Use of a T Cell–Specific Monoclonal Antibody, T10B9, in a Novel Allogeneic Stem Cell Transplantation Protocol for Hematologic Malignancy High-Risk Patients

John S. Thompson,1 Claire Pomeroy,2 Richard J. Kryscio,3,4 Stephen A. Brown,1 Donna Reece,5 Rita Kramer,5 Dianna S. Howard,5 Gary vanZant,5 Suzanne Humphries,2 Gordon Phillips5

1Division of Allergy and Immunology; 2Division of Infectious Diseases; 3Department of Biostatistics, The Markey Cancer Center, University of Kentucky; 4Veterans Affairs Medical Center; 5Blood and Marrow Transplant Program, The Markey Cancer Center, University of Kentucky, Lexington, Kentucky

Correspondence and reprint requests: John S. Thompson, MD, VA Medical Center 151, 1101 Veterans Dr., Lexington, KY 40502-2236 (e-mail: jsthom1@ukyk.edu).

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ABSTRACT

To reduce the toxicity of traditional conditioning regimens for allogeneic stem cell transplantation (allo-SCT), we used single-agent chemotherapy conditioning with either busulfan (total cumulative dose, 16 mg/kg) or melphalan (200 to 240 mg/m²), followed by the anti–T cell–specific monoclonal antibody T10B9 (MEDI-500) daily for 3 days. T cell–replete SCT was performed from HLA-identical sibling donors. Acute graft-versus-host disease (aGVHD) prophylaxis consisted of 7 additional days of T10B9 and delayed onset of cyclosporine (ie, on day +4 or +5). Twenty-six high-risk hematologic malignancy patients were entered onto this study. All 24 patients who survived longer than 8 days engrafted, although 1 patient experienced late graft failure. Deaths occurred in 21 of 26 patients because of infection (n = 7), progression/recurrence of primary disease (n = 6), aGVHD (n = 4), regimen-related toxicity (n = 1), and other causes (n = 3). Five of these patients are enjoying disease-free survival with a median survival of 1193 days after allo-SCT. The conditioning regimen induced modulation of surface expression of CD3 (but not CD4 or CD8) and was associated with decreasing tumor necrosis factor-α (but not interleukin-6) serum levels. In conclusion, single-agent chemotherapy conditioning with T10B9 produced durable engraftment and long-term survival in some patients who would not have qualified for a traditional allo-SCT.

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KEY WORDS

Stem cell transplantation • Anti–T cell monoclonal antibody MEDI-500 (T10B9) • Regimen-related toxicity • Acute graft-versus-host disease • IL-6 • TNF-α

INTRODUCTION

The curative benefits of allogeneic stem cell transplantation (allo-SCT) have been muted by the toxicity of traditional regimens, including high-dose radiation and chemotherapy [1,2]: graft-versus-host disease (GVHD) [3,4], an increased incidence of severe infection [5], disease recurrence, and the risk of second malignancies [6]. As a result, allo-SCT has usually been restricted to younger and more fit patients with well-matched donors and to primary transplantations. Patients who are older, who have comorbid disease, or who have received a previous transplant are at higher risk for nonrelapse mortality [7,8]. New approaches are needed to extend allo-SCT to these so-called high-risk patients.

There is now clear evidence indicating that commonly used conditioning regimens not only cause increased regimen-related toxicities (RRT), but also are a major factor in the pathogenesis of acute GVHD (aGVHD) [9-15]. Both experimental and clinical studies performed by ourselves [13,14] and others [9-12,15] have established that pretransplantation conditioning with myeloablative doses of total body
irradiation (TBI) and certain cytotoxic drugs is a major factor in the incidence and severity of aGVHD. These experimental and clinical studies demonstrated that pretransplantation TBI acts not only to reduce the host/antigraft reaction, but also to augment the allogeneic aGVHD response [13]. Although de-escalation of the doses of TBI and/or cytotoxic drugs might address this issue, a reduction might also reduce the anticancer benefit. Moreover, it now seems that the role of conditioning to ablate tumor cells may be no more important than its role to provide sufficient immunosuppression to allow allogeneic engraftment.

