Marrow transplantation from HLA-matched unrelated donors has been established as a treatment option for patients with hematologic malignancies who have no suitable related donor. In reports from multicenter groups and registries, survival after unrelated donor marrow transplantation has been 39% to 55% for patients with early leukemia and 13% to 23% for those with advanced disease. Regimen-related complications appear to be similar for recipients of matched related or unrelated donor marrow, but the risks of infections, graft failure and acute graft-versus-host disease (GVHD) are clearly higher in the latter group.

Following unrelated donor marrow transplantation, grades 2-4 acute GVHD occurs in 59% to 79% of patients and grades 3-4 acute GVHD in 36% to 47% with most centers using the standard combination of cyclosporine and short methotrexate (15 mg/m² on day 1 and 10 mg/m² on days 3, 6, and 11) as prophylaxis. GVHD accounted for 29% to 33% of the mortality. The incidence of acute GVHD can be reduced by T-cell depletion of the marrow, but any potential benefit has been offset by higher incidences of graft failure, relapse and infection, and there was no improvement in survival. The addition of polyclonal or monoclonal anti-T-cell antibodies to the standard combination of cyclosporine and methotrexate for GVHD prophylaxis has not effectively reduced the incidence of GVHD, and results using the triple combination of cyclosporine, methotrexate and methylprednisolone have been inconsistent.

Tacrolimus (FK506) is an immunosuppressive macrolide lactone that blocks the earliest steps of T-cell activation by inhibiting the calcium-dependent signal transduction pathway. Although the mechanism of action, pharmacokinetics, and side-effect profile of tacrolimus are similar to those of cyclosporine, its immunosuppressive potency in vitro is 50 to 200 times greater than that of cyclosporine. In animal models that evaluated marrow transplantation with major disparities in histocompatibility, tacrolimus was active in preventing and treating acute GVHD.

The preliminary clinical experience with tacrolimus in marrow transplantation has been encouraging. The initial reports suggested that tacrolimus could be used as salvage therapy for patients with chronic or acute GVHD even when resistant to cyclosporine and corticosteroids. In phase II studies of HLA-identical marrow transplant recipients, the incidence of grades 2-4 acute GVHD was 41% to 44% with tacrolimus alone or in combination with short methotrexate or methylprednisolone. Herein we report the results of a study of tacrolimus in combination with a reduced dose schedule of methotrexate for prevention of acute GVHD in recipients of marrow transplants from HLA-matched unrelated donors.

**MATERIALS AND METHODS**

**Patients.** From January 1994 through December 1995, 30 adults with HLA-matched unrelated donors underwent transplantation using tacrolimus and minidose methotrexate for prevention of acute GVHD. In each case, the donor was serologically matched with the patient at HLA-A, -B, and -DR, and HLA-DR was further evaluated by sequence-specific primer polymerase chain reaction. All patients are at least 100 days from transplantation. Eligibility criteria for transplantation were as described. Patient characteristics are shown in Table 1. All patients had hematologic malignancies. The majority of the patients (77%) were not in remission or first chronic phase, and 37% had disease resistant to conventional therapy. The three...
patients with chronic myelogenous leukemia in first chronic phase were 3.5, 5.5, and 8.5 years from diagnosis, respectively. Two patients had undergone autologous marrow transplantation and one patient had undergone unrelated donor marrow transplantation (from a different donor) at least 1 year previously. The protocol was approved by the Institutional Review Board of the M.D. Anderson Cancer Center, and written informed consent was obtained from all participants.

Preparative regimens and transplantation. The preparative regimens were administered as described previously. Patients received either thiopeta (THIO) 5 mg/m² intravenously (IV) on day -7, cyclophosphamide (CYC) 60 mg/kg IV on days -6 and -5, and total body irradiation (TBI) 3 Gy on days -3, -2, -1 and 0; CYC 60 mg/kg IV on days -7 and -6 followed by TBI 3 Gy on days -4, -3, -2, -1 and 0; or THIO 250 mg/m² IV on days -9, -8, and -7, busulfan (BU) 1 mg/kg orally q 6 hours for 12 doses on days -6, -5, -4, -3, and 0 CYC 60 mg/kg IV on days -3 and -2. Patients with high-grade lymphoma, acute lymphoblastic leukemia, or a history of central nervous system involvement also received methotrexate 12 mg intrathecally or 6 mg intravenicularly with leucovorin rescue monthly from the 3rd through 12th months posttransplant. Day 0 is the day of marrow infusion. Marrow harvests contained a median of 3.9 × 10⁸ nucleated cells/kg (range 1.2 to 5.9) and were processed for ABO-incompatibility by standard measures when necessary. None of the marrows was depleted of T cells.

