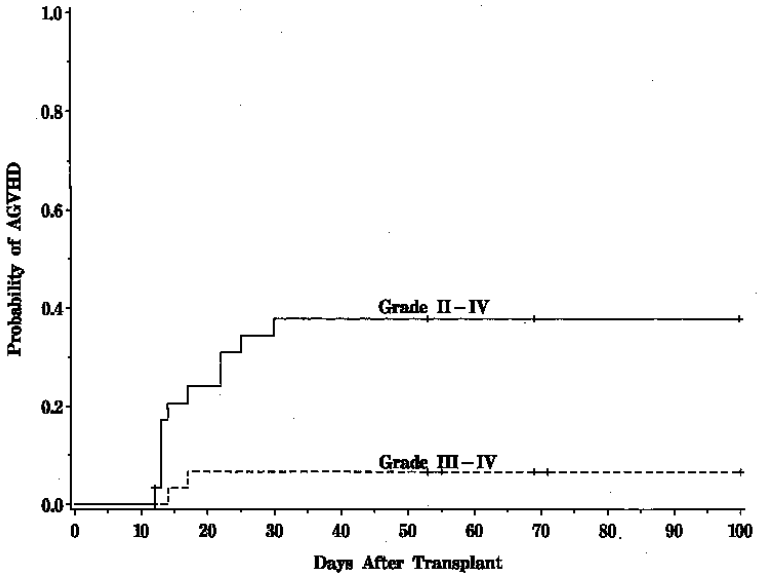

Figure 1. Actuarial probability of developing grades II–IV or grades III–IV acute GVHD for the entire patient population
Tick marks represent patients who died before developing either ≥ grade II GVHD or ≥ grade III GVHD, respectively.

as having relapsed. Confidence limits for the Kaplan-Meier estimate were based on the arcsine transformation, using Greenwood's formula for the standard error of their survival estimator [24]. Comparisons of time-to-event distributions were made using the logrank test and a significance level of 0.05. Immune function parameters were compared using an independent two-tailed Student's *t*-test. $p \leq 0.01$ was considered significant in immune function assays.

RESULTS

Engraftment

The mean bone marrow inoculum administered after T cell depletion was $8.6 \pm 4.8 \times 10^7$ cells/kg. Limiting dilution assays were performed in 23 of 31 patients to determine the degree of T cell depletion. In these patients, the mean log T cell depletion was 1.6 ± 0.3 and the mean number of administered T cells was $4.4 \pm 6.2 \times 10^5$/kg. All 31 patients were evaluable for engraftment. Durable engraftment was achieved in 29 of 31 patients (94%). The median time to an ANC $\geq 500/mm^3$ for 3 consecutive days was 15 days (range, 9–23 days) in engrafting patients. Two patients had graft rejection. One patient rejected after having received a marrow graft from a phenotypically matched donor, whereas the second patient rejected after transplantation with a graft from a donor who was mismatched at one HLA locus (HLA-B).

GVHD

Twenty-nine of 31 patients were evaluable for the development of acute GVHD. The probability of developing grade II–IV acute GVHD was 39.2% (95% confidence interval [CI] = 23.2–54.9), and the probability of grade III–IV acute GVHD was 7.1% (95% CI = 5.7–8.9) in this patient population (Fig. 1). There was no difference in the incidence of grade II–IV acute GVHD between patients transplanted with marrow grafts from phenotypically matched donors (38.1%) vs. those transplanted from mismatched unrelated donors (40%, $p = 0.99$) (Fig. 2). Twenty-three of 31 patients survived until day 100 and were evaluable for chronic GVHD. Limited chronic GVHD occurred in 12 patients; extensive chronic GVHD developed in four patients. Seven patients had no evidence of chronic GVHD. The overall probability of developing extensive chronic GVHD in this patient population was 18.3% (95% CI = 12–25.6).

