1083-8791/04/1004-0004$30.00/0
doi:10.1016/j.bbmt.2003.11.005

A Pilot Study of Tacrolimus and Mycophenolate Mofetil Graft-versus-Host Disease Prophylaxis in Childhood and Adolescent Allogeneic Stem Cell Transplant Recipients

Ifeyinwa Osunkwo,[1] Olga Bessmertny,[2] Lauren Harrison,[1] Ying-Kuen Cheung,[3] Carmella Van De Ven,[1] Gustavo del Toro,[1] James Garvin,[1] Diane George,[1] M. Brigid Bradley,[1] Karen Wolownik,[1] Cheryl Wischhover,[1] Joseph Levy,[1] Donna Skerrett,[4] Mitchell S. Cairo[1]

[1]Department of Pediatrics, [2]Department of Pharmacy, [3]Division of Biostatistics, and [4]Department of Pathology, Children's Hospital of New York-Presbyterian, Columbia University, New York, New York

Correspondence and reprint requests: Mitchell S. Cairo, MD, Columbia University, 161 Fort Washington Ave., 7th Floor, Room 754, New York, NY 10032 (e-mail: mc1310@columbia.edu).

Received July 28, 2003; accepted November 19, 2003

ABSTRACT

Tacrolimus (FK506)/mycophenolate mofetil (MMF) has been demonstrated to be an effective salvage therapy for steroid-resistant chronic graft-versus-host disease (GVHD), but its effectiveness as prophylaxis for acute GVHD (aGVHD) is unknown. We investigated the safety and efficacy of FK506/MMF in preventing aGVHD and sparing the use of methotrexate and methylprednisolone in childhood and adolescent allogeneic stem cell transplant (AlloSCT) recipients. Thirty-four childhood and adolescent patients (median age, 7 years; range, 0.5-21 years; 24 males and 10 females) undergoing 37 AlloSCTs for malignant (n = 22) and nonmalignant (n = 12) disorders received FK506 (0.03 mg/kg/d by continuous intravenous infusion) and MMF (15 mg/kg per dose orally or intravenously twice daily). Stem cell sources included 22 umbilical cord blood donors (21 unrelated and 1 related), 6 related bone marrow donors, and 9 related peripheral blood donors. Malignant diagnoses included 7 acute lymphoblastic leukemias, 3 acute myeloid leukemias, 1 acute promyelocytic leukemia, 2 non-Hodgkin lymphomas, 4 Hodgkin diseases, 3 chronic myeloid leukemias, and 2 neuroblastomas; nonmalignant diagnoses included 2 β-thalassemias, 1 sickle cell disease, 4 aplastic anemias, 1 Wiskott-Aldrich syndrome, 1 Hurler syndrome, 2 hemophagocytic lymphohistiocytoses, and 1 myelodysplastic syndrome. The probability of developing grade ≥II aGVHD was 45.4% ± 9.7% (7 related bone marrow/related peripheral blood; 5 umbilical cord blood), and for chronic GVHD it was 38.1% ± 19.7%. FK506/MMF was well tolerated. Three patients had grade III to IV neurotoxicity (disorientation and leukoencephalopathy); 4 patients developed grade III to IV nephrotoxicity (all received concomitant nephrotoxins). Patients who achieved target mycophenolic acid levels (1.0-3.5 μg/mL) before day +30 had a significantly reduced incidence of developing grade ≥II aGVHD (16.7% ± 15.2% versus 100%; $P < .02$). These results suggest that FK506/MMF is well tolerated and may be a safe and effective methotrexate- and methylprednisolone-sparing alternative GVHD prophylaxis regimen after AlloSCT. Further pharmacokinetic and pharmacodynamic studies are ongoing in pediatric and adolescent AlloSCT recipients to define optimal MMF dosing.

KEY WORDS

Tacrolimus • Mycophenolate mofetil • Graft-versus-host disease • Allogeneic stem cell transplantation

INTRODUCTION

Acute graft-versus-host disease (aGVHD) is a potentially fatal complication after allogeneic hematopoietic stem cell transplantation (AlloSCT) and results in large part from alloreactive donor T cells reacting against HLA-disparate host antigens [1]. The incidence of clinically significant aGVHD ranges from 9% to 50% among HLA-matched sibling AlloSCT recipients and varies with the degree of histoincompatibility, the recipient and donor ages, the source and quality of donor T lymphocytes, the incidence of

cytomegalovirus (CMV) infection, and the type of GVHD prophylaxis strategy [2]. Despite the use of prophylactic immunosuppressive agents such as cyclosporine (CsA), methylprednisolone (PDN), methotrexate (MTX), and antithymocyte globulin, approximately 60% to 80% of patients who undergo HLA-identical but unrelated adult donor marrow transplantation without T-cell depletion and 40% to 60% of patients who receive HLA-disparate unrelated umbilical cord blood (UCB) transplants will develop grade II to IV aGVHD [3-5]. Furthermore, we and others have demonstrated that recipients of HLA-disparate unrelated UCB transplants experience a 40% to 60% risk of developing grade ≥II aGVHD [6-9].

Historically, CsA and MTX (with or without PDN) have been used for aGVHD prophylaxis after matched related and unrelated allogeneic donor transplantation and have been shown in randomized trials to be superior to either drug alone in preventing severe grade II to IV aGVHD [10,11]. Although the efficacy of these regimens (MTX and CsA with or without PDN) in preventing moderate to severe aGVHD after AlloSCT ranges from 20% to 50%, each of these agents is also associated with significant organ toxicity. CsA is associated with renal, hepatic, and central nervous system toxicity; unpleasant cosmetic side effects (hirsutism and gingival hyperplasia); and multiple drug-drug interactions [12-15]. MTX enhances conditioning regimen–induced mucositis; may cause hepatotoxicity, nephrotoxicity, and myelosuppression; and may secondarily delay engraftment [16]. PDN may precipitate glucose intolerance, hypertension, and osteopenia [17,18].

Tacrolimus (FK506), a heterocyclic macrolide antibiotic isolated from the broth of *Streptomyces tsukubaensis*, is a new GVHD prophylaxis agent similar to but 50 to 200 times more potent an immunosuppressant than CsA [19,20]. FK506 inhibits calcineurin, resulting in inhibition of interleukin-2 production and decreased proliferation of antigen-specific T lymphocytes [19,20]. In 3 randomized human trials, FK506 in combination with standard-dose MTX was associated with a significantly lower incidence of aGVHD compared with CsA/MTX [21-23].