Supportive care. The patients were hospitalized in laminar air-flow rooms through engraftment. Filgrastim 5 µg/kg/d SC was given from day 7 through engraftment. Infection prophylaxis during the peritransplant period consisted of nonabsorbable antibiotics orally, vancomycin 1 g/m² IV daily, fluconazole 200 mg IV every 12 hours, and acyclovir 5 mg/kg IV every 8 hours. All patients received broad spectrum antibiotics for neutropenic fever and hyperalimentation when needed. Blood products were irradiated at 25 Gy and filtered to remove leukocytes. Intravenous immunoglobulin 200 to 500 mg/kg was given weekly through day 100 and monthly thereafter through 1 year. Once engrafted, the patients also received twice-weekly trimethoprim-sulfamethoxazole orally or pentamidine by inhalation every 3 weeks. Cytomegalovirus (CMV)-seropositive patients received prophylactic ganciclovir 5 mg/kg IV 5 days per week from engraftment through day 100. Foscarnet was given to patients unable to tolerate ganciclovir. Urine and blood buffy coats were tested for CMV by the shell vial assay before transplantation and weekly through day 100. CMV-seronegative patients with seronegative donors received acyclovir 400 mg orally twice daily through day 100.

GVHD prophylaxis and treatment. Methotrexate 5 mg/m² IV was given on days 1, 3, 6, and 11. The dose was reduced or omitted for severe mucositis (oral ulceration or significant edema), reduction in creatinine clearance by more than 50%, or weight gain more than 10 kg. Tacrolimus was administered at 0.03 mg/kg/d IV by continuous infusion from day -2. Following engraftment when the patient was able to take medications orally, the 24-hour dose of tacrolimus was converted 1 IV:4 po and given orally in a twice-daily divided dose. Doses were adjusted to maintain whole blood steady state or trough levels at 5 to 15 ng/mL by an automated microparticulate enzyme immunoassay (Abbott Laboratories, Abbott Park, IL). Tacrolimus was discontinued or reduced in dose when blood levels were elevated or the serum creatinine was increased. For the first 10 patients, the dose of tacrolimus was tapered 33% at week 9 and at week 17, and tacrolimus was discontinued on day 180. For the remainder of the patients, tacrolimus was administered at full-dose through day 180 and tapered by 20% every 2 weeks thereafter. Patients were observed prospectively for development of acute GVHD. The diagnosis of GVHD was based on clinical evidence with histologic confirmation, and GVHD was graded according to the consensus criteria. Patients who developed grade 2-4 GVHD were treated initially with methylprednisolone at 0.5 mg/kg IV q 6 hours.

Toxicity grading. Early toxicity related to the preparative regimen (RRT) was graded according to the criteria of Bearman et al. In this system, grade 1 toxicity is reversible without treatment, grade 2 is not life-threatening but requires treatment, grade 3 requires life-support intervention, and grade 4 is fatal. RRT in each organ system was scored as the highest grade observed in that organ system through day 28, except that deaths after day 28 as a result of RRT occurring before day 28 were also scored as grade 4. Adverse events that could be attributed to infection (culture-documented), bleeding, or other medications were not scored as RRT. The maximum toxicity score was the highest grade recorded in any individual organ system, and the cumulative toxicity score was the sum of the highest grades recorded for all eight organ systems.

Assessment of engraftment. Neutrophil recovery was defined as the first of 3 consecutive days that the absolute neutrophil count (ANC) exceeded the target number (0.5 or 1.0 × 10⁹/L), and platelet recovery was defined as the day that the platelet count exceeded the target number (20 or 50 × 10⁹/L) with no platelet transfusions the following week. Marrow biopsies and aspirates were examined at 1, 3, 6, 12, 18, and 24 months after transplantation. Hematopoietic chimerism was evaluated by restriction fragment length polymorphisms at the AY-29 or YNH24 loci as described.