Relapse

The 2-year probability of relapse for the entire cohort was 29.4% (95% CI = 16.7–43.3) (Fig. 3). Five patients in accelerated phase have relapsed while three patients in chronic phase developed disease recurrence. One of the latter patients who had rejection of the marrow graft subsequently had autologous reconstitution with return of CML. There was a significantly higher likelihood of relapse for patients transplanted in accelerated phase (60%) than for those in chronic phase (13.8%, $p = 0.027$). Two patients who relapsed in accelerated phase were treated with donor lymphocyte infusions. One patient failed to respond and died of recurrent disease; the second patient is currently in remission. One patient who relapsed after undergoing transplantation for chronic-phase disease is currently receiving therapy with donor lymphocyte infusions but has not yet responded. There was no difference in relapse rates for patients transplanted with matched (27%) vs. mismatched (36%) marrow grafts ($p = 0.92$).

All of the 14 patients who were alive and in clinical remission had samples of either bone marrow or peripheral blood tested by PCR for the presence of the *bcr/abl* RNA transcript. PCR testing was performed to assess the presence of minimal residual disease in patients who were otherwise in remission. In 13 of 14 patients, this assay was performed within 6 months of the date of last contact. Twelve of these 14 patients were documented to be negative for *bcr/abl* RNA. One of these patients

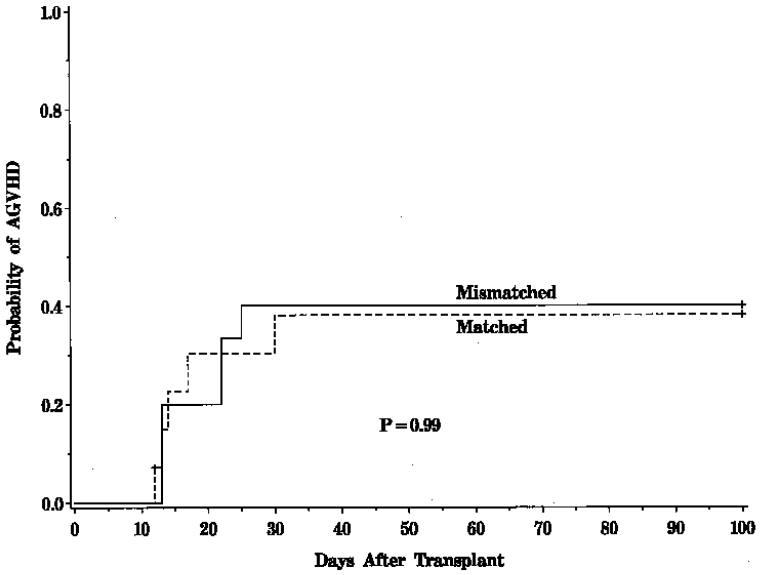

Figure 2. Actuarial probability of developing grades II–IV acute GVHD for patients transplanted with matched or mismatched marrow grafts
Tick marks represent patients who died before developing ≥ grade II acute GHVD.

was in remission after the administration of donor lymphocyte infusions for treatment of relapsed disease. The remaining two patients tested were found to be positive for *bcr/abl* RNA after two rounds of nested primer PCR but have not yet demonstrated any clinical evidence of relapse.

Survival

The 2-year probability of survival was 56% (95% CI = 29.2–75.9) for the entire cohort of patients. Overall survival for patients transplanted in accelerated phase (57.1%) was similar to that for patients transplanted in chronic phase (54.5%, $p = 0.81$). While survival for patients receiving phenotypically matched marrow grafts was higher (70%) when compared with patients receiving mismatched marrow grafts (42%), this difference was not significant ($p = 0.29$) (Fig. 4). Two year disease-free survival was 45% (95% CI = 25.2–62.8) for the entire study population. Disease-free survival at two years was 50 and 28.6% for patients transplanted in chronic

