FK506 (Tacrolimus) Monotherapy for Prevention of Graft-Versus-Host Disease After Histocompatible Sibling Allogeneic Bone Marrow Transplantation

FK506 (Tacrolimus) is an immunosuppressive drug that blocks the activation of antigen-specific T lymphocytes, a major component in the pathogenesis of graft-versus-host disease (GVHD). This study was designed to obtain first estimates of the safety and efficacy of FK506 monotherapy in the prevention of GVHD following HLA-identical sibling marrow transplantation. Additionally, a subset of patients was studied to define the pharmacokinetic profile of FK506. Twenty-seven adult patients with leukemia or myelodysplasia received FK506 starting the day before transplant at a dose of 0.04 mg/kg/d by continuous intravenous infusion. When clinically possible, FK506 was given orally in two divided doses starting at five times the daily intravenous dose. FK506 doses were adjusted to target a steady state or trough blood level between 10 to 30 ng/mL. These patients were followed for 6 months posttransplant. All patients had sustained marrow engraftment. Frequent adverse events included reversible renal dysfunction, diarrhea, fever, nausea, vomiting, and headache. Most patients required FK506 dose reductions associated with elevated serum creatinine. Two (7%) patients relapsed, one of whom died of the disease within the 6-month study period. A second patient died due to pulmonary mucor. Whole blood pharmacokinetic parameters indicated a half-life of 18.2 ± 12.1 hours; volume of distribution of 1.67 ± 1.02 L/kg; clearance of 71 ± 34 mL/h/kg; and bioavailability of 32 ± 24%. Eleven of 27 (41%) patients developed grade II to IV acute GVHD, including grade III to IV acute GVHD rates of 54% and 33%, grade III to IV acute GVHD rates of 26% and 17%, and chronic GVHD rates of 58% and 50% in the CSA monotherapy groups. In a retrospective review of 833 adult recipients of cyclosporine monotherapy prophylaxis after HLA-identical sibling BMT, the overall incidence of acute GVHD was 43 ± 3%.1 Additionally, other studies of cyclosporine monotherapy for GVHD prophylaxis have reported rates of grade II to IV acute GVHD ranging from 27% to 47% and chronic GVHD rates ranging from 30% to 73%.4-6 Although clinical studies have demonstrated that the combination of cyclosporine with methotrexate or cyclosporine with methotrexate and prednisone is more effective than monotherapy regimens for the prevention of acute GVHD,1-5-11 the development of new agents may permit a further reduction in the incidence or severity of GVHD or a reduction in the toxicity associated with posttransplant immunosuppression.

FK506 did not impair marrow engraftment and was effective in the prevention of GVHD in preclinical studies of allogeneic marrow transplantation.11-14 In addition, FK506 prevented both graft rejection and GVHD in preclinical studies of limb and small-bowel allografting.15-17

In preliminary clinical trials, FK506 demonstrated efficacy in the prevention and treatment of acute GVHD and in reversing or stabilizing chronic GVHD following allogeneic marrow transplantation.18-20 FK506 has been effective in the prevention and treatment of graft rejection in solid organ transplantation.21-25

Based on these observations, we conducted a multicenter phase II study to evaluate the safety and efficacy of FK506 monotherapy for the prevention of GVHD following genotypically identical allogeneic BMT therapy for leukemia or myelodysplasia. In addition, a subset of patients was studied to define the pharmacokinetic profile of FK506 in this patient population.

