Allogeneic stem cell transplantation (ASCT) has been widely used in the treatment of hematologic malignancies. High-dose myeloablative chemotherapy followed by allogeneic bone marrow or peripheral blood stem cell transplantation has successfully treated many otherwise incurable patients [1,2]. However, regimen-related toxicities (RRTs) can be severe and sometimes fatal [3-6]. ASCT in patients

Phase II Study of a Moderate-Intensity Preparative Regimen with Allogeneic Peripheral Blood Stem Cell Transplantation for Hematologic Diseases: The Texas Transplant Consortium Experience


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ABSTRACT

Conventional preparative regimens for allogeneic stem cell transplantation are associated with excessive regimen-related toxicity (RRT) in some patients because of underlying comorbidities, advanced age, or prior treatment. We studied a preparative regimen designed to reduce RRT, yet allow for adequate engraftment and development of a graft-versus-malignancy effect. Thirty patients (median age, 57 years) were entered on study. Twenty-nine patients received stem cells from HLA-identical siblings and 1 from a sibling mismatched for 1 antigen at the A locus. Sixteen patients had received previous stem cell transplants (6 allogeneic and 10 autologous). The preparative regimen consisted of fludarabine 30 mg/m² per day IV on day –10 to day –5, busulfan 1 mg/kg per dose PO (n = 6) or 0.8 mg/kg per dose IV (n = 24) for 8 doses every 6 hours on day –6 to day –5, and horse-derived antithymocyte globulin 5 mg/kg per day IV (n = 12) or 15 mg/kg per day IV (n = 18) on day –4 to day –1. GVHD prophylaxis consisted of cyclosporine (CYA) 3 mg/kg BID PO starting on day –3 (n = 13) or CYA and methotrexate 15 mg/m² IV on day +1 and 10 mg/m² IV on day +3 and day +6 (n = 17). The median number of CD34⁺ cells transplanted was 3.19 × 10⁶/kg. All patients demonstrated recovery of hematopoietic function. Twenty-six (89%) of 29 evaluable patients achieved greater than 90% donor cell chimerism before day 100. Three patients never achieved greater than 90% donor chimerism, and another 3 patients subsequently lost donor chimerism. All 6 of these patients had autologous reconstitution with progressive disease. RRT was minimal; 7 patients had greater than grade II nonhematologic toxicity and there were no toxic deaths attributable to the conditioning regimen. Transplantation-related mortality was 7% (95% confidence interval [CI], 6%-8%) at 3 months and 28% (95% CI, 23%-34%) at 12 months after transplantation. Non-relapse-related mortality was most often due to infection. Grade II or greater GVHD developed in 56% of evaluable patients, and all patients with disease response developed GVHD. Actuarial estimates of overall and disease-free survival at 12 months were 52% (95% CI, 43%-63%) and 30% (95% CI, 24%-37%), respectively. Although this preparative regimen allowed adequate engraftment with minimal RRT, GVHD and infectious complications caused significant morbidity and mortality. Further study to define appropriate patient populations for this regimen, while limiting GVHD and infection risks, is needed.

KEY WORDS

Allogeneic stem cell transplantation • Regimen-related toxicity • Graft-versus-malignancy • Reduced intensity preparative regimen
older than 45 years is associated with a higher incidence of graft-versus-host disease (GVHD), end organ damage, and decreased survival compared to younger patients [5-6]. Patients who receive an ASCT after previous high-dose chemotherapy may also have excessive morbidity and mortality [7,8]. RRT has generally limited the use of ASCT to younger patients with good performance status. A significant number of patients have diseases that may be amenable to treatment with ASCT but are at high risk for severe RRT because of underlying comorbidities.