The adverse effects of aggressive conditioning are mediated, in part, by the induction of high levels of proinflammatory cytokines [9-15]. Therefore, considerable emphasis has been placed in recent years on designing conditioning regimens that achieve immunosuppression while reducing RRT and the propensity to induce proinflammatory cytokines [16-23]. We report herein the results of a pilot trial for high-risk patients in which conditioning was limited to single-agent therapy with busulfan or melphalan followed by the pretransplantation and posttransplantation intravenous administration of MEDI-500, a clone of the T cell–specific monoclonal antibody T10B9 [24,25]. To determine whether this protocol reduced the levels of inflammatory cytokines, tumor necrosis factor (TNF)-α and interleukin (IL)–6 serum levels were obtained before the onset of conditioning, before transplantation, and 1 week after transplantation.

MATERIALS AND METHODS

Inclusion Criteria

A diagnosis and disease status appropriate for allo-SCT but considered to be ineligible for a standard conditioning regimen were required. For patients ≤50 years of age, additional high-risk criteria were required, eg, serious comorbid medical conditions, an Eastern Cooperative Oncology Group performance status of ≥2, advanced disease status, or a previous transplantation.

Clinical Characteristics

The median age of the 26 patients was 49.8 years (range, 22-72 years); 15 were >50 years old, and 1 was 72 years old. Clinical diagnoses are listed in Table 1. All but 1 patient had previously undergone extensive chemotherapy. Two patients were in first remission, and 2 were in second remission. All others were in relapse or had an advanced disease status. Five patients had undergone prior transplantations; 3 had received autologous transplants for Hodgkin lymphoma and multiple myeloma, and 2 had undergone prior allo-SCT for acute myelogenous leukemia (AML).

Donor Criteria

Donors were required to be hematologically normal HLA-identical siblings without contraindications for granulocyte colony-stimulating factor (G-CSF) or anesthesia. There were no age limitations.

Conditioning and GVHD Prophylaxis Regimens

Four patients were conditioned with busulfan 4 mg/kg intravenously in divided doses from day −7 to day −4 (total dose, 16 mg/kg), and the remaining patients were conditioned with intravenous melphalan administered on day −5. All drug doses were based on the lesser of actual or corrected ideal body weight. During the study period, the dose of melphalan was decreased from an original dose of 240 to 220 mg/m² and finally to 200 mg/m² intravenously to reduce toxicity. The monoclonal antibody T10B9 (clone MEDI-500; MedImmune Corp., Gaithersburg, MD) was administered as a single intravenous injection of 42 mg/d from day −3 to day +7. Although T10B9 had been given as a 14-mg twice-daily dosage in earlier clinical kidney and cardiac transplantation studies,
pharmacokinetics and a pilot study in kidney/pancreatic transplantation suggested that 42 mg would ensure an excess of T10B9 throughout the 24-hour period (unpublished data). Cyclosporine 3.0 mg/kg intravenously was started on day +5 and was scheduled to continue by mouth until day +180 with target blood levels of <250 pg/L.

**Stem Cell Source**

Initially, bone marrow was used in 13 patients. One of these 13 bone marrow recipients received G-CSF–mobilized marrow [25]. Later in this study, 13 patients received G-CSF–mobilized peripheral blood stem cells. The mean number of CD34+ cells infused was 5.68 ± 6.65 × 10^6, and the median was 4.3 × 10^6. One patient with chronic myelogenous leukemia in blast crisis received 36.23 × 10^6 CD34+ cells, but this resulted in no significant difference in the day of absolute neutrophil count >500/μL or platelet independence. This patient did not develop GVHD and survived for 423 days.

**Donor Leukocyte Infusion**

Donor leukocyte infusion was optional for patients who did not achieve complete remission and did not have grade II or greater aGVHD or extensive chronic GVHD (cGVHD). When administered, the initial dose was 1 × 10^7/kg CD3+ cells. If no GVHD occurred within 2 more months, patients were eligible for a second dose of 5 × 10^7 cells per kilogram. Only 2 patients in this series received donor leukocyte infusions.

**Supportive Care**

Allopurinol, intravenous fluids, empiric antibiotics, and prophylactic and/or empiric antifungal drugs were used routinely. Acyclovir was given prophylactically to herpes simplex virus–seropositive patients from day +1 to day +30 or longer if they required steroids or if GVHD was present. Ganciclovir was given empirically to cytomegalovirus (CMV)–seropositive patients. Total parenteral nutrition was not routinely used.