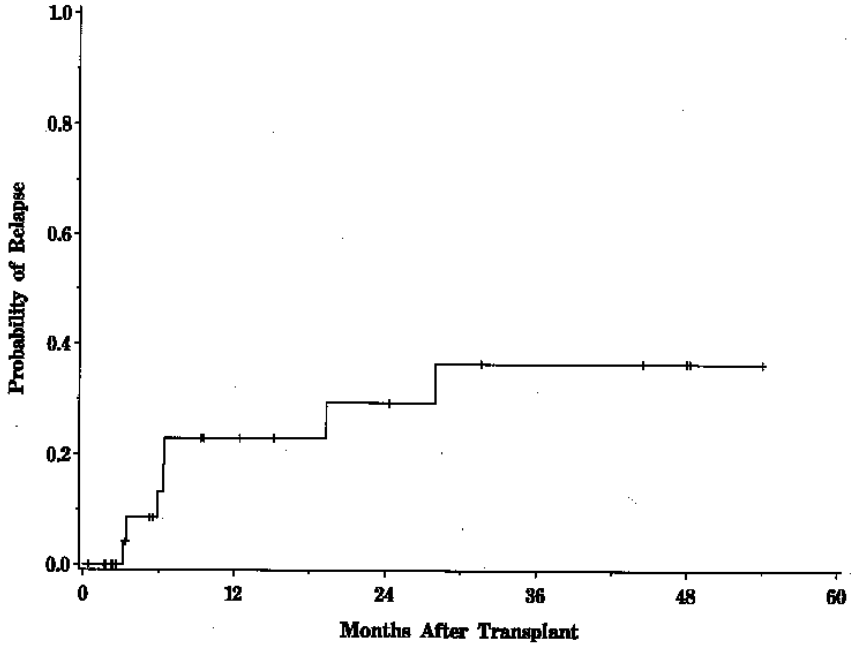

Figure 3. Actuarial probability of leukemia relapse for the entire patient population
Tick marks represent patients currently in continuous complete remission.

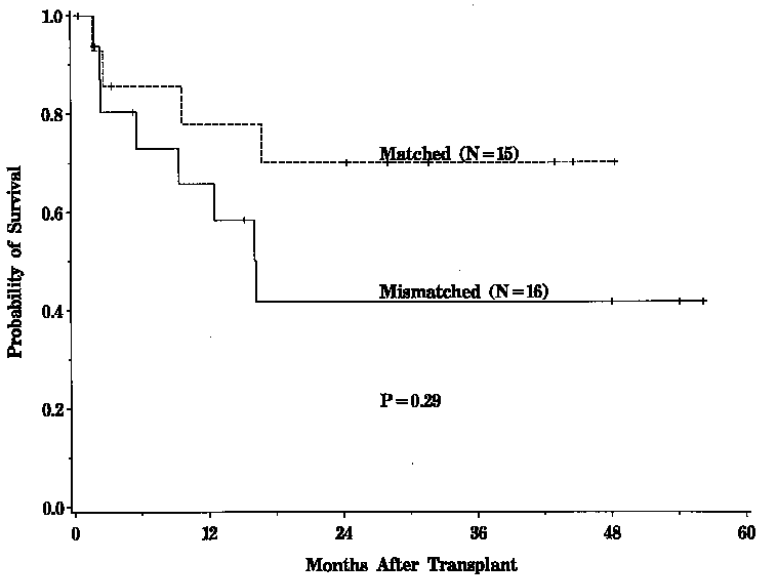

Figure 4. Actuarial probability of survival for patients transplanted with either matched or mismatched marrow grafts
Tick marks represent patients currently alive.

phase and accelerated phase, respectively (p = 0.47). When comparing patient populations as to whether they received phenotypically matched or mismatched marrow grafts, disease-free survival rates were 55 vs. 35%, respectively (p = 0.41) (Fig. 5). The performance status of surviving patients is shown in Table 2.

Cause of death

Infection was the major cause of death (Table 3). Two patients died of disseminated aspergillosis, one from CMV, and two from bacterial infections (*Pseudomonas bacteremia* and staphylococcal pneumonia). The other primary cause of death was disease relapse (four patients). No patient died directly from GVHD; however, all patients who succumbed to infection had a history of GVHD and were on immunosuppressive medications at the time of death. Thus, GVHD was a likely contributory factor to death in these patients. Death due to pulmonary hemorrhage, interstitial pneumonitis, veno-occlusive disease, and adult respiratory distress syndrome occurred in one patient each.