Conditioning regimens for ASCT were initially designed to destroy the underlying malignancy and immunosuppress the host to allow donor engraftment and prevent graft rejection. Data from murine stem cell transplantation models suggested that some hematologic malignancies were not curable even with the most intensive and otherwise lethal preparative regimens [1]. More recently, it has been demonstrated that the antitumor effect of the transplanted cells may be a major factor in the success of ASCT in eradicating malignancy. Weiden et al. described the GVH and graft-versus-leukemia effect in 1979, demonstrating that the allograft might have a role in eradicating the malignant clone beyond just rescuing the hematopoietic function of the bone marrow [9]. The immunologic power of ASCT is further supported by the results of donor lymphocyte infusion (DLI) data in patients with relapsed leukemias after ASCT [10-18]. DLI can result in complete and sustained remissions of disease in some patients without the use of cytotoxic drugs. This graft-versus-leukemia effect, which may more broadly be referred to as a graft-versus-malignancy (GVM) effect, may also be active, although to a lesser extent, in multiple myeloma [19-22], non-Hodgkin’s lymphoma [23], and renal cell carcinoma [24]. The curative effect of ASCT may, therefore, be a result of the GVM effect rather than the cytotoxic properties of the conditioning regimen. Decreasing the cytotoxicity and maximizing the immunosuppressive effects of the preparative regimen may allow for adequate engraftment and development of the GVM effect, while ameliorating RRT.

Studies of reduced-dose conditioning regimens and ASCT have been reported by several centers [25-41]. One of the first reports by Slavin et al. described a reduced-intensity preparative regimen for hematologic diseases that demonstrated acceptable engraftment rates, disease control, and survival, with decreased RRT [25]. Based on this experience we designed a similar phase II multi-institutional protocol to evaluate the feasibility of using a similar preparative regimen and ASCT in patients at high risk for RRT.

**MATERIALS AND METHODS**

**Patients and Donors**

Thirty patients were entered on study between June 1998 and May 2001. The protocol was conducted through the Texas Transplant Consortium, and all patients were recruited through the following member institutions in San Antonio, Texas: Wilford Hall Medical Center, University of Texas Health Science Center at San Antonio, Audie L. Murphy Veterans Administration Hospital, and the Texas Transplant Institute. Institutional review boards of participating centers approved protocols and informed consent docu-

ments. All study patients were judged not to be candidates for standard myeloablative preparative regimens because of comorbid illnesses, age greater than 55 years, prior high-dose chemotherapy, or combinations of the above. All patients gave informed consent.

All donors were HLA-identical siblings except one donor who had a mismatch with the recipient for 1 antigen at the A locus. Donor peripheral blood stem cells were collected by apheresis after 4 to 5 days of treatment with granulocyte colony-stimulating factor (GCSF) (5 to 10 µg/kg per day) with a goal of collecting 2 × 10^6 CD34+ cells per kg recipient weight.

**Preparative Regimen**

The preparative regimen consisted of fludarabine 30 mg/m² per day IV on day –10 to day –5, busulfan 1 mg/kg per dose orally (PO) (n = 6) or 0.8 mg/kg per dose intravenously (IV) (Busulfax, Orphan Biomedical, Minnetonka, MN) (n = 24) for 8 doses every 6 hours on day –6 to day –5, and horse-derived antithymocyte globulin (ATG) (ATGAM; UpJohn, Kalamazoo, MI) 5 mg/kg per day IV (n = 12) or 15 mg/kg per day IV (n = 18) on day –4 to day –1. GVHD prophylaxis consisted of cyclosporine (CYA) 3 mg/kg twice a day (BID) PO starting on day –3 (n = 13), or CYA and methotrexate (MTX) 15 mg/m² IV on day +1, and 10 mg/m² IV on day +3 and day +6 (n = 17). Intravenous busulfan was substituted for oral busulfan when it became available at study sites. The change in dose of equine ATG and the addition of MTX for GVHD prophylaxis as described above were made by protocol amendment because of grade III and IV acute GVHD observed in patients who received CYA alone.