Mycophenolate mofetil (MMF), another immunosuppressive agent, works through its active metabolite mycophenolic acid (MPA) on proliferating lymphocytes by noncompetitively inhibiting both isoforms of inosine monophosphate dehydrogenase, the rate-limiting enzyme in de novo purine synthesis [24]. MMF augments the efficacy of standard immunosuppressants without overlapping toxicities. MMF has been demonstrated to be effective in the treatment of adults with aGVHD and chronic GVHD (cGVHD) [25], results in reduced use of PDN-based therapies, and can be safely combined with MTX and PDN [26]. FK506 in combination with MMF has been used effectively as salvage therapy for steroid-resistant cGVHD in adult AlloSCT recipients [27], as well as in solid organ transplant (SOT) recipients. FK506/MMF, in comparison with CsA/MMF, seems to be superior in preventing allograft rejection, with improved graft function at 2 years after renal cadaveric transplantation [28].

We postulate that this combination may be an effective alternative for aGVHD prophylaxis and may additionally allow sparing of MTX, PDN, or both. We report preliminary results of MPA steady-state trough concentration monitoring and its correlation with the development of aGVHD in this population.

MATERIALS AND METHODS

Patients

Between October 2000 and April 2003, we investigated the safety and efficacy of FK506/MMF for GVHD prophylaxis in 37 AlloSCT patients by using both related and unrelated donors in 34 pediatric and adolescent recipients (≤22 years old; Table 1). All patients were enrolled on Columbia University Institutional Review Board–approved protocols. All patients and/or parents signed an appropriate institutional review board–approved informed consent before study entry. Poor-risk patients were defined as patients in malignant relapse, with progressive malignant disease, and in third or greater complete malignant response (CR ≥3) and/or those receiving a second AlloSCT. All other patients were defined as average risk.

HLA Typing and Allogeneic Donors

HLA typing was performed by serology for HLA-A and -B and by high resolution DNA typing for -DRB1. Matched family donors (MFD) were required to be a 6/6 or 5/6 HLA match, whereas UCB (related and unrelated) donors had to be 6/6, 5/6, or 4/6 HLA matched. UCB donor units were also required to have a minimum of 1.5×10^7 nucleated cells of cryopreserved UCB units per kilogram recipient body weight.

Conditioning Regimens

Conditioning regimens were diverse; 57% of patients received myeloablative conditioning regimens, and 43% received fludarabine-based reduced-intensity conditioning regimens (Table 2). Eleven patients (31%) received total body irradiation.

GVHD Prophylaxis

FK506 was administered either intravenously (IV) at 0.03 mg/kg/d by continuous infusion or orally (PO)

Table 1. *Patient Characteristics*

Variable	Data
No. patients	**34 (37 AlloSCT)**
Median age, y (range)	**7 (0.5-21)**
Sex	
Male	**24 (70%)**
Female	**10 (30%)**
Diagnosis (n = 34)	
Malignant (n = 22)	
ALL (5 CR2, 2 CR3)	**7**
AML (1 CR1, 1 CR2, 1 refractory)	**3**
APL (CR2)	**1**
NHL (1 CR2, 1 PR2)	**2**
HD (2 CR2, 1 PD, 1 PR2)	**4**
CML-CP	**3**
Neuroblastoma (1 CR1, 1 PD)	**2**
Nonmalignant (n = 12)	
β-Thalassemia	**2**
SCD	**1**
Aplastic anemia	**4**
WAS	**1**
Hurler's syndrome	**1**
HLH	**2**
MDS	**1**
Transplant history	
Autologous	**7**
Allogeneic	**3**
Transplant source (allogeneic)	
Cord blood	**22 (57%)**
Marrow	**6 (18%)**
PBSC	**9 (25%)**
Preparative regimen	
Reduced intensity	**16 (43%)**
Myeloablative	**21 (57%)**
HLA disparity	
None (6/6)	**16**
Class I (5/6)	**6**
Class II (5/6)	**1**
Double class I (4/6)	**9**
Class I and II (4/6)	**3**
Double class II (4/6)	**2**

ALL indicates acute lymphoblastic leukemia; AML, acute myelogenous leukemia; APL, acute promyelocytic leukemia; NHL, non-Hodgkin lymphoma; HD, Hodgkin disease; CML-CP, chronic myelogenous leukemia in chronic phase; SCD, sickle cell disease; WAS, Wiscott-Aldrich syndrome; HLH, hemophagocytic lymphohistiocytosis; MDS, myelodysplastic syndrome; PBSC, peripheral blood stem cell; PD, progressive disease; CR, complete response; PR, partial response.

at 0.12 mg/kg/d in 2 divided doses starting on day −1 or on the first day of conditioning (protocol dependent). Doses were adjusted to maintain FK506 random (IV) or trough (PO) concentrations between 5 and 20 ng/mL (whole-blood enzyme-linked immunosorbent assay) [29]. Patients were converted from IV to PO FK506 at a ratio of 4:1 (PO/IV dose) when they were able to take medications orally. FK506 was tapered after day +60 if patients had grade ≤II aGVHD. MMF was administered daily starting on day +1 at a dose of 15 mg/kg per dose IV or PO twice daily. Beginning in January 2002, MMF doses were adjusted to maintain MPA trough concentrations within a reference range of 1 to 3.5 μg/mL. Patients were initiated on IV MMF and were converted to a PO formulation when this was clinically appropriate or when the IV formulation was not available. MPA/MPA glucuronide (MPAG) trough concentrations were obtained after 3 consecutive days of dosing at each MMF dose level and upon switching from an IV to PO formulation. MPA/MPAG trough concentrations were monitored every 2 weeks in patients who achieved target levels. MMF was discontinued or tapered after day +28 (per each individual protocol) if patients had grade ≤II aGVHD.

GVHD was graded according to the Seattle consensus criteria [30]. The "rule of 9" or a burn chart was used to estimate the extent of skin rash. Patients were staged and graded once a week for the occurrence of aGVHD. Once a clinical diagnosis of aGVHD or cGVHD was determined, histologic confirmation was obtained if possible.

FK506/MPA Trough Concentrations

FK506 whole-blood concentrations were measured with the IMx Tacrolimus II assay (Abbott Laboratories, Abbott Park, IL) [29] based on the macroparticle enzyme immunoassay technique. Blood samples for the FK506 assay were drawn through indwelling catheters not used for infusion of FK506.