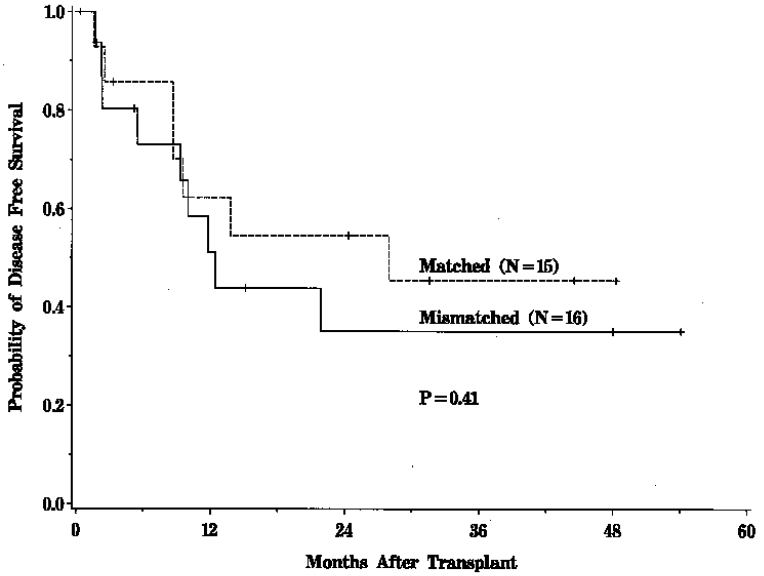

Figure 5. Actuarial probability of disease-free survival for patients transplanted with marrow grafts from either phenotypically matched or mismatched donors
Tick marks represent patients currently alive.

Table 2. *Karnokfsky performance status of surviving patients*

Score	Number of patients (%)
100	6 (35)
90	6 (35)
80	4 (24)
≤70	1 (6)

Table 3. *Causes of death*

	Number of occurrences
Graft rejection	1
Relapse	4
Infection	5
Aspergillus	2
Cytomegalovirus	1
Bacterial	2
Pulmonary hemorrhage	1
Interstitial pneumonitis	1
Veno-occlusive disease	1
Acute respiratory distress syndrome	1

Immunological reconstitution

Immune reconstitution data was available for 25 of the >40-year-old patients with CML. These results were compared with those from 36 patients with CML who received unrelated donor marrow transplanted over the same time period. Not all patients were tested at every time point, which resulted in similar numbers during each testing interval of patients over and under 40 years of age. The data shown in Figure 6 represent the median value at each of the indicated intervals of the patients tested. Total number of $CD3^+$ T cells as well as the $CD4^+$ and $CD8^+$ T cell subsets tended to be fewer in the older patient group; however, these differences were not significant at any of the intervals tested. T cells recovered to the lower limits of normal by 12–18 months with a slightly faster recovery of $CD8^+$ T cells compared with $CD4^+$ T cells. $CD56^+$ NK cells recovered quickly in both groups and stayed at normal levels, and B cell numbers normalized at 1 year and overshot the normal range during subsequent testing intervals. The functional activity of T cells was measured by assessing the proliferative response to T cell mitogens (PHA and ConA) and to a T and B cell mitogen (PWM). The results were similar for all three stimuli in that a low normal proliferative response required 18 or more months to recover (PHA data shown in Fig. 6; ConA and PWM data are qualitatively the same as PHA data and are not shown). Results were not significantly different between the two age groups.

DISCUSSION

The major obstacle to successful unrelated marrow transplantation is the increased risk of transplant-related mortality when compared with HLA-identical sibling BMT. Transplant-related mortality has been shown to correlate with increasing recipient age, signifying that older recipients are at highest risk from regimen-related toxicity [5,25]. For these patients to benefit from unrelated BMT, therefore, reductions in the incidence of graft rejection and severe GVHD are necessary. The aim of this study was to determine whether this goal could be accomplished using a transplant regimen that was designed to mitigate the severity of GVHD without compromising alloengraftment.

The results of this study demonstrate that unrelated marrow transplantation can be effective therapy for CML patients over the age of 40. Overall survival and disease-free survival at 2 years in this older cohort of patients were approximately 55 and 45%, respectively. These data compare favorably with previously published reports on unrelated BMT for CML. For example, a recent analysis from the National Marrow Donor Program reported a 3-year disease-free survival of 40% for patients of all ages who were transplanted in chronic phase [26]. In this study, older patients had significantly worse outcomes. Furthermore, a multinational European study recently reported 2–3-year disease-free survival rates of 55% for pediatric patients transplanted in the chronic phase of CML [27]. The largest single center analysis by Anasetti *et al.* [7], which comprised both adult and pediatric patients, documented 2.5 year disease-free survival rates of 52 and 42% for patients transplanted in chronic and accelerated phase, respectively. Thus, older patients in the current study did not appear to do significantly worse than younger patients.