**Supportive Care**

The conditioning regimen initially was designed to be administered in the outpatient clinic. Patients were hospitalized for administration of frequent IV medicines, neutropenic fever, inability to maintain adequate oral intake, or at the discretion of the attending physician. All patients seropositive for herpes simplex virus received acyclovir 400 mg PO 3 times a day (TID) beginning day –10 through engraftment and then 200 mg PO TID until day 60. Fluconazole 400 mg PO once daily was begun on day –10 and given through day 60. A fluoroquinolone was added when the absolute neutrophil count (ANC) fell below 1.0 × 10^9/L and was continued until first neutropenic fever, or the ANC recovered to greater than 0.5 × 10^9/L for 2 consecutive days with no other documented infection. Cytomegalovirus (CMV) reactivation was treated with ganciclovir 5 mg/kg IV BID for 7 days, then every day afterward until resolution of viremia. Trimethoprim-sulfamethoxazole was administered daily on day –10 to day –1 and then 2 or 3 times weekly after engraftment, or pentamidine by inhalation every 4 weeks was given for prophylaxis against *Pneumocystis carinii* infection. Phenytoin, 300 mg PO TID given on days –11 through –8 and then 300 mg PO once daily on day –7, was administered for antiseizure prophylaxis and then discontinued. GCSF 5 µg/kg subcutaneously daily (rounded to either 300 µg or 480 µg) starting on day +7 was given to 23 patients and continued until the ANC exceeded 1.5 × 10^9/L for 2 consecutive days or the ANC was greater than 3.0 × 10^9/L for 1 day.
Engraftment, Chimerism Analysis, and Donor Lymphocyte Infusion

Hematopoietic cell engraftment was defined as the first of 3 consecutive days on which an ANC greater than 0.5 × 10^9/L was achieved. All chimerism analysis was done on total peripheral blood nucleated cells. Red blood cells were lysed prior to DNA extraction. At Wilford Hall Medical Center, Audie L. Murphy Veterans Administration Hospital, and University of Texas Health Science Center in San Antonio, chimerism analysis was done by a quantitative assay that uses the polymerase chain reaction (PCR) to amplify polymorphic short tandem repeat loci. Primer pairs complementary to conserved sequences on either side of the variable length regions were used to amplify fragments that were of different lengths for different individuals. Primers were synthesized with a Cy5.5 label attached to the 5’ direction (forward) primer. The fragment sizes were measured by their migration distance in a sequencing gel and were compared to known alleles and to labeled molecular weight markers using Visible Genetics software (Toronto, Ontario, Canada).

Genotypes of the donor and recipient were assigned for each genetic locus by comparing PCR product sizes with commercially prepared size standards. In posttransplantation samples, the relative quantities of donor and recipient DNA were calculated from measurements of the areas under informative peaks. Whenever possible, postransplantation recipient samples were tested in parallel with a donor sample, a pretransplantation recipient sample, and known mixtures of the two. The mixtures were selected to approximate the expected results and to determine the level of sensitivity for detection for each test system. When recipient pretransplantation samples were not available, buccal mucosal cells were collected with the caveat that they frequently contain an admixture of donor cells in engrafted patients. Chimerism analysis at the Texas Transplant Institute was performed by Lab Corp (Laboratory Corporation of America, Burlington NC) using similar methods. Variable and short tandem repeat DNA regions were amplified and allele sizes were analyzed after electrophoresis in vertical gels and detected by silver staining. Allele sizes were compared with size standard ladders and previously tested standard samples.

Chimerism analysis of peripheral blood was done on days 28, 56, and 80. CYA was stopped immediately if there were signs of disease progression or there was less than 90% donor chimerism. CYA was tapered after the day 28 analysis if there was no evidence of acute GVHD. The dose was decreased by 25% in weekly intervals and discontinued by day 56. CYA could be re instituted or the dose increased if GVHD occurred. DLI was administered if there was less than 90% donor chimerism and CYA had been discontinued, or if there was evidence of disease progression and no active GVHD. Donor lymphocytes were collected without cytokine mobilization by leukopheresis at the time DLI was deemed necessary. DLI was administered in a single infusion or in stepwise increments at the discretion of the attending physician. Cell doses ranged from 1 × 10^7 to 5 × 10^9 CD3+ cells per kg recipient body weight.

Data Analysis

Complete remission was determined by standard disease-specific criteria as defined by the International Bone Marrow Transplant Registry (IBMTR). Acute and chronic GVHD were scored according to published guidelines [42,43]. Toxicity was graded by World Health Organization classification of Common Toxicity Criteria. Venoocclusive disease (VOD) of the liver was diagnosed by standard criteria [44]. Pearson's chi-square test was used to compare GVHD prophylaxis groups. Unadjusted probabilities of transplantation-related mortality (TRM), overall survival (OS), and disease free survival (DFS), defined as the number of months from the day of stem cell transplantation, were estimated using the Kaplan-Meier method [45].