Table 2. *Conditioning Regimens*

Regimen	n
Myeloablative (n = 21)	
TBI/Cy	**3**
TBI/Cy/Etopophos	**2**
TBI/Cy/thiotepa/ATG	**1**
TBI/Mel	**3**
TBI/Mel/CAMPATH	**1**
TBI/Mel/ATG	**1**
Cy/ATGAM	**1**
Bu/Cy	**2**
Bu/Cy/CAMPATH	**2**
Bu/Mel/ATG	**2**
Bu/Cy/ATG/VP-16	**1**
Flu/Cy/ATG	**2**
Nonmyeloablative (n = 16)	
Flu/Bu/ATG	**7**
Flu/Cy/ATG	**5**
Flu/Bu/CAMPATH	**2**
Flu/Cy/Etopophos/ATG	**1**
Flu/Bu	**1**

TBI indicates total body irradiation (1200 cGy); Bu, busulfan (6.4-16 mg/kg); Flu, fludarabine (150-180 mg/m^2); Cy, cyclophosphamide (120 mg/kg); Mel, melphalan (135 mg/m^2); ATG, rabbit antithymocyte globulin (4.5-8 mg/kg); ATGAM, horse antithymocyte globulin (90 mg/kg); CAMPATH, alemtuzumab (54 mg/m^2); Etopophos, etoposide phosphate (40 mg/kg); VP-16, etoposide (30 mg/kg).

Serum levels of MPA and MPAG were determined by using reverse-phase high-performance liquid chromatography (Shimadzu, Kyoto, Japan) [31]. A total of 2 to 3 mL of peripheral blood was drawn into a red-topped (plain) tube, and plasma was separated and shipped to Mayo Clinical Laboratories (Rochester, MN) for analysis.

Supportive Care

All patients were hospitalized in protective isolation, defined as private/single rooms with a high-efficiency particulate air filtration system and reverse isolation with mask and strict hand washing. Broad-spectrum antibiotics were used to treat initial episodes of febrile neutropenia. All patients received anti-infective prophylaxis against *Pneumocystis carinii* pneumonia, fungal infections, herpes simplex virus, and CMV (seropositive recipients and/or donors only). Trimethoprim/sulfamethoxazole for *Pneumocystis carinii* pneumonia prophylaxis was given daily during the preparative regimen until day −2 and was resumed after transplantation at 5 mg/kg/d PO divided twice daily 3 times a week upon myeloid engraftment (absolute neutrophil count [ANC] $\geq$500/mm^3 for 2 consecutive days). Patients who did not engraft by day +42 were given pentamidine 4 mg/kg IV every 2 weeks. All but 2 patients received fungal prophylaxis with liposomal amphotericin B at 3 mg/kg/d IV over 2 hours starting on day 0 until day +100, as we have previously described [32]. Acyclovir 250 mg/m^2 per dose IV was administered every 8 hours for herpes simplex virus prophylaxis to all patients until myeloid engraftment and resolution of mucositis (grade <II). Patients who were CMV seropositive or who received allogeneic stem cells from a CMV-seropositive donor were given a prophylactic alternate-day regimen of IV foscarnet (90 mg/kg per dose every 48 hours) alternating with IV ganciclovir (5 mg/kg per dose every 48 hours) starting after myeloid engraftment until day +100 if no GVHD was evident, as we have previously described [33].

All patients received hematopoietic growth factors starting on day 0 with sargramostim (granulocyte-macrophage colony-stimulating factor) at 250 μg/m^2/d IV over 2 hours within 3 hours of stem cell infusion until the white blood cell count was $\geq$300/mm^3 for 2 consecutive days. Patients were then switched to filgrastim (granulocyte colony-stimulating factor; G-CSF) at 10 μg/kg/d IV over 15 to 30 minutes. This was tapered by 50% when ANC reached $\geq$2500/mm^3 for 2 days.

Irradiated, leukodepleted packed red blood cell transfusions of 10 to 15 mL/kg were given to maintain hemoglobin $\geq$8.0 g/dL. Patients received transfusions of 1 random unit of irradiated leukodepleted platelets per 10 kg body weight to maintain platelet counts >20000/mm^3. All blood products were leukodepleted by using a 170- to 250-μm filter and irradiated with 25 Gy. All CMV-seronegative patients with CMV-negative donors received leukocyte-reduced or CMV-seronegative blood products to prevent transfusion-associated CMV infection. Patients were also followed up clinically for the development of veno-occlusive disease, which was graded for severity by using the criteria of Bearman et al. [34].

Statistical Analysis

Results are presented as medians with specified ranges of data sets. Probabilities of survival and incidences of aGVHD or cGVHD were calculated with the Kaplan-Meier method performed with the Prism statistical program (Graph Pad, San Diego, CA).

RESULTS

Patients and Donors

From October 2000 to April 2003, 34 children and adolescents with malignant and nonmalignant disease received 37 AlloSCT MFD or UCB transplants at the Children's Hospital of New York-Presbyterian. Patient characteristics are summarized in Table 1. The median age was 7 years (range, 0.5-21 years). The sex distribution was 24 males and 10 females. The disease distribution was as follows: 7 patients with acute lymphoblastic leukemia (5 in CR2 and 2 in CR3), 3 with acute myeloid leukemia (1 in CR1, 1 in CR2, and 1 refractory), 3 with chronic myeloid leukemia (CML; chronic phase), 4 with Hodgkin disease (2 in CR2, 1 in second partial response, and 1 with progressive disease), 2 with non-Hodgkin lymphoma (1 in CR2 and 1 in second partial response), 2 with neuroblastoma (1 in CR1 with aplasia and 1 with progressive disease), 4 with aplastic anemia (severe), 2 with hemophagocytic lymphohistiocytosis (HLH), and 2 with β-thalassemia. One patient each had sickle cell disease, Wiskott-Aldrich syndrome, Hurler syndrome, acute promyelocytic leukemia (CR2), and myelodysplastic syndrome (MDS) without malignant transformation.

Three patients underwent a second AlloSCT because of graft failure. Two (1 each with B thalassemia and Hurler's syndrome) had undergone a prior reduced-intensity transplantation and underwent retransplantation on days +124 and +62. One patient with HLH had received a prior fully ablative transplant and underwent retransplantation on day +183. None of these patients was evaluable for aGVHD or cGVHD before receiving their second transplant.