While selection of patients with favorable prognostic features can be responsible for encouraging results, that was not a plausible explanation in this study. Rather, most of the patients presented herein had other adverse prognostic characteristics in addition to advanced age. For example, the majority of chronic-phase patients were transplanted more than 1 year after diagnosis, which has been associated with reduced survival when compared with patients transplanted within 1 year of diagnosis [1,3,5]. Furthermore, approximately 50% of the patients in this study received marrow grafts from one antigen-mismatched donors, which has been shown to result in a significantly increased incidence of severe acute GVHD, chronic GVHD, and treatment-related mortality when compared with transplantation with phenotypically matched unrelated marrow grafts [28–30]. When all these factors are considered, we believe it is unlikely that the observed results could be ascribed to favorable patient selection.

Although there was no statistical difference in survival between patients transplanted with matched vs. mismatched marrow grafts, several points deserve emphasis. First, the number of patients in this series was small, and therefore the lack of difference may have been obscured by the small sample size. Second, more patients who received mismatched marrow grafts had accelerated-phase disease (7 of 16 vs. 3 of 15) and were therefore at higher risk for adverse outcome. Third, it is likely that as higher resolution retrospective DNA sequencing is performed on HLA class I and II alleles, additional HLA disparities will be revealed, which may magnify the survival differences between the two groups. To that end, incorporation of the four additional donor/recipient disparities revealed by IEF or DNA sequencing resulted in an improvement in 2-year disease-free survival from 45 to

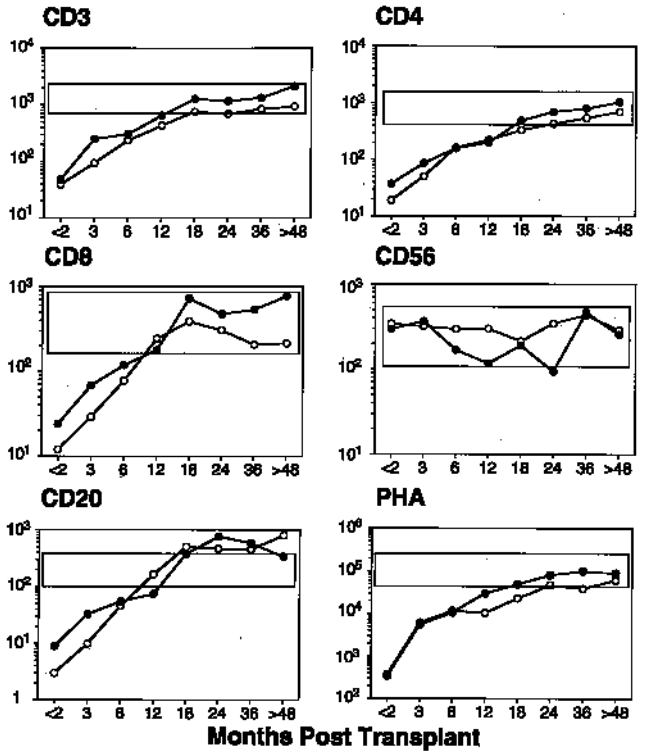

Figure 6. Immune reconstitution after unrelated donor transplantation for CML

Shown are data from 25 patients >40 years (○) and 36 patients <40 years (●), transplanted with T cell-depleted marrow from an unrelated donor for the treatment of CML. If a patient was sampled more than once during the indicated interval, only the sample closest to the target time point was included. Not all patients were tested at each target time point. Data are presented as the median cells per mm^3 for the indicated lymphocyte subset and the median counts per minute of ^{3}H-thymidine for cultures stimulated with PHA. The 5th and 95th percentile of simultaneously tested normal control individuals are shown as the open rectangles. The number of patients sampled during each interval for patients <40 and >40, respectively, were: <2 months, 16 and 22; 3 months, 21 and 22; 6 months, 18 and 17; 12 months, 15 and 15; 18 months, 9 and 7; 24 months, 9 and 6; 36 months, 7 and 7; 48 months, 5 and 5.