RESULTS

Patient Characteristics

Thirty patients underwent ASCT on this protocol. There were 23 men and 7 women. The median age was 57 years (range, 27-69 years). All patients had relapsed or persistent disease at the time of transplantation and had failed to respond to at least one prior therapy. The median number of prior therapies was 3. Three chronic-phase chronic myelogenous leukemia (CML) patients failed interferon as their only prior treatment. Sixteen patients had undergone previous stem cell transplantation (6 allogeneic and 10 autologous). Patient and donor characteristics, as well as variations in the preparative regimen, are summarized in Table 1.

Toxicities

Seven (23%) of the patients had greater than grade II nonhematologic RRT. Three patients had grade III and 1 patient had grade IV nausea and vomiting. Two patients had grade III and 2 patients had grade IV mucositis. One patient had grade III diarrhea. Grade IV pulmonary toxicity was seen in 2 patients, both of whom had received prior high-dose therapy. Patient 6 received interleukin (IL)-2 off protocol after engraftment and developed capillary leak, adult respiratory distress syndrome, and pulmonary effusions requiring mechanical ventilation. Patient 9 developed respiratory failure requiring mechanical ventilation 3 days postransplantation. An open lung biopsy on day 28 revealed bronchiolitis obliterans and organizing pneumonia (BOOP). Six of the 7 patients who developed greater than grade II nonhematologic toxicity had had prior high-dose chemotherapy. Serum sickness was diagnosed in 11 (37%) of the patients. The diagnosis of serum sickness was made clinically based on a temporal relationship between ATG administration and presence of 2 or more of the following symptoms: arthralgia, myalgia, erythematous rash, and fever. VOD was not diagnosed in any patient.

Engraftment

All patients had hematologic recovery with and ANC greater than 0.5 × 10^9/L at a median of day 13 (range, 9-25 days). The protocol was amended to allow the use of GCSF as described above because of prolonged neutrophil nadirs in the first 6 patients treated. Three patients who failed to achieve platelet engraftment had progressive disease with bone marrow involvement. Six patients did not have platelet nadirs below 20 × 10^9/L. Packed red blood cells and platelet support were not required in 7 and 8 patients, respectively, during the first 30 days after transplantation. Twenty-nine
Moderate-Intensity Allogeneic PBSCT

patients were evaluable for chimerism analysis (Table 2); chimerism studies were not performed in 1 patient. Twenty-six (89%) of the patients had greater than 90% donor chimerism before day 100. Three patients never achieved greater than 90% donor chimerism, and another 3 patients lost donor chimerism before day 100. All 6 patients had disease involving the bone marrow at the time of their transplantation (3 CML, 2 myelofibrosis, 1 chronic myelomonocytic leukemia) and subsequently had autologous reconstitution and disease progression. Five of the 6 patients received DLI and 1 patient refused further therapy. All 5 of these patients failed to respond to the DLI and continued to manifest progressive disease and decreased donor chimerism. Ten patients relapsed or had progressive disease despite greater than 90% donor chimerism. Of these 10 patients, 4 refused or were too ill to receive further therapy; 3 had active GVHD at the time of disease progression and, therefore, did not receive DLI; 2 received DLI but failed to respond; and 1 received DLI and appeared to be responding, but died of a cerebral hemorrhage.

Graft-versus-Host Disease

Grades II to IV acute GVHD occurred in 8 (62%) of 13 patients who received CYA alone and 9 (52%) of 17 patients who received CYA/MTX (*P* = not significant) (Table 2). Twenty patients were evaluable for chronic GVHD, which occurred in 4 (50%) of 8 patients in the CYA-alone group and 5 (42%) of 12 patients in the CYA/MTX group (*P* = NS) (Table 2). In the CYA-alone GVHD prophylaxis group, 2 patients had grade IV acute GVHD. In the CYA/MTX group, 1 patient had grade III and no patients had grade IV acute GVHD. Five patients (17%) died with complications related to GVHD. One patient died of steroid refractory acute GVHD, and 4 others died of infection while their GVHD was quiescent on systemic corticosteroids.