Patients received AlloSCTs from either MFD (n = 15) or UCB (n = 22) (Table 3). Eight 6/6 and one 5/6 HLA-matched MFD received G-CSF–mobi-

Table 3. *Patient Demographics, Allogeneic Stem Cell Source, HLA Match, Cell Dose, and Preparative Regimen*

Patient No.	Age (y)	Sex	Disease Status	HLA/Donor	Mismatch	TNC (kg × 10^7)	CD34 (kg × 10^5)	Preparative Regimen (RI versus M)
599-068	2	M	ALL CR2	4/6 UCB	Class II × 2	6.5	3.76	TBI/Mel (M)
599-072	16	F	CML-CP	6/6 PBSC	0	30.9	46.6	Flu/Cy/ATG (RI)
599-073	14	M	AML PD*	5/6 UCB	Class I	1.12	0.6	TBI/Mel (M)
599-075	12	M	AA	6/6 BM	0	51.4	78.6	Cy/ATGAM (M)
599-077	6	F	ALL CR2	6/6 PBSC	0	5.1	7.6	TBI/Cy/Etop (M)
599-079	18	M	AML CR2	4/6 UCB	Class I/II	1.42	0.43	TBI/Cy (M)
599-080	10	F	NHL CR2	4/6 UCB	Class I/II	3.67	3.1	TBI/Cy (M)
599-081	8	F	ALL CR2	4/6 UCB	Class II × 2	3.81	2.8	TBI/Cy (M)
599-083	20	M	HD PD*	4/6 UCB	Class I × 2	1.64	0.89	Flu/Cy/ATG (RI)
599-085	5	M	NBL PD*	4/6 UCB	Class I × 2	6.8	1.65	Flu/Bu/ATG (RI)
599-086	3	M	Sickle cell	6/6 BM	0	22.5	51.3	Bu/Cy (M)
599-088	2	M	WAS	5/6 UCB	Class I	6.78	2.14	Flu/Cy/ATG (RI)
599-091	11	M	ALL CR3*	5/6 UCB	Class I	2.13	1.28	TBI/Mel/ATG (M)
501-001	17	F	HD CR2	4/6 UCB	Class I × 2	2.85	1	Flu/Bu/ATG (RI)
501-002	21	M	HD CR2	6/6 PBSC	0	47.0	53.3	Flu/Bu (RI)
501-003	18	F	HD PR2*	4/6 UCB	Class I × 2	4.9	4.4	Flu/Bu/ATG (RI)
501-004	11	M	NHL PR2	4/6 UCB	Class I × 2	3.77	1.96	Flu/Bu/ATG (RI)
503-001	0.5	F	B-Thal	5/6 UCB	Class I	9.5	5.32	Flu/Bu/ATG (RI)
503-002	1.5	M	B-Thal	6/6 BM	0	154.8	63.4	Fu/Bu/Alem (RI)
599-095	0.75	M	HLH	6/6 UCB	0	10.4	2.26	Bu/Cy/ATG/VP-16 (M)
599-093	3	M	ALL CR2	6/6 PBSC	0	4.9	5.8	TBI/Mel (M)
599-098	16	M	APL CR2	6/6 PBSC	0	63.9	51.1	Bu/Cy (M)
599-101	14	F	ALL CR2	6/6 PBSC	0	67.4	50.6	TBI/Cy/Etop (M)
505-001	14	M	CML-CP	4/6 UCB	Class I × 2	1.61	0.77	Flu/Bu/ATG (RI)
505-002	16	M	CML-CP	6/6 PBSC	0	78.2	46.1	Flu/Bu/Alem (RI)
599-099	1.5	M	AML	4/6 UCB	Class I × 2	11	8.48	Bu/Mel/ATG (M)
599-100	1.5	M	Hurler's	6/6 BM	0	67.3	23.8	Flu/Cy/ATG (RI)
503-001	1	F	B-Thal*	4/6 UCB†	Class I × 2	16.9	5.15	Bu/Cy/Alem (M)
599-095	1.5	M	HLH*	6/6 UCB†	0	6.3	4.1	TBI/Mel/Alem (M)
599-100	1.5	M	Hurler's*	6/6 PBSC†	0	164.8	97.7	Bu/Cy/Alem (M)
599-106	4	F	NBL CR1	6/6 UCB	0	7.96	2.96	Bu/Mel/ATG (M)
599-102	6	M	ALL CR3*	5/6 PBSC	Class I	64.3	50.8	TBI/Thio/Cy/ATG (M)
509-001	7	F	AA	5/6 BM	Class II	143.8	50.4	Flu/Cy/ATG (M)
509-003	4	M	AA	5/6 UCB	Class I	5.5	3.6	Flu/Cy/ATG (M)
509-004	12	M	AA	6/6 BM	0	189.7	64.1	Flu/Cy/ATG (RI)
509-005	14	M	HLH	4/6 UCB	Class I/II	201	36	Flu/Cy/Etop/ATG (RI)
504-001	1	M	MDS	4/6 UCB	Class I × 2	7.99	2.88	Flu/Bu/ATG (RI)

ALL indicates acute lymphoblastic leukemia; CML, chronic myeloid leukemia; AML, acute myeloid leukemia; AA, aplastic anemia; NHL, non-Hodgkin lymphoma; HD, Hodgkin disease; NBL, neuroblastoma; WAS, Wiskott-Aldrich syndrome; B-Thal, β-thalassemia; HLH, hemophagocytic lymphohistiocytosis; CR, complete response; PR, partial response; PD, progressive disease; CP, chronic phase; UCB, umbilical cord blood; PBSC, peripheral blood stem cells; BM, bone marrow; RI, reduced intensity; M, myeloablative; TBI, total body irradiation; Mel, melphalan; Flu, fludarabine; Cy, cyclophosphamide; ATG, rabbit antithymocyte globulin; ATGAM, horse antithymocyte globulin; Etop, etoposide phosphate; Bu, busulfan; Alem, alemtuzumab; VP-16, etoposide; Thio, thiotepa; TNC, total neutrophil count; APL, acute promyelocytic leukemia.

*Poor-risk patients.

†Second AlloSCT.

lized peripheral blood stem cells (PBSC); five 6/6-matched and one 5/6-matched MFD received G-CSF–mobilized bone marrow. There were three 6/6, five 5/6, and fourteen 4/6 HLA-matched UCB recipients. Mismatches for the UCB units were as follows: 6 class I, 1 class II, 3 class I and II, 2 double class II, and 9 double class I.

For all patients, the median nucleated cell dose was 7.99 × 10^7/kg (range, 1.12-201 × 10^7/kg), and the median CD34 cell dose was 5.15 × 10^5/kg (range, 0.43-97.7 × 10^5/kg). For all MFD recipients, the median nucleated cell dose was 64.3 × 10^7/kg (range, 4.9-189.7 × 10^7/kg), and the median CD34 cell dose was 51.1 × 10^5/kg (range, 0.58-97.7 × 10^5/kg). The median total neutrophil count and CD34 counts for matched family bone marrow recipients were 105.55 × 10^7 (range, 22.5-189.7 × 10^7) and 65.4 × 10^5 (range, 23.8-78.6 × 10^5), respectively, and for matched family PBSC recipients they were 63.9 × 10^7 (range, 4.9-164.8 × 10^7) and 50.6 × 10^5 (range, 5.8-97.7 × 10^5), respectively. For recipients of UCB, the median nucleated cell dose was 5.9 × 10^7/kg (range, 1.12-201 × 10^7/kg), and the median CD34 cell dose was 2.84 × 10^5/kg (range, 0.43-36 × 10^5/kg).