MATERIALS AND METHODS

Patients. Adult patients undergoing allogeneic BMT therapy using genotypically matched marrow donors for leukemia or myelodysplasia were enrolled in the study from four participating centers: the Sammons Cancer Center, Baylor University Medical Center (Dallas, TX); the University of Minnesota (Minneapolis, MN); Emory University (Atlanta, GA); and the Brigham and Women's Hospital, Harvard University (Boston, MA). The protocol and consent forms were approved by each center's Institutional Review Board for Human Investigation. Written informed consent was obtained from all patients. Patients were excluded from the study if they had a prestudy Karnofsky score < 80%, estimated creatinine clearance < 60 mL/min, total bilirubin above the upper limit of normal, SGOT or SGPT > 1.5 × upper limit of normal, FVC or FEV1 < 75% predicted, cardiac ejection fraction < 50%, or known hypersensitivity to Cremophor or related products. In addition, patients were...
excluded if they were carriers of any of the human immunodeficiency viruses, recipients of T-cell depleted marrow, or pregnant.

After study eligibility was established, patients received pretransplant cytotoxic conditioning per each institution's protocol as shown in Table 1. The bone marrow was infused on day zero. The use of hematopoietic growth factors, chronic phenytoin or phenobarbital, prophylactic fluconazole, and pentoxifylline were prohibited.

GVHD prophylaxis. The prophylactic GVHD immunosuppressive regimen in all patients was FK506 monotherapy. The initial dose of FK506, based on lean or actual body weight (whichever was less), was 0.04 mg/kg/d intravenously as a continuous infusion starting on the day before marrow transplant. When oral administration was feasible, patients were switched to oral FK506 at five times the mean whole blood levels ranging from 16 to 8 to 37 ± 38 ng/mL at 24 hours posttransplant. Beyond day 7, intravenous FK506 doses were lowered primarily due to the elevated serum creatinine to approximately 0.03 mg/kg/d resulting in pooled mean whole blood levels ranging from 23 ± 17 to 23 ± 14 ng/mL. The median duration on intravenous therapy was 26 (6 to 39) days. Subsequent oral adjusted FK506 doses, before taper, were between 0.12 ± 0.07 and 0.15 ± 0.07 mg/kg/d, with corresponding pooled mean whole blood levels ranging from 16 ± 10 to 23 ± 13 ng/mL.

Adverse events. Nephrotoxicity was the primary adverse event associated with FK506. Twenty-five (92.6%) patients had a doubling of their baseline serum creatinine levels during the 6-month study period, with a mean peak serum creatinine of 2.6 mg/dL. Renal function improved when FK506 doses were reduced or temporarily held. The mean serum creatinine to approximately 2.3 mg/dL on day 147, and FK506 was inadvertently not resuming. This patient did not receive subsequent immunosuppressive therapy and did not develop acute or chronic GVHD. A second patient developed an elevated serum creatinine of 2.3 mg/dL on day 147, and FK506 was discontinued.

### Table 1. Patient Demographics and Disease Characteristics

<table>
<thead>
<tr>
<th>Patient</th>
<th>27</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary diagnosis, N (%)</td>
<td>27</td>
</tr>
<tr>
<td>CML, 1st chronic phase</td>
<td>13 (48)</td>
</tr>
<tr>
<td>AML, 1st or 2nd complete remission</td>
<td>6 (22)</td>
</tr>
<tr>
<td>Relapse</td>
<td>1 (4)</td>
</tr>
<tr>
<td>MDS</td>
<td>6 (22)</td>
</tr>
<tr>
<td>ALL, 1st complete remission</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>40</td>
</tr>
<tr>
<td>Range</td>
<td>18-53</td>
</tr>
<tr>
<td>Preparative regimen, N (%)</td>
<td>9 (33)</td>
</tr>
<tr>
<td>Cyclophosphamide, TBI</td>
<td>8 (30)</td>
</tr>
<tr>
<td>Etoposide, cyclophosphamide, TBI</td>
<td>8 (30)</td>
</tr>
<tr>
<td>Busulfan, cyclophosphamide</td>
<td>7 (26)</td>
</tr>
<tr>
<td>Cyclophosphamide, cytosine arabinoside, TBI</td>
<td>2 (7)</td>
</tr>
</tbody>
</table>

| Donor-recipient matching, N (%) | 18 (67) |
| For sex | 9 (33) |
| Match | 10 (37) |
| Mismatch | 5 (19) |
| For sex and parity* | 3 (11) |
| Male/female | 7 (26) |
| Parous female/male | 4 (14) |
| Parous female/female | 1 (4) |

Abbreviations: AML, acute myelogenous leukemia; ALL, acute lymphocytic leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplasia; TBI, total body irradiation.