Infections

Febrile neutropenia occurred in 19 (63%) of 30 patients in the immediate transplantation period (before day 30); however, 4 patients had fever only during the administration of the ATG. Five patients died of infectious causes between

### Table 1. Patient, Donor, and Transplantation Characteristics*

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Disease</th>
<th>Patient Age/Sex</th>
<th>Prior Therapies</th>
<th>High-Risk Characteristics</th>
<th>Donor Age/Sex</th>
<th>Prep Regimen Variations†</th>
<th>GVHD Prophylaxis</th>
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* CML indicates chronic myelogenous leukemia; CP, chronic phase; IV Bu, intravenous busulfan, ATG, antithymocyte globulin; CYA, cyclosporine; coag, coagulopathy; PXE, pseudoxanthoma elasticum; PO Bu, oral busulfan; MDS, myelodysplastic syndrome; CMMML, chronic myelomonocytic leukemia; NHL, non-Hodgkin’s lymphoma; MC, mantle cell; AML M-7, acute myelogenous leukemia FAB type M-7; allo, allogeneic stem cell transplantation; MM, multiple myeloma; auto, autologous stem cell transplantation; MTX, methotrexate; CLL, chronic lymphocytic leukemia; BP, blast phase; MI, myocardial infarction; RA, refractory anemia; DLI, diffuse large cell; HD, Hodgkin’s disease; MF, myelofibrosis; RAEBT, refractory anemia with excess blasts in transformation; AP, accelerated phase; age, age greater than 55 years.

†All patients received the same dose of fludarabine.
2 and 21 months posttransplantation. Four of the 5 had responding disease but were being treated with systemic corticosteroids for acute or chronic GVHD. Another patient died of sepsis during corticosteroid treatment for refractory multiple myeloma posttransplantation. Fungal infections were diagnosed in 3 patients who were receiving corticosteroids for treatment of GVHD. In 1 patient *Mucor* sp was identified on a single surveillance blood culture with no identifiable source and no subsequent positive cultures. The 2 other patients died of disseminated *Aspergillus* sp infection.

Nine patients developed CMV reactivation. Eight of the 9 patients were on systemic corticosteroids for GVHD. One patient not receiving corticosteroids developed a CMV infection that occurred prior to neutrophil engraftment and involved the lungs, gastrointestinal tract, and bladder.

**Survival**

Disease response and survival data are summarized in Table 2. Actuarial OS and DFS at 12 months were 52% (95% confidence interval [CI], 43%-63%) and 30% (95% CI, 24%-37%), respectively (Figure 1). Of the 6 patients who died of disease progression did not develop GVHD. One patient, who developed grade II acute GVHD, had disease progression after initiation of treatment with systemic corticosteroids. There were 16 surviving patients, 8 in complete response (CR), 1 in partial response (PR), and 7 with relapsed or persistent disease. All patients alive with CR or PR developed acute or chronic GVHD. Two surviving patients with relapsed disease also developed chronic GVHD.

Two patients who had a previous ASCT were alive as of this report, but both had persistent disease and had lost donor chimerism. Three of the 4 patients who had undergone previous autologous stem cell transplantation and obtained a CR had non-Hodgkin’s lymphoma, and 1 patient with multiple myeloma had a PR. Two other patients with multiple myeloma who had undergone previous autologous stem cell transplantation initially responded, but their disease later progressed despite the development of extensive chronic GVHD.

**DISCUSSION**

Slavin and colleagues reported the use of a busulfan, fludarabine, and antithymocyte-containing regimen to treat
standard-risk ASCT patients [25]. In their initial experience with this regimen it appeared to be sufficiently immunosuppressive and cytotoxic to allow for adequate engraftment and control of disease, yet have greater potential to reduce RRT than standard preparative regimens. In our current study we expanded the use of a similar regimen to treat patients at high risk for RRT in a multi-institutional setting.