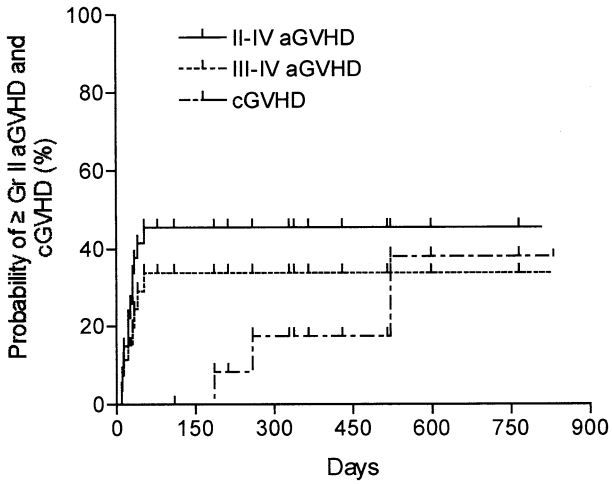

Figure 1. The probability of patients developing grade II to IV or III to IV aGVHD and cGVHD. The probability of patients developing grade II to IV (——) or III to IV aGVHD (----) or cGVHD (- · - · -) was determined by the method of Kaplan and Meier. GVHD was graded according to the Seattle consensus criteria, and patients were staged and graded prospectively once a week for the occurrence of aGVHD.

Incidence and Severity of GVHD (Acute and Chronic)

Twenty-seven patients were evaluable for aGVHD and 21 for cGVHD. Twelve patients developed grade ≥II aGVHD. Eight of these were grade III or IV. During the study period, the Kaplan-Meier probability of developing grade II to IV and grade III to IV aGVHD was 45.4% ± 9.7% and 33.8% ± 9.9%, respectively (Figure 1). Four patients developed limited cGVHD (2 PBSC, 1 bone marrow, and 1 UCB). None developed extensive cGVHD (Table 4). The probability of developing limited cGVHD for all 21 evaluable patients was 38.1% ± 19.7% (Figure 1). The probability of developing grade ≥II aGVHD was higher with related PBSC (62.5% ± 17.1%) compared with UCB (37.1% ± 13.2%) or related bone marrow (40.0% ± 21.9%; Figure 2). The probability of developing grade III to IV aGVHD was higher with related PBSC (50.0% ± 17.7%) compared with UCB (22.6% ± 11.5%) or related bone marrow (20.0% ± 17.9% (Figure 3).

MPA Trough Concentrations and aGVHD

During the second half of the study, we assessed steady-state MPA/MPAG trough concentrations in 14 patients. Of these, 8 patients (57%) received reduced-intensity conditioning regimens. Two of 14 patients developed primary graft failures and were not evaluable for GVHD. All patients had MPA and MPAG below the target range (MPA ≤0.6 μg/mL and MPAG ≤17 μg/mL) during the initial dosing period at 15 mg/kg IV or PO every 12 hours (Table 5). MMF doses were increased to 15 mg/kg IV every 6 to 8 hours in 5 patients without significant changes in MPA or MPAG trough concentrations (Figure 4A). Six patients subsequently received MMF at 600 mg/m^2 PO or IV every 6 hours. Two (33%) of 6 patients at 600 mg/m^2 every 6 hours achieved the targeted MPA concentrations (mean, 1.15 ± 0.15 μg/mL). MMF doses were further increased to 900 to 1200 mg/m^2 IV or PO every 6 hours in the next 8 patients, 5 of whom achieved MPA within the target range (Figure 4A). Six of 7 patients with grade ≥II aGVHD did not achieve targeted MPA trough concentrations before GVHD onset (Table 5). Five of these 6 patients received full-intensity conditioning.

Five of 14 patients achieved target MPA trough concentrations within 30 days after AlloSCT and had grade ≤I aGVHD (Figure 4B). Four of these 5 patients received reduced-intensity conditioning. Five (62.5%) of the 8 patients who received related PBSCs developed grade II to IV aGVHD. We had assessed MPA trough concentrations in only 4 of these 8 patients (1 achieved the target MPA trough before day +30 and developed grade I skin aGVHD; 3 patients never achieved target MPA trough concentrations and developed grade III-IV aGVHD). Ten of 12 patients were initiated on IV MMF on day +1 and were subsequently switched to the PO formulation when it became clinically appropriate. We were unable to detect any differences in MPA trough concentrations between PO and IV MMF formulations, possibly because of a small sample size, wide variability in dosing, and good oral bioavailability of the drug.

Failure to achieve target MPA levels before day 30 after AlloSCT was associated with a higher probability of developing moderate to severe aGVHD. The probability of developing grade ≥II aGVHD in patients who did not achieve an MPA trough level ≥1 μg/mL was significantly higher compared with recipients who achieved a level ≥1 μg/mL (100% versus 16.7% ± 15.2%; $P = .02$; Figure 4B).

Hematologic Recovery

Twenty-six patients were evaluable for myeloid recovery (ANC ≥500/mm^3 for 2 days). Two patients died too early to be evaluable for myeloid engraftment. Eight patients in our study experienced graft failure and had autologous myeloid reconstitution. Seven of these patients developed primary graft failure (1 each with β-thalassemia, MDS, and Hurler syndrome and 2 each with HLH and CML [in chronic phase]). One patient with β-thalassemia had secondary graft failure with a maximum donor chimerism of 65% on day +30, which decreased to 0% by day +100. Of the 8 patients with graft failure, 7 received reduced-intensity conditioning, with only 1 patient (HLH) receiving full ablation. Seven of these patients