55% in patients transplanted with phenotypically matched marrow grafts and a decline of 45 to 35% in patients who received mismatched grafts. Thus, these data would lead one to predict that more definitive HLA typing will lead to a further demarcation of these patient cohorts.

The major cause of treatment-related mortality in unrelated BMT is GVHD. Conventional prophylaxis for GVHD in unmodified grafts has typically been with methotrexate and cyclosporine. With this regimen, severe grades III–IV acute GVHD have been reported to occur in approximately 50% of patients transplanted with phenotypically matched grafts and in 70% of recipients transplanted with one antigen-mismatched marrow grafts [28]. To ameliorate the severity of GVHD, we used *ex vivo* T cell depletion, which resulted in a substantial reduction in the incidence and severity of GVHD. The reduced incidence in GVHD due to T cell depletion, however, was not counterbalanced by a marked increase in graft failure. Graft rejection occurred in only 6% of patients, and the remaining patients had durable engraftment with no instances of late graft failure. Marrow graft rejection has been shown to be increased in unrelated marrow transplantation for CML when compared with that observed in HLA-identical sibling BMT [31]. Registry analyses have noted a further increase in non-engraftment when patients are transplanted with T cell-depleted marrow grafts [32]. This is thought to be due to radioresistant host T cells that survive the conditioning regimen and are then capable of rejecting the graft [33]. To overcome graft resistance, we used a relatively intensive preparative regimen that was based on prior clinical studies and animal models, which have shown that graft resistance can be overcome by augmenting the intensity of the conditioning regimen [34,35]. This resulted in an incidence of graft rejection that was somewhat higher than but still comparable with that observed in patients receiving unmodified grafts from mismatched related and unrelated donors [28].

While there was an approximate 30% 1-year treatment-related mortality in this patient population, this rate was not appreciably different from that observed in non–age-selected or younger patient populations [5,7]. The major cause of death was due to infection similar to that observed in other series of unrelated BMT. All patients with infection in this study were on immunosuppressive medications for the treatment of GVHD, thus implicating GVHD as having a contributory role in patient death. Relapse was the other major cause of mortality and occurred with higher frequency than noted in recipients of non–T cell-depleted grafts [26]. Disease relapse, however, was much lower in chronic-phase as opposed to accelerated-phase patients in whom there appeared to be relative preservation of an antileukemic effect [22]. That the vast majority of chronic-phase patients who were in clinical remission were also in molecular remission supports this contention. Based on our previous observation that PCR testing is highly predictive for subsequent relapse in these patients [36], we predict that the majority of these patients would remain in remission. For accelerated-phase patients, additional strategies such as posttransplant cellular immunotherapy [37,38] may be warranted to reduce the higher relapse rate. The use of mismatched marrow grafts did not confer an additional graft-vs.-leukemia effect, but patient numbers may not have been sufficient to detect such a difference.

Successful outcomes after unrelated BMT are contingent on effective immunological reconstitution, which is necessary to prevent the development of opportunistic infections. Since older age has been associated with delayed recovery of immune function [39], we compared parameters of immune reconstitution between the present cohort and a group of similarly transplanted younger patients with CML. We observed no significant differences between these two groups, indicating that transplantation of older patients did not result in a further delay in immune recovery. Thus, although T cell depletion has been associated with a delay in immune reconstitution [40], the use of T cell depletion in these older patients did not preclude the normalization of these parameters, nor did the tempo lag behind that observed in younger patients.

In summary, we conclude from these data that patients between the ages of 40 and 50 need not be *a priori* excluded

from receiving unrelated marrow grafts because of concern that they will have excessive transplant-related toxicity. While regimen-related mortality is higher than in HLA-identical sibling marrow transplantation, patients who lack HLA-identical sibling donors can be considered suitable candidates for unrelated marrow transplantation, and a sizable portion can be expected to have long-term survival. The use of T cell depletion allowed for a reduction in the severity of GVHD and may be advantageous in older patients, particularly those transplanted with HLA-disparate marrow grafts.

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