Statistical considerations. At the time of analysis, median inter-
occlusive disease (VOD), infection, nephrotoxicity, relapse and death were calculated according to the method of Kaplan and Meier. 38

RESULTS

Engraftment. Neutrophil recovery was delayed to day 35 in one patient because of autoimmune neutropenia, 39 and the remainder engrafted by day 30 posttransplant. The median times to neutrophil recovery were 16 days (range, 11 to 35) for an ANC \( \geq 0.5 \times 10^9/L \) and 17 days (range, 12 to 36) for an ANC \( \geq 1.0 \times 10^9/L \). Platelet recovery occurred in 84% of the patients by day 100. The median times to platelet recovery were 32 days (range 13 to 100+) for a platelet count \( \geq 20 \times 10^9/L \), and 42 days (range, 16 to 100+) for a platelet count \( \geq 50 \times 10^9/L \).

GVHD. Ten patients developed grades 2-4 acute GVHD. Five patients had acute GVHD of the skin alone, and five had visceral involvement. The actuarial rate of grade 2-4 acute GVHD was 34% (95% CI, 17% to 52%), and that of grade 3-4 acute GVHD was 17% (95% CI, 3% to 31%) (Fig 1). The rates of grades 2-4 GVHD did not differ significantly between patients with molecularly matched donors and those without (28% \( v \) 50%, \( P = .15 \)). Six patients had steroid-resistant acute GVHD. Twenty-two patients survived at least 100 days posttransplant; the actuarial rate of chronic GVHD at 1 year was 59% (95% CI, 8% to 74%).

GVHD prophylaxis compliance and toxicity through day 100. The full scheduled dose of methotrexate was administered to 100% of patients on day 1, 100% on day 3, 93% on day 6, and 43% on day 11. The most common reason for omitting the methotrexate was mucositis. Twenty-three (77%) patients developed grade 2 mucositis, and the remainder had grade 1 mucositis.

For patients who were converted from IV to oral tacrolimus, the median last IV dose was 0.016 mg/kg (53% of the scheduled dose). The median oral dose of tacrolimus on day 100 was 0.049 mg/kg (41% of the scheduled dose). Nine patients discontinued tacrolimus prematurely: 1 for nephrotoxicity, 2 for relapse, and 6 for terminal care or early death unrelated to nephrotoxicity. Before day 100, 63% had a doubling of the creatinine from baseline, and 52% has a peak creatinine that exceeded 2.0 mg/dL (Fig 2). The median peak creatinine was 2.1 mg/dL (range, 1.3 to 4.2). One patient was dialyzed. Other adverse events potentially related to tacrolimus included hyperkalemia (serum potassium >5.5 mEq/dL) (40%), tachycardia (3%), headache (3%), and seizure (3%). None of these events was life-threatening or fatal. Of the patients who were discharged from hospital, all required magnesium replacement, 59% required treatment of hypertension, and 5% required insulin. No patient developed hemolytic-uremic syndrome before day 100.

Transplant-related complications through day 100. Seventy-seven percent of patients were discharged from hospital, and the median time of discharge was day 24 (range days 15 to 55). The incidence of grades 3-4 RRT was 3%. The median maximum RRT was 2, and the median cumulative RRT was 4 (range 2 to 8). Three (10%) patients developed VOD with a bilirubin exceeding 6 mg/dL, and for one patient this was fatal. Eleven patients (41%) developed hemorrhagic cystitis. Other life-threatening or fatal events included nonbacterial thrombotic endocarditis complicated by a myocardial infarction in one patient and toxic epidermal necrolysis presumably caused by an antibiotic in a second patient.

Twenty-five patients had documented infections before day 100. These included 20 with gram positive bacteremia, 3 with gram negative bacteremia, 1 with anaerobic bacteremia, 1 with fungemia, 2 with invasive Aspergillus, and 11 with single or multiple viral infections (4 herpes simplex virus, 6 CMV, and 3 respiratory viruses). The actuarial incidence of CMV infection was 26% for seropositive patients or patients with seropositive donors. The CMV infections included 2 patients with viruria, 3 with pneumonia, and 2

**Fig 1.** Actuarial risk of grades 2-4 (solid line) and grades 3-4 (dashed line) acute GVHD.

**Fig 2.** Actuarial risk of doubling serum creatinine from baseline (solid line) or serum creatinine exceeding 2 mg/dL (dashed line).
with enteritis. The CMV infections were diagnosed within the first 6 weeks posttransplant and contributed to 3 deaths.