Table 4. *Incidence and Severity of Acute and Chronic GVHD and Survival*

Patient No.	Age (y)	Sex	Disease Status	Donor Source	HLA Match	Maximum Grade of aGVHD	Day of Onset	Maximum Grade of cGVHD	Day of Onset	Survival (d)
599-068	2	M	ALL CR2	UCB	4/6	0	N/A	0	N/A	D +337
599-072	16	F	CML-CP	PBSC	6/6	NE	N/A	NE	N/A	A +984
599-073	14	M	AML Rel*	UCB	5/6	NE	N/A	NE	N/A	D +17
599-075	12	M	AA	BM	6/6	0	N/A	Limited	258	A +989
599-077	6	F	ALL CR2	PBSC	6/6	II	27	0	N/A	D +469
599-079	18	M	AML CR2	UCB	4/6	0	N/A	NE	N/A	D +28
599-080	10	F	NHL CR2	UCB	4/6	II	13	0	N/A	A +924
599-081	8	F	ALL CR2	UCB	4/6	IV	14	NE	N/A	D +41
599-083	20	M	HD PD*	UCB	4/6	0	N/A	NE	N/A	D +78
599-085	5	M	NBL PD*	UCB	4/6	III	40	NE	N/A	D +40
599-086	3	M	Sickle cell	BM	6/6	0	N/A	0	N/A	A +766
599-088	2	M	WAS	UCB	5/6	III	53	0	N/A	D +223
599-091	11	M	ALL CR3*	UCB	5/6	0	N/A	0	N/A	D +212
501-001	17	F	HD CR2	UCB	4/6	0	N/A	0	N/A	A +831
501-002	21	M	HD CR2	PBSC	6/6	0	N/A	Limited	186	A +832
501-003	18	F	HD PR2*	UCB	4/6	0	N/A	Limited	521	D +630
501-004	9	M	NHL PR2	UCB	4/6	0	N/A	0	N/A	A +516
503-001	0.5	F	B-Thal	UCB	5/6	NE	N/A	NE	N/A	A +656
503-002	1.5	M	B-Thal	BM	6/6	III	34	0	N/A	A +500
599-095	0.75	M	HLH	UCB	6/6	0	N/A	0	N/A	D +217
599-093	3	M	ALL CR2	PBSC	6/6	III	10	Limited	311	A +689
599-098	16	M	APL CR2	PBSC	6/6	0	N/A	0	N/A	A +599
599-101	14	F	ALL CR2	PBSC	6/6	III	23	0	N/A	D +186
505-001	14	M	CML CP	UCB	4/6	NE	N/A	NE	N/A	A +575
505-002	16	M	CML-CP	PBSC	6/6	I	28	0	N/A	A +429
599-099	1.5	M	AML	UCB	4/6	NE	N/A	NE	N/A	D +417
599-100	1.5	M	Hurler's	BM	6/6	NE	N/A	NE	N/A	D +210
503-001	1	F	B-Thal*	UCB†	4/6	II	23	0	N/A	A +532
599-095	1.5	M	HLH*	UCB†	6/6	NE	N/A	N/E	N/A	D +34
599-100	1.5	M	Hurler's*	PBSC†	6/6	III	30	0	N/A	D +148
599-106	4	F	NBL CR1	UCB	6/6	I	24	0	N/A	D +110
599-102	6	M	ALL CR3*	PBSC	5/6	IV	11	NE	N/A	D +44
509-001	7	F	AA	BM	5/6	II	31	NE	N/A	D +59
509-003	4	M	AA	UCB	5/6	0	N/A	0	N/A	A +365
509-004	12	M	AA	BM	6/6	0	N/A	0	N/A	A +328
509-005	14	M	HLH	UCB	4/6	NE	N/A	NE	N/A	A +283
504-001	1	M	MDS	UCB	4/6	NE	N/A	NE	N/A	A +218

ALL indicates acute lymphoblastic leukemia; CML, chronic myelogenous leukemia; AML, acute myeloid leukemia; AA, aplastic anemia; NHL, non-Hodgkin lymphoma; HD, Hodgkin disease; NBL, neuroblastoma; WAS, Wiskott-Aldrich syndrome; B-Thal, β-thalassemia; HLH, hemophagocytic lymphohistiocytosis; MDS, myelodysplastic syndrome; CR, complete response; PR, partial response; PD, progressive disease; CP, chronic phase; UCB, umbilical cord blood; PBSC, peripheral blood stem cells; BM, bone marrow; A, alive; D, dead; N/A, not applicable; NE, not evaluable; Rel, relapsed; APL, acute promyelocytic leukemia.

*Poor-risk patients.

†Second AlloSCT.

received unrelated cord blood as the source of donor stem cells. The median time to ANC recovery was 16 days (range, 3-79 days). The median time to recovery for evaluable MFD was 13.5 days (range, 11-17 days), and that for UCB was 23 days (range, 3-79 days; Figure 5A). Myeloid engraftment occurred earlier with MFD AlloSCT compared with UCB donors. Seventeen patients were evaluable for platelet recovery (platelet count $\geq$20000/mm^3 untransfused for 7 days), 7 had primary graft failures, 7 died too early to be evaluable, and 4 did not achieve platelet recovery. The median time to platelet recovery was 19 days (range, 1-206 days). Similarly, platelet engraftment occurred earlier with evaluable MFD donors (12.5 days; range, 8-26 days) compared with UCB donors (28 days; range, 6-206 days; Figure 5B).

Toxicity and Complications

Opportunistic Infections. There were 16 documented opportunistic infections in 37 AlloSCT recipients. One patient had central venous catheter fungal infections with *Candida parapsilosis* that were effectively treated by increased doses of liposomal amphotericin B and removal of the infected indwelling catheter. One patient with a history of sinopulmonary

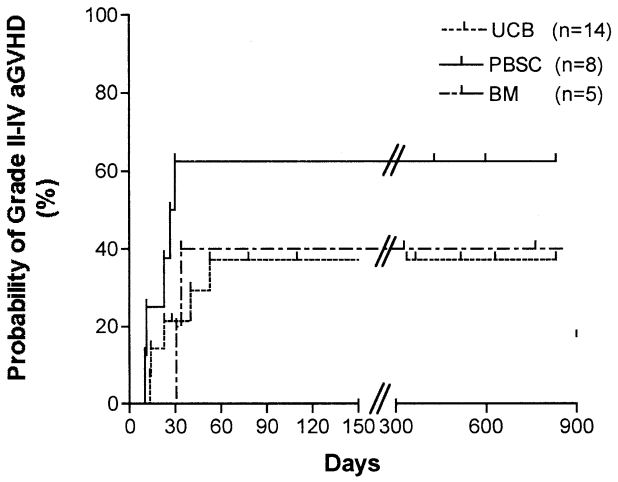

Figure 2. Probability of developing grade II or higher or grade III to IV aGVHD. The probability of developing grade II or higher or grade III to IV aGVHD in patients who received AlloSCT from peripheral blood (PBSC; ——), bone marrow (BM; - · - · -), or unrelated cord blood units (UCB; ----) was determined by the method of Kaplan and Meier.

aspergillosis had a reactivation of *Aspergillus* infection after AlloSCT and subsequently died from cGVHD. Two other patients developed pulmonary aspergillosis after UCB AlloSCT: 1 on day +42 before myeloid engraftment and 1 on day +209. Both of these patients died: 1 from disseminated CMV and 1 from progressive disease. One other patient also developed disseminated CMV and died of multiorgan failure on day +41. During the study, there was a 6-month period of increased nosocomial infections with adenovirus in our institution. Six patients with disseminated adenovirus disease died, mostly secondary to multiorgan failure and/or disease, whereas 2 with localized disease were alive and free of adenoviral infection at the last follow-up.