**Serum Cytokine Assays**

Peripheral blood was obtained by venipuncture for cytokine analysis for 21 of the 26 patients. Samples were drawn before the onset of conditioning, on pretransplantation days −1 or 0, and 6 to 8 days after transplantation. Serum was separated and stored at −70°C. All samples were analyzed simultaneously. Cytokine assays for TNF-α and IL-6 were performed by enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN) by following the manufacturer’s instructions. All samples were tested in duplicate or triplicate and compared with a standard curve, and the results were expressed as the average of the replicates.

**Peripheral Blood Immunophenotyping**

To determine the effect of the conditioning regimen, aliquots of the same blood samples were obtained for leukocyte phenotyping. Using specific phenotyping antibodies, absolute CD3, CD4, CD8, CD20, and CD56/16 counts were determined by flow cytometry at the indicated times. The mean of samples obtained before conditioning was compared with the means of the samples obtained before and after transplantation.

**Statistical Analysis**

The absolute numbers of CD3+, CD4+, and CD8+ cells were compared by using an analysis of variance for a repeated-measures design with post hoc comparison of means depending on the Fisher protected least significant differences procedure.

To determine the effect of the conditioning regimen on inflammatory cytokines, 3 time periods were considered: period 1, baseline (before conditioning); period 2, before transplantation (days −1 or 0), and period 3, after transplantation (days +6 to +8). A linear mixed model was used to compare mean response across periods for each cytokine value. Because not all patients had measurements in all 3 periods, the dataset was unbalanced. Post hoc comparison of means was based on the least-squares means to account for missing responses that were assumed to have occurred at random. Satterwhaite’s approximation was used to estimate the degrees of freedom associated with the t tests based on these least-squares means. Statistical significance was determined at the .05 level throughout all analyses.

**RESULTS**

**Early Deaths**

Two patients died of infectious complications on days +3 and +8 (Table 2).

**Engraftment**

The remaining 24 patients engrafted with an absolute neutrophil count ≥500/μL on median day +10 (range, day 8-14) and with platelet recovery ≥20 000/μL on median day +14 (range, day 7-100). The median day of the last erythrocyte transfusion was day +23 (range, day 7-60). Chimerism determined by variable number of tandem repeats analysis demonstrated >95% donor hematopoiesis in 17 of 19 patients and partial chimerism in 2 others by day +30. One of the partial-chimerism patients later converted spontaneously to full chimerism. In the other, residual and progressive malignant disease...
complicated the assessment of chimerism analysis. Two other patients, assayed only on day +100, were fully chimeric. One patient became aplastic during treatment for human herpes virus-6 with acyclovir and foscarnet but underwent successful retransplantation from his original donor and remained disease free at day +1418.

Graft-versus-Host Disease

All 24 patients who engrafted were evaluable for GVHD. Of these, 11 (46%) had grade 0 or I, 6 (25%) had grade II, and 7 (29%) had grade III or IV aGVHD. Four deaths in the group with grade III or IV disease were directly attributable to aGVHD. This included 2 of 4 patients who received busulfan and 2 of 3 who received melphalan 240 mg/m², 1 of 2 who received melphalan 220 mg/m², and only 1 in 17 who received melphalan 200 mg/m². One patient, who received melphalan 220 mg/m², died on day 31 with diffuse alveolar damage. These toxic manifestations were one of the factors that led to the reduction in melphalan dosage.

Infections

Two patients died early: 1 with an unidentified septic syndrome on day 3 and another with Staphylococcus aureus and Candida albicans bacteremia on day 8. Three patients (13%) developed adenovirus infection, and 1 died of disseminated infection on day +72. One patient developed disseminated varicella-zoster virus within the first 30 days but recovered. CMV was cultured from the nasopharynx in 3 patients and from the bowel in 3 patients. Four of the 6 were asymptomatic, but 2 patients (8%) developed CMV colitis within the first 100 days. In both, the colitis resolved, but 1 patient died of recurrent AML on day +457; the