The incidence of RRT was minimal for this regimen. Several reports have indicated high mortality rates because of RRT in patients that have received previous high-dose therapy [7,8]. Other reports have found that patients with multiple myeloma treated with ASCT have excessive TRM as well [21]. We observed no toxic deaths within the first 30 days of the transplantation and overall acceptable RRT in this high-risk group of patients. The low incidence of nonhematologic RRT that we observed was comparable to that reported by others using a similar regimen [30]. TRMs at 3 and 12 months were 7% and 28%, respectively, and were most often due to infection. Infectious complications were a major source of morbidity and the leading cause of nonrelapse mortality in this study. The less intensive cytotoxic properties of this conditioning regimen resulted in fewer serious early infections, but its profound and prolonged immunosuppressive properties appeared to have predisposed patients to later infectious complications. The addition of systemic corticosteroids to control acute and chronic GVHD appeared to contribute to the infectious mortality in these high-risk patients who were already severely immunosuppressed.

We observed no clinical evidence of VOD in any patient, in contrast to the 15% incidence of severe or moderate VOD observed by Slavin et al. [25]. The absence of VOD in this study may be related to different grading criteria or the incorporation of intravenous versus oral busulfan in our protocol. Pulmonary toxicity was observed in 1 patient with the occurrence of diffuse interstitial infiltrates and ventilator dependence beginning 3 days posttransplantation. An open lung biopsy later confirmed the diagnosis of BOOP. This patient had received a prior allogeneic transplant and, upon relapse with CML blast crisis, was treated with induction chemotherapy prior to treatment on protocol. Pulmonary toxicity was also seen in another patient who received IL-2 off protocol after engraftment. This patient...
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was included in the overall assessment of outcomes because the preparative regimen was given on study. At the completion of the IL-2 infusion the patient developed capillary leak syndrome and hypotension requiring mechanical ventilation and vasopressor support. The patient subsequently recovered but developed grade IV acute GVHD.

Serum sickness has not been described as a significant problem in other reports. We found that 37% of patients exhibited some form of this syndrome and it was the only reason for a hospital admission in 4 patients. Serum sickness was differentiated from GVHD by the constellation of symptoms involving fever, arthralgias, and myalgias with an erythematous rash that was more serpiginous than macular/papular. Skin biopsies were done to confirm histologic evidence of GVHD if a rash was the predominant finding. Patients with isolated myalgias or arthralgias were given narcotics for pain relief, and isolated rashes were treated with topical steroids or observation (after skin biopsy). Patients with more severe symptoms or combinations of symptoms were treated with a pulse of 1 mg/kg of corticosteroids for 1 to 5 days. It is possible that this constellation of signs and symptoms could still represent engraftment syndrome or early acute GVHD. Skin biopsies may not be diagnostic of GVHD at this early point posttransplantation; the clinical characteristics can overlap and improvement on systemic corticosteroid treatment can be seen for each diagnosis.

Six patients (20%) required no blood product transfusion support, but the median number of days to neutrophil and platelet recovery was similar to that reported for transplantation employing standard preparative regimens. Febrile neutropenia occurred in more than half of the patients in this study, necessitating hospitalization and the administration of intravenous broad-spectrum antibiotics. This outcome was disappointing, as we initially intended for this regimen to be administered and managed in the outpatient setting. Ultimately, 29 of the 30 patients were hospitalized at some point during the first 30 days for febrile neutropenia, administration of intravenous busulfan, and/or ATG toxicity.

Minimal GVHD prophylaxis and a rapid taper of immunosuppression after engraftment were employed to expedite the development of a GVM response. Severe GVHD and the use of systemic corticosteroids to control GVHD appeared to contribute to excess morbidity and mortality in the first cohort of patients. MTX was added to the CYA for prophylaxis, and subsequently no patients had grade IV acute GVHD. The dose of ATG was also increased in 15 of the 17 patients who received CYA/MTX, possibly affecting the incidence of GVHD. Four of the 6 patients who died of progressive disease were in the group of patients who received CYA/MTX and the higher dose of ATG, and these 4 patients did not develop GVHD. This observation suggests that the improvement in immunosuppression may have contributed to a decreased GVM effect. Three patients had full donor chimerism and acute or chronic GVHD at the time of disease progression, demonstrating that some malignant clones are immunoresistant and progress too rapidly for, or are not responsive to, the GVM effect.