* Parity status of one female donor to male patient is unknown.

RESULTS

From April 1992 to September 1992, 27 adult patients were enrolled in this trial. Patient characteristics are shown in Table 1.

**Drug administration/blood levels.** The initial intravenous FK506 dose of 0.04 mg/kg per day was maintained through 7 days posttransplant. In the patients as a group, the mean whole blood levels of FK506 ranged from 21 ± 8 to 37 ± 38 ng/mL from day 1 through day 7 posttransplant. Beyond day 7, intravenous FK506 doses were lowered primarily due to the elevated serum creatinine to approximately 0.03 mg/kg/d resulting in pooled mean whole blood levels ranging from 23 ± 17 to 23 ± 14 ng/mL. The median duration on intravenous therapy was 26 (6 to 39) days. Subsequent oral adjusted FK506 doses, before taper, were between 0.12 ± 0.07 and 0.15 ± 0.07 mg/kg/d, with corresponding pooled mean whole blood levels ranging from 16 ± 10 to 23 ± 13 ng/mL.

Adverse event monitoring. Patients were clinically evaluated throughout the 6-month study period. All adverse events, whether or not considered related to the administration of FK506, were recorded and assessed for purposes of drug safety and subsequent tabulation. In addition, laboratory studies, including a complete blood count and serum chemistries, were obtained routinely. Renal dysfunction was defined as a doubling of serum creatinine over baseline. All concomitant medications were recorded and were used to assess treatment requirements for hyperglycemia, hypertension, and GVHD.

**Pharmacokinetics.** In 12 patients, serial blood samples were obtained over 24 hours following a single intravenous dose of FK506 at 0.02 mg/kg infused over a 4-hour period to determine the terminal half-life, total body clearance, and volume of distribution of FK506. Blood samples were drawn before and at 1, 2, 4, 2.5, 4.5, 4.75, 5, 6, 7, 8, 10, 12, 16, and 24 hours subsequent to initiation of the intravenous infusion. In 11 of the 12 patients, serial blood samples were obtained over 12 hours following consistent oral dosing for 4 consecutive days to assess bioavailability. Blood samples were drawn before and at 0.33, 0.66, 1, 1.5, 2, 3, 4, 6, 8, 10, and 12 hours after dosing.

**Grading of GVHD.** Acute and chronic GVHD were graded and staged by clinicians at the study centers using previously described criteria. Biopsies were obtained when indicated to corroborate the clinical diagnosis of GVHD, but were not required.

**Statistical analysis.** The primary efficacy end point was to measure the incidence of moderate-to-severe acute GVHD (grade II to IV) and chronic GVHD within the 6-month study period. Results are reported for all patients enrolled in the study (intention-to-treat analysis). Patients were censored for GVHD end points at the time of relapse or death. Kaplan-Meier curves were generated for relapse-free survival and patient survival.
Eleven of the 27 patients developed grade II to IV acute GVHD with a cumulative incidence of 41% (95% CI = 22% to 59%) (Fig 1). One patient (4%) had grade III and none had grade IV acute GVHD. The median time to onset of grade II to IV acute GVHD was 16 (11 to 43) days. Organ involvement associated with grade II to IV acute GVHD included three patients with only skin involve-
ment, one patient with isolated involvement of the gastrointestinal tract, four patients with skin and gastrointestinal involvement, and three patients with skin and liver involvement. GVHD was confirmed histologically by biopsy in eight of 10 patients with skin involvement and four of five patients with gastrointestinal involvement. No liver biopsies were obtained. All 10 patients who developed grade II acute GVHD responded to systemic steroids. The one patient who developed grade III acute GVHD received systemic steroids as first-line therapy with partial response and subsequently received azathioprine.