Relapse and survival. Seven patients had documented relapse posttransplant. The actuarial relapse rate at 1 year was 28% (Fig 3). Sixteen patients have expired. The causes of death include 6 infection, 2 GVHD and infection, 2 GVHD, 4 relapse, 1 VOD, and 1 nonbacterial thrombotic endocarditis. Day-100 survival is 73% (95% CI, 57% to 89%), and the survival at 1 year is 47% (95% CI, 27% to 66%) (Fig 3).

DISCUSSION

Tacrolimus is a highly potent inhibitor of T-cell activation effective in the treatment and prevention of solid organ allograft rejection and in the treatment of selected autoimmune disorders. Phase II studies also suggested that tacrolimus is effective in the prevention of acute GVHD after HLA-identical marrow transplantation. In this study, we found that use of the combination of tacrolimus and minidose methotrexate resulted in a 34% incidence of grades 2-4 GVHD and a 17% incidence of grades 3-4 GVHD in adult recipients of HLA-matched unrelated donor marrow transplants. The incidence of severe GVHD is lower than reported with the marrow transplant patients, block this enzyme and are potent inhibitors of tacrolimus metabolism. Thus, the reduction in tacrolimus clearance by these drugs could account for the higher blood concentrations and lower dose requirements. Appropriate dosing of tacrolimus for the marrow transplant patients treated with fluconazole or other inhibitors of the cytochrome P450 system remains to be determined.

The dose-schedule of short methotrexate (15 mg/m² on day 1 and 10 mg/m² on days 3, 6, and 11) used in combination with cyclosporine was determined in a canine marrow transplantation model, and the efficacy of the combination was established by randomized studies. Combining cyclosporine with methotrexate did not reduce the amount of methotrexate that could be administered, and reducing or omitting the methotrexate on days 6 or 11 was associated with a significant increase in the risk of acute GVHD shortly thereafter.

The minidose methotrexate regimen (5 mg/m² IV was given on days 1, 3, 6, and 11) was developed at the M.D. Anderson Cancer Center in an effort to reduce mucosal and hepatic complications, and it has proved effective in combination with cyclosporine for prevention of acute GVHD in HLA-identical marrow transplant recipients. Our results show that minidose methotrexate is also effective in combination with tacrolimus as GVHD prophylaxis in the unrelated donor marrow transplant recipients. The efficacy of the reduced dose-schedule of methotrexate was not due to an increase in the area under the time-concentration curve...
(AUC), because no differences in methotrexate AUC were found in a randomized study comparing tacrolimus to cyclosporine,\textsuperscript{23} nor does methotrexate alter the clearance of tacrolimus.\textsuperscript{24} In view of the apparent reduction in risk of early morbidity without loss of efficacy, further evaluation of the minidose methotrexate schedule is warranted.

Infections were clearly a problem in our patients. We found a 26% incidence of CMV infection in the patients at risk, and only half responded to treatment. These infections were not necessarily a failure of ganciclovir prophylaxis, since most developed during the initial period of neutropenia or just after engraftment but before initiation of ganciclovir prophylaxis. These findings may be related to the small population in this study.

In a preliminary evaluation, we recently noted that the risks of acute GVHD and early survival for HLA-nonidentical marrow transplant recipients using tacrolimus and minidose methotrexate were similar to those for patients receiving partially T-cell-depleted marrow transplants and a cyclosporine-based GVHD prophylaxis regimen.\textsuperscript{33} The ability to achieve control of GVHD comparable to that seen with partial T-cell depletion represents an advance for pharmacologic GVHD prophylaxis. Whether the increased risks of infection and relapse seen with T-cell depletion can be avoided by using tacrolimus without manipulations of the marrow is unknown. Controlled trials will be required to firmly determine the comparative activity of this new GVHD prophylaxis regimen.

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