Six of 7 patients who were alive in complete remission had lymphoid malignancies. This study was not designed to detect differences between disease entities, and it is unclear whether this observation is due to bias because of the small number of patients studied, or there is an intrinsic advantage of the GVM effect with lymphoid malignancies. It is possible that the busulfan and fludarabine in this regimen allowed for more effective cytotoxic debulking of lymphoid diseases and, therefore, more time to develop a meaningful GVM effect. Other studies in patients with lymphoid malignancies have reported similar favorable responses using different preparative regimens [27], suggesting that the composition of the preparative regimen may be less important than the development of GVM.

Our disappointing results in patients with myeloid malignancies may have several explanations in addition to the small sample size and selection bias. Patients with myeloid malignancies in our study tended to have undergone very heavy pretreatment, and this group of patients included all of the 6 patients who had undergone a previous ASCT. It is possible that the fludarabine, busulfan, and ATG regimen was not the optimal regimen for these patients. For example, Giralt et al. at MD Anderson Cancer Center have reported a 64% CR rate in patients with acute myelogenous leukemia, CML, and myelodysplastic syndrome using preparative regimens containing melphalan and purine analogs [40]. In our patients with CML, 2 patients had disease that had progressed beyond chronic phase at the time of transplantation, and both had undergone a previous ASCT. Of the 4 patients with CML chronic phase, 1 had undergone a previous allogeneic transplantation using a donor who was mismatched at 1 HLA antigen, and the other 3 patients were greater than 1 year from diagnosis and had other comorbidities.

Patients who had previously undergone a conventional ASCT did poorly in this study, irrespective of other factors. RRT was acceptable in these patients, but disease control was not achieved. Three of the 6 patients who had less than 90% donor chimerism at day 80 had undergone a previous ASCT. Five of the 6 patients used the same donor, and 1 patient, whose donor was mismatched for 1 antigen at the A locus, used another sibling. It is possible that minor histocompatibility antigen sensitization may have contributed to the lack of donor engraftment and eventual disease progression in these patients.

Actuarial overall survival (52%) and disease free survival (30%) at 12 months were much less for patients in this study than for those reported by Slavin et al. [25]. We believe this result to be due to significant differences in the patient population under study. Unlike the heavily pretreated patients in our study, patients in the previous report had not undergone previous ASCT, and many would have been eligible for conventional preparative regimens. Moreover, our patients were older and had more comorbidities. However, our protocol and that used by Slavin et al. had differences that preclude direct comparisons. The equine ATG used in our study and the rabbit ATG (Fresenius AG, Munich, Germany) used in the previous report may have different immunosuppressive properties that would have affected engraftment and GVHD rates. Substituting IV busulfan for oral busulfan may have also changed the toxicity profile, and both changes could have affected survival outcomes. Our data compare more favorably with other reports of using reduced-intensity preparative regimens in similar patient populations [26-40]; however, comparisons are difficult because of the heterogeneity of patients,
diseases, and preparative regimens. In the largest series reported to date of high-risk patients (n = 86) treated with reduced-intensity preparative regimens, the OS and DFS at 2 years were 28% and 23%, respectively [40]. The Seattle group has reported an OS of 67% at a median follow-up period of 417 days in 45 patients with relative contraindications to conventional ASCT who were treated with low-dose total body irradiation; however, this study primarily included patients with more indolent hematologic diseases and acute leukemia in CR [41].

In conclusion, a reduced-intensity preparative regimen consisting of fludarabine, busulfan, and ATG can be safely administered in a multicenter trial to high-risk, heavily pretreated patients, including those who have received prior high-dose chemotherapy, with minimal RRT and acceptable engraftment. Disease response and long-term control occurred only in patients who developed GVHD, suggesting that GVHD and GVM are tightly linked. Control of GVHD with additional immunosuppression led to serious morbidity and several deaths from infection in this study. The same risk factors that preclude patients from ASCT because of RRT may also preclude patients because of excessive GVHD and infectious risks. Further study is needed to prevent and treat GVHD and infectious complications in these high-risk patients. Ultimately, the most effective way to reduce toxicity of ASCT is to continue efforts to separate the GVH and GVM effects. In the future, larger collaborative efforts will be needed to perform randomized, prospective, disease-specific studies with reduced-intensity preparative regimens to optimize patient populations that are best served by this treatment.

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REFERENCES