Noninfectious Toxicity. Sixteen patients (43%) developed grade II to III mucositis. The incidence of mucositis was higher after fully ablative conditioning regimens (14 [67%] of 21 patients) versus reduced-intensity regimens (2 [12.5%] of 16 patients). No patient developed moderate to severe veno-occlusive disease, but 1 patient developed mild to moderate veno-occlusive disease. Three patients developed neurotoxicity secondary to FK506 (1 patient recovered fully, 1 recovered partially, and 1 died of leukoencephalopathy). Eighteen (48.6%) of 37, 12 (32.4%) of 37, and 4 (10.8%) of 37 AlloSCT recipients developed grade III to IV hyperkalemia, hypokalemia, and hypomagnesemia, respectively. Four patients developed grade III to IV nephrotoxicity (10.8%) while also receiving other nephrotoxic drugs, including liposomal amphotericin B, foscarnet, ganciclovir, vancomycin, and aminoglycosides. One patient developed grade III pulmonary toxicity (radiation pneumonitis) that required prolonged steroid therapy.

Survival

Sixteen patients have died with a median follow-up of 333 days (range, 17-989 days) for survival and cGVHD. Immediate causes of death were as follows: 1 pulmonary hemorrhage, 1 idiopathic pneumonic syndrome, 4 multiorgan failures, 6 progressive diseases, 1 disseminated adenovirus, 1 FK506 neurotoxicity, 1 GVHD, and 1 systemic CMV. The probability of 1-year overall survival (OS) for all patients was 47.1% (95% confidence interval, 27.6%-66.6%). The probability of 1-year OS for average-risk patients was 55.7% ± 12.0% (95% confidence interval, 32.3%-79.1%), and for poor-risk patients it was 37.5% ± 17.1% (95% confidence interval, 4.0%-71.0%; Figure 6).

Two of the 3 patients who underwent a second AlloSCT died. One died on posttransplantation day +148 without evidence of cGVHD, and the second died on day +34, before hematologic recovery. The third patient is alive and free of cGVHD at 532 days (17 months) after transplantation. Only 2 of these 3 patients with follow-up >100 days after retransplantation were considered evaluable for cGVHD.

DISCUSSION

The results of this study demonstrate that the combination of FK506 and MMF seems to be a safe and effective regimen for GVHD prevention after myeloablative and reduced-intensity AlloSCT in childhood and adolescent recipients. Potential advantages of this regimen are that it spares the prophylactic use of corticosteroids (which have been associated with glucose intolerance, hypertension, and osteopenia) and MTX (which may enhance conditioning reg-

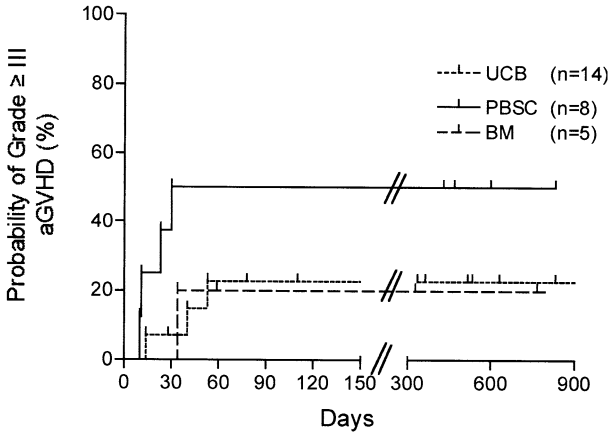

Figure 3. Probability of developing grade III to IV or higher aGVHD. The probability of developing grade III to IV or higher aGVHD in patients who received AlloSCT from peripheral blood (PBSC; ——), bone marrow (BM; - · - · -), or unrelated cord blood units (UCB; ----) was determined by the method of Kaplan and Meier.

Table 5. *Patient Demographics, MMF Doses, MPA Concentrations, and Acute GVHD**

Patient No.	Age (y)	Sex	Disease Status	Donor Source	Mismatch	Conditioning	First MMF Dose	Frequency	Route	Target MPA Reached	Acute GVHD (Grade)
503-001	1	F	B-thal	UCB	Class I	Ablative	15 mg/kg	q12h	IV	No	II
503-002	1.5	M	B-thal	BM	0	Ablative	15 mg/kg	q12h	IV	Yes	III
599-100	1.5	M	Hurler's	PBSC	0	Ablative	15 mg/kg	q8h	IV	No	III
599-088	2.5	M	WAS	UCB	Class I	Reduced intensity	15 mg/kg	q12h	PO	No	III
599-102	6	M	ALL CR3	PBSC	Class I	Ablative	15 mg/kg	q8h	IV	No	IV
501-004	9	M	NHL PR	UCB	2 class I	Reduced intensity	15 mg/kg	q12h	PO	Yes	No
599-101	14	F	ALL CR2	PBSC	0	Ablative	15 mg/kg	q12h	IV	No	III
505-002	16	M	CML	PBSC	0	Reduced intensity	600 mg/m^2	q6h	IV	Yes	I
599-106	4	F	NBL CR1	UCB	0	Reduced intensity	900 mg/m^2	q6h	IV	Yes	I
509-001	7	F	AA	BM	Class II	Ablative	900 mg/m^2	q6h	IV	No	II
509-003	4	M	Severe AA	UCB	Class I	Ablative	900 mg/m^2	q6h	IV	Yes	No
509-004	12	M	Severe AA	BM	0	Reduced intensity	900 mg/m^2	q6h	IV	Yes	No

UCB indicates umbilical cord blood; BM, bone marrow; PBSC, peripheral blood stem cells; B-thal, β-thalassemia; WAS, Wiskott-Aldrich syndrome; ALL, acute lymphoblastic leukemia; CR, complete response; NHL, non-Hodgkin lymphoma; PR, partial response; CML, chronic myelogenous leukemia; NBL, neuroblastoma; AA, aplastic anemia; IV, intravenous; PO, oral.

*Two patients who had their MPA trough concentrations monitored developed primary graft failure after reduced-intensity conditioning and therefore were not evaluable for GVHD and were excluded from the table.

imen-induced mucositis, liver and renal toxicity, and myelosuppression and may secondarily delay engraftment) [16]. The safety profile of MMF is superior to that of corticosteroids or MTX: mild myelosuppression and gastrointestinal toxicity (diarrhea) are the most commonly reported adverse events.