Table 2. Outcomes

<table>
<thead>
<tr>
<th>Variable</th>
<th>Conditioning Drug</th>
<th>Busulfan</th>
<th>Melphalan</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>4 mg/kg/d × 4</td>
<td>240 mg/m²</td>
<td>220 mg/m²</td>
</tr>
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<td>Total no. Patients</td>
<td>4</td>
<td>3</td>
<td>2</td>
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<tr>
<td>ANC &gt;500 μL²</td>
<td>12 (8-14)</td>
<td>9 (8-10)</td>
<td>11 (10-12)</td>
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<td>14 (9-20)</td>
<td>30 (11-48)</td>
<td>63 (27-100)</td>
</tr>
<tr>
<td>Last RBC*</td>
<td>21 (0-54)</td>
<td>18 (12-23)</td>
<td>61 (21-100)</td>
</tr>
<tr>
<td>aGVHD</td>
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<td></td>
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<tr>
<td>No. at risk†</td>
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<td>0</td>
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</tr>
<tr>
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</tr>
<tr>
<td>RRT§</td>
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<td>1</td>
</tr>
<tr>
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<tr>
<td>Follow-up (914-1532 d)</td>
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<td>Survival</td>
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</tr>
<tr>
<td>DFS§</td>
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</table>

RBC indicates red blood cells.

*Engraftment: mean (range) days after transplantation.
†Excludes patients dying on days 3 and 8.
‡Includes all patients who underwent transplantation.
§Regimen-related toxicity.
||Disease-free survival.

Regimen-Related Toxicity

Veno-occlusive disease did not occur in any of the patients, but 8 developed mucositis. Although the numbers are small, the incidence tended to correlate with a more toxic chemotherapeutic regimen, including 4 of 4 patients who received busulfan and 2 of 3 who received melphalan 240 mg/m², 1 of 2 who received melphalan 220 mg/m², and only 1 in 17 who received melphalan 200 mg/m². One patient, who received melphalan 220 mg/m², died on day 31 with diffuse alveolar damage. These toxic manifestations were one of the factors that led to the reduction in melphalan dosage.

Infections

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Survival (Follow-up 914-1532 Days)

Twenty patients (77%) survived to day +50, and 16 (62%) survived to day +100 (Figure 1). Three of the 6 deaths in the early (>50 days) period were attributed to aGVHD on day +43; 1 patient had aGVHD but died of fungal and bacterial infection on day +47; and the sixth died of diffuse alveolar damage (RRT) on day +31. An additional 4 patients died by day +100 because of progressive disease (day +51), recurrence (day +100), disseminated adenovirus infection (day +72), and grade IV aGVHD (day +56). Of the 16 patients who survived for >100 days, 1 AML patient developed extramedullary relapse approximately 1 year after transplantation and died on day +428. Another 3 patients died of recurrence on days +423, +457, and +611. One of these developed grade III aGVHD after discontinuation of GVHD prophylaxis but succumbed to recurrent disease. Complete remission persisted until death in 7 more patients, who died of the following causes: aGVHD on day +103, infection on day +134, renal failure on day +196, sepsis and systemic fungal disease on day +352, acute respiratory distress syndrome and renal failure on day +423, heart failure on day +426, and disseminated herpes zoster on day +700. Five patients (19%) were disease free beyond 914 days: 1 patient with myelodysplastic syndrome, indeterminate stage (aged 53 years); 1 patient with AML in second complete remission (aged 72 years); 1 patient with AML in first relapse (aged 55 years); and 2 patients with non-Hodgkin lymphoma in advanced disease stage after primary induction failure (aged 44 and 51 years). One received busulfan induction and a bone marrow graft, and 4 received melphalan (200 mg/kg) induction followed by primed peripheral blood stem cells.

Laboratory Results

To test the effect of a single daily infusion of 42 mg of T10B9 on T lymphocytes, the absolute numbers of cells expressing CD3, CD4, and CD8 were determined in 14 of the 26 patients before the onset of the infusion and on the day of the last T10B9 infusion. There was a marked decrease in absolute numbers of CD3+ T cells from 1340/μL at the onset of treatment to 5/μL at the end of treatment. Figure 2 illustrates the results in log 10 function. CD4 cells decreased from 618 to 32/μL, and CD8 cells decreased from 777 to 65/μL. Although there was a decrease in CD4+ and CD8+ T cells at the end of T10B9 treatment, there were substantially more T cells bearing these surface markers than could be accounted for by the number of CD3+ cells. Comparison of mean CD3, CD4, and CD8 values on the last day of T10B9 treatment (after log transformation) showed that geometric means varied significantly by type of count ($F_{2,22} = 7.40; P = .0035$). Post hoc analysis demonstrated that the geometric mean for CD3+ cells was significantly smaller than the corresponding means for the CD4+ ($P = .004$) and CD8+ ($P = .002$) cells but that the CD4 and CD8 counts were not different ($P = .78$). Thus, T10B9 induces modulation of the surface expression of CD3, but not CD4 or CD8, molecules during administration to bone marrow transplant recipients as it does in recipients of solid organs.