Chronic GVHD. Six of 24 patients alive and relapse free at 100 days posttransplant developed chronic GVHD during the 6-month follow-up period. The median time to onset of chronic GVHD was 129 days. Five were categorized as having extensive disease and one as having limited disease. The 6-month Kaplan-Meier estimate of chronic GVHD is 30.6% (95% CI = 9% to 53%) and is shown in Fig 2. One patient developed de novo chronic GVHD, and one patient developed chronic GVHD 6 weeks after being inadvertently tapered off FK506 by day 145. All six patients with chronic GVHD were treated with systemic steroids as first-line therapy.

Four patients were managed with CSA or CSA and azathioprine after FK506 had been discontinued or the study had been completed. One patient with extensive chronic GVHD received treatment with CSA, azathioprine, and psoralen-ultraviolet A light.

Two patients experienced recurrent leukemia posttransplant during the study period. One patient transplanted in first complete remission with acute lymphocytic leukemia and the Philadelphia chromosome relapsed on day 119 posttransplant. The second patient with acute myelogenous leukemia transplanted in first relapse developed recurrent disease on day 41 posttransplant. The 6-month Kaplan-Meier estimate of relapse-free survival is 89% (95% CI = 77% to 100%) and is shown in Fig 3.

Two patients died during the study period. One patient with myelodysplasia died on day 53 due to pulmonary mucormycosis. The patient with acute myelogenous leukemia...
who relapsed on day 41 died on day 186 posttransplant. FK506 had been discontinued at the time of relapse on day 41. The 6-month Kaplan-Meier estimate of patient survival was 91% (95% CI = 78% to 100%).

**Pharmacokinetics.** The terminal half-life, total body clearance, and volume of distribution of FK506 in 12 patients and the bioavailability of oral FK506 in 11 patients were determined by noncompartmental analysis. For oral FK506, these studies were performed between 23 and 43 days posttransplant. Following intravenous administration, the terminal half-life of FK506 was 18.2 ± 12.1 hours, the total body clearance was 71 ± 34 mL/h/kg, and the volume of distribution was 1.67 ± 1.02 L/kg. Following oral administration, the mean peak concentration of FK506 in whole blood was 49.8 ± 49.4 ng/mL, and was achieved in a mean time of 2.0 ± 1.6 hours. Based on a comparison of intravenous AUC0.12 at steady state, the bioavailability of FK506 in bone marrow transplant patients was 32 ± 24%.

**DISCUSSION**

The primary objective of the study was to obtain estimates of the safety and efficacy of FK506 in preventing GVHD after allogeneic BMT. The design of the trial, using FK506 as a single agent, enabled a clear estimate of these parameters.

FK506 is a heterocyclic macrolide (molecular weight 822 daltons) with potent immunosuppressive activity determined both in vitro and in vivo. FK506 was approximately 100 times more potent than CSA in inhibiting the generation of murine and human cytotoxic T lymphocytes and the mixed lymphocyte reaction assay. The pathophysiology of GVHD involves antigen activated T cells and monocytes leading to direct and indirect cytotoxicity of epithelial cells of the skin, gut, and liver. Activated T cells release interleukin (IL)-2 and interferon-gamma, which activate macrophages to produce inflammatory proteins like tumor necrosis factor-alpha and IL-1, resulting in nonspecific tissue injury and the clinical picture of acute GVHD. FK506 effectively inhibited T-lymphocyte activation, the formation of IL-2 by T lymphocytes, and the formation of other soluble mediators that are responsible, in part, for the development of acute GVHD.