There was a 9% incidence of grade III to IV neurotoxicity associated with FK506 use in this study. FK506 can cause mild to severe neurotoxicity that can be concentration independent, whereas dose reduction or drug discontinuation often leads to regression of symptoms. Mild neurotoxicity associated with the use of FK506 (headache, tremors, paresthesia, and insomnia) has been reported to occur in 19% to 64% of SOT recipients [35,36]. Moderate to severe neurotoxicity (seizures, confusion, hallucinations, leukoencephalopathy, and cortical blindness) can occur in up to 32% of patients [37,38].

Preliminary data from Pavletic et al. [39] suggest that FK506/MMF possesses a superior safety profile, is associated with a lower incidence of aGVHD, and is associated with earlier myeloid/platelet engraftment compared with a CsA/low-dose MTX regimen. In their study, 23 adults with hematologic malignancies underwent fully ablative matched related donor AlloSCT and received FK506 at 0.03 mg/kg/d IV continuously until day +60 plus MMF 15 mg/kg IV or PO every 12 hours until day +28 for GVHD prophylaxis. Outcomes were compared with those of 23 matched historical controls who received CsA/MTX

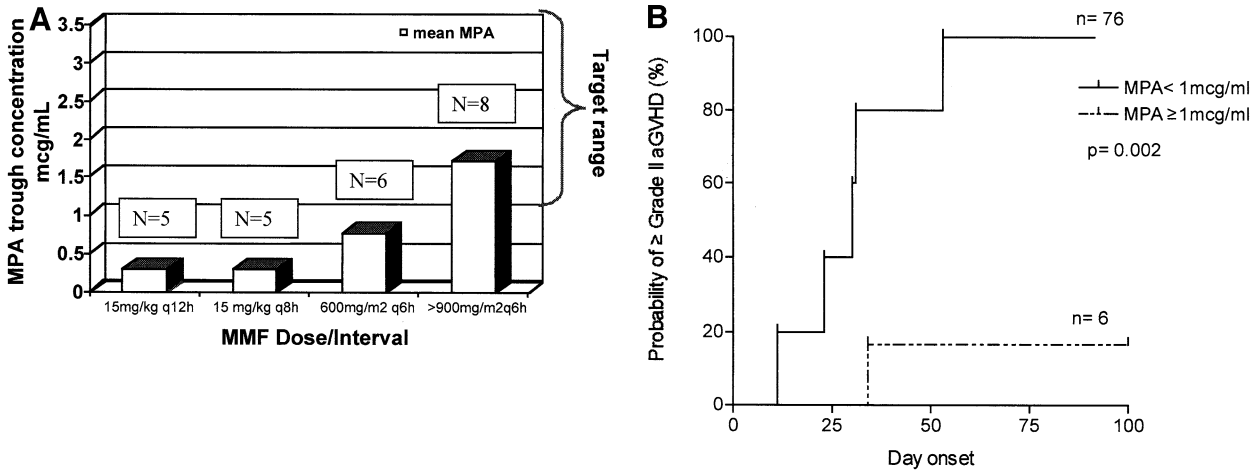

Figure 4. A, Relationship between MMF dose and steady-state MPA trough concentrations. B, Probability of developing grade II or higher aGVHD with regard to MPA trough levels. The probability of developing grade II or higher aGVHD in patients who did not achieve an MPA trough level ≥1 μg/mL (——) compared with those recipients who achieved a level ≤1 μg/mL (— - - —) was determined by the method of Kaplan and Meier.

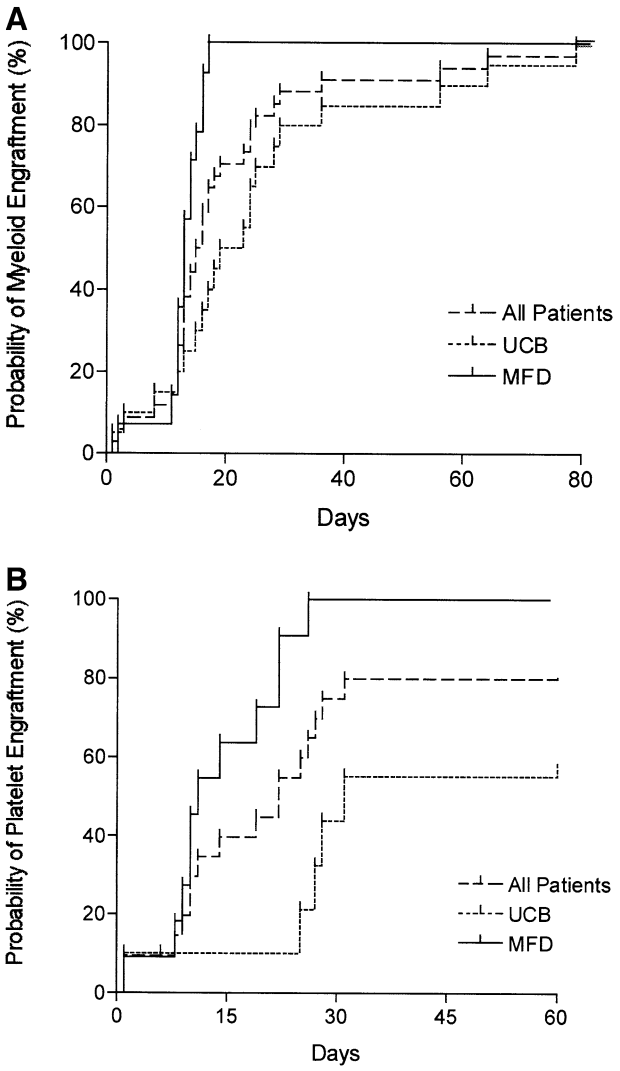

Figure 5. A, Probability of myeloid engraftment (ANC ≥500/mm^3 for 2 days) determined by the Kaplan-Meier method in all patients (----) and patients who received either umbilical cord blood (UCB; — —) or matched family donor (MFD; ——) allogeneic stem cell transplants. Patients who did not engraft were excluded from data analysis. B, Probability of platelet (Plt) engraftment (Plt count ≥20000/mm^3 for 7 days) was determined by the Kaplan-Meier method in all patients (---) and in patients who received either UCB (— —) or MFD (——) allogeneic stem cell transplants. Patients who did not engraft were excluded from the data analysis.

GVHD prophylaxis at the same institution. The cumulative incidence of grades II to IV and III to IV aGVHD in FK506/MMF versus CsA/MTX at 100 days after transplantation was 15% versus 62% (P = .0003) and 6% versus 25% (P = .025), respectively. The median time to neutrophil and platelet engraftment in study patients versus controls was 10 versus 15 days (P = .001) and 13 versus 19 days (P = .11), respectively. There