Previous in vivo studies have demonstrated that T10B9 does not cause a major increase in serum TNF-α and IL-6 when used to treat transplantation rejection or to prevent rejection in cardiac transplan-

![Figure 1](image1.png)

**Figure 1.** Patient survival: Kaplan-Meier plot of survival of 26 high-risk patients receiving single-agent chemotherapy plus T10B9 anti–T cell conditioning.

![Figure 2](image2.png)

**Figure 2.** Effect of MEDI-500 on absolute CD3+, CD4+, and CD8+ peripheral blood T-cell counts (log 10). Data are mean ± SEM. Note the number of cells before initiation of MEDI-500 and at the end of 10 days of treatment. Also note that at the end of treatment, the CD3 count was significantly lower than either the CD4 or CD8 counts.
In recent years, a number of alternative regimens have been explored to reduce or eliminate the myeloablative agents used in conventional protocols and, by so doing, to include patients who ordinarily cannot undergo transplantation because of age, disease status, prior transplantation, or comorbid conditions [20,26-28]. Although many different regimens have been designed to reduce myeloablation, common elements have included combinations of the following for immunosuppression: fludarabine [16,21], low-dose TBI [20,22,27], antithymocyte globulin [16,17,28,29], and alemtuzumab (Campath-1H; Berlex Laboratories, Seattle, WA) [30-32]. In addition to the added immunosuppressive effects of fludarabine and TBI, cyclophosphamide [29,33], busulfan [16], melphalan [21,30], and other drugs have been added for their specific antineoplastic properties. In some regimens, mycophenolate mofetil has been added to conventional cyclosporine [22,27] for post-transplantation GVHD control and to help with engraftment [27].

The central theme behind all of the reduced myeloablative regimens is the concept that immunosuppression of host T cells, rather than making space by myeloablation, is the critical requirement for successful engraftment of allo-SCT [26-32,34,35]. Several years ago, we developed a powerful anti-T cell monoclonal antibody, T10B9. Basic and preclinical studies [24,25,36] demonstrated that T10B9 is an immunoglobulin Mκ protein that specifically targets cells expressing the αβ chains of the T-cell receptor and spares cells expressing the γδ T-cell receptor [25,37]. The result is modulation of the receptor of the surface of the targeted T cells and apoptosis. An important characteristic of T10B9 is that it is not mitogenic and has a reduced capacity to stimulate the production of inflammatory cytokines [24,25]. Another potential advantage for T10B9, as a conditioning agent, is its short half-life of <24 hours. Therefore, T-cell immunosuppression can be localized to a specific period without the disadvantage of prolonged suppression.

T10B9 has been extensively used for ex vivo T-cell depletion [38,39] and as one of the T-cell depletion arms in the National Heart, Lung, and Blood Institute–sponsored multicenter unrelated bone marrow transplant study [40]. In vivo, T10B9 has also been found to very successfully treat kidney allograft rejection [41] and prevent solid-organ rejection [42] and to do so with minimal toxicity. It targets the αβ chains of the T-cell receptor and induces modulation of the T-cell receptor of the cell surface and apoptosis. The infusion results in a dramatic decrease in circulating CD3+ T cells within 10 to 30 minutes after infusion. After 3 to 5 days of daily infusion with T10B9, new CD4+ and CD8+ T cells begin to reappear in the circulation, but they continue to express low levels of CD3 on their surface by flow cytometry as long as the antibody is continued [24,25]. Within 48 hours after cessation of treatment, CD3 reappears on the surface of circulating lymphocytes. On the basis of this exten-
sive background, T10B9 was considered an attractive agent for the immunosuppression component of conditioning in a single-agent allo-SCT trial. However, this raised the question of whether T10B9 would have the same effect on circulating lymphocytes and cytokines in this protocol as compared with that reported in solid-organ transplantation. The data demonstrate quite clearly that T10B9 induced modulation of surface CD3 in patients undergoing allo-SCT similar to that previously observed in solid-organ transplantation.