FK506 was reasonably well tolerated in this study. Renal dysfunction was the most consistent adverse event associated with FK506, resulting in dose reduction. Renal function improved with dose reductions or a temporary hold in the administration of FK506. Renal dysfunction in the postmarrow transplant setting often is exacerbated by the concomitant use of other nephrotoxic drugs frequently used in the management of marrow transplant recipients; thus, monitoring serum creatinine and making appropriate dose adjustments while maintaining adequate blood levels are essential. The spectrum of renal dysfunction associated with FK506 in this trial was similar to CSA monotherapy trials. The mean peak serum creatinine in two CSA monotherapy trials was 2.4 mg/dL, percent of patients with doubling of their serum creatinine from baseline ranged from 58% to 92%, and the percent of patients requiring hemodialysis ranged from 6% (2 of 36 patients) to 16% (8 of 50 patients). Whether there is a difference in renal toxicity between FK506 and CSA-based immunosuppressive regimens following transplantation can only be determined by a prospective, randomized, controlled trial.

Hepatotoxicity, as evidenced by a median peak total bilirubin of 1.5 mg/dL, was lower than the 2.6 mg/dL reported in a CSA monotherapy trial. The incidence of hypertension was similar to reported rates of 39% and 60% of patients in CSA monotherapy trials. Eleven patients in this trial required antihyperglycemic therapy, three of whom remained insulin-dependent at 6 months posttransplant. The incidence of hyperglycemia with CSA monotherapy is not well documented for comparison. Headaches were commonly reported in our patients, but none were considered severe. However, the dose of FK506 was reduced in one patient with subsequent improvement. Nausea, vomiting, and diarrhea noted in our patients may be secondary to FK506, the pretransplant cytotoxic regimen, or GVHD. Fever is a common consequence of neutropenia after BMT and was expected in this patient population. The 48-year old female patient with myelodysplasia who developed TTP posttransplant received high-dose busulfan and cyclophosphamide before BMT. This patient had full recovery from TTP with plasma-exchange transfusion therapy. TTP or TTP-like syndromes have been associated with FK506 and CSA following solid organ transplantation and with cyclosporine following allogeneic BMT. The incidence in each clinical setting is uncommon, and the precise pathophysiology is obscure.

FK506 monotherapy was effective in the prevention of GVHD. The overall incidence of acute GVHD in this trial was comparable to that reported in previous studies of CSA monotherapy, and the incidence of grade III to IV acute GVHD was less. One patient (9%) required second-line therapy for acute GVHD in this trial, compared with 31% and 45% in CSA monotherapy studies. The rate of chronic GVHD was relatively low compared with reported rates with cyclosporine monotherapy. However, the follow-up period in this noncomparative study was limited to 210 days posttransplant.

Pharmacokinetic-parameter estimates are heterogeneous...
as reflected in the large standard deviations. However, the overall results suggest a larger volume of distribution and higher oral bioavailability and clearance than reported in liver transplant patients. Therefore, dosing regimens must be specific to the BMT population and cannot be generalized from other patient groups.

We conclude that FK506 monotherapy has an acceptable safety profile and is effective in the prevention of acute GVHD following HLA-identical sibling marrow transplantation for leukemia and myelodysplasia. No deleterious effects on marrow recovery following transplantation or early (6 months) posttransplant relapse and survival have been observed and follow-up continues. In preclinical studies using a canine model with major histocompatibility complex mismatched donors, methotrexate added to FK506 reduced the incidence of GVHD and improved survival. In Phase II trials with patients receiving marrow transplantation from unrelated donors, FK506 combined with methotrexate or corticosteroids was effective in the prevention of GVHD. Whether FK506 in combination with methotrexate will result in an improvement in the prevention of GVHD compared with cyclosporine in combination with methotrexate following allogeneic sibling-matched marrow transplantation is the subject of an ongoing phase III study. The initial intravenous dose in these subsequent trials has been reduced from 0.04 mg/kg/d to 0.03 mg/kg/d to improve tolerability, particularly dose-limiting nephrotoxicity.

REFERENCES