We continued treatment with T10B9 for 6 to 7 days after transplantation for 2 reasons. The first was simply to delay the necessity for cyclosporine and thereby reduce the incidence and severity of nephrotoxicity and neurotoxicity in this early post-allo-SCT period. The second was based on experimental evidence that established that host T cells were the predominant source of TNF-α during the first 3 to 5 days in a murine model of allogeneic bone marrow transplantation [43,44]. We believe that this accounts for the observed progressive decrease in TNF-α levels from the pretransplantation samples to the pretransplantation samples taken after 3 days of T10B9 and to the samples obtained at the end of T10B9 therapy on day 6 to 8 after transplantation. In contrast, note that IL-6, which is not produced by T cells, increased progressively over these time periods.

Our initial choice for an antineoplastic agent during conditioning was too toxic. Two of the 4 patients treated with busulfan (4 mg/kg/d × 4 days for a total dose of 16 mg/kg; Table 2) died of aGVHD on days +43 and +56. Another died of disease recurrence in the presence of grade II aGVHD. The fourth was alive at +1246 days. All 4 developed stomatitis. Because of a protocol exclusion for prior use and these results with busulfan, we used the protocol-specified alternative, ie, a single dose of melphalan, as the study progressed. However, we found that the initial choice of melphalan doses of 220 to 240 mg/m² were too toxic in these patients. Two of the 3 who received 240 mg/m² died with sepsis on day 8 and 50, and 1 of 2 who received 220 mg/m² died of diffuse alveolar damage and infection on day +31. Three of 5 patients who received 240 or 220 mg/m² developed stomatitis. Therefore, the dose of melphalan was reduced to 200 mg/m² administered on day −5. Of the 17 patients treated with this dosage of melphalan, 4 were alive and disease free at follow-up at +914 - 1, 532 days.

Several important points distinguish the results of this trial from those of other published reports. (1) Failure to engraft and graft failure have been significantly increased with some of the reduced myeloablative protocols [19,22,27,29]. All evaluable patients in this study engrafted promptly. Three patients never required red blood cell support. Furthermore, >95% donor chimerism was demonstrated in all but 1 of 21 patients in whom it was tested. We believe that this strongly supports the conclusion that the daily injection of T10B9 provided adequate host immunosuppression. (2) In some other protocols, a high incidence of infection has been reported. In our study, CMV was cultured in 6 (23.1%) of 26 patients. Four were asymptomatic, and only 2 patients (8%) developed CMV colitis. This is contrast to the higher incidence of CMV reactivation in 46.6% of patients treated with Campath-1H for aGVHD prophylaxis [31,32]. The difference in the CMV infection rate may relate to the more prolonged immunosuppression that results from humanized Campath-1H or to its effect on non-T cells and T cells. (3) The incidence of aGVHD was higher in our patients than in those treated with Campath-1H [30,35] but was generally comparable with the overall incidence when mycophenolate/cyclosporine [22,27] or cyclosporine/prednisone [16,21] was used for GVHD prophylaxis. Although mycophenolate delayed the onset, aGVHD often occurred after drug cessation.

We [13,14] have demonstrated increased levels of serum TNF-α, IL-1β, and IL-6 in mice after TBI, whereas there was much less of an increase after either busulfan or cyclophosphamide conditioning. Therefore, we reasoned that deletion of a myeloablative dose of TBI from the conditioning regimen might reduce the induction of high levels of these proinflammatory cytokines and, correspondingly, reduce the incidence of RRT and aGVHD. Our pilot trial does not confirm or refute this theory because there was no comparable high-risk control group that received traditional conditioning with TBI. Taken in the context that all of the patients entering in this trial were considered to be at too high a risk for a traditional allo-SCT, the fact that 5 of 26 patients are enjoying long-term disease-free survival is encouraging. However, 2 patients died of sepsis within 8 days of transplantation. The ability to select more favorable patients from the high-risk pool could be an important factor, not only for entry into this protocol, but also perhaps as a general guide for patients being considered for other reduced-myeloablative-conditioning protocols.

In conclusion, the use of the T cell–specific monoclonal antibody T10B9 in a conditioning regimen with single-agent chemotherapy achieved durable engraftment and long-term survival in high-risk patients who would not have qualified for a conventional SCT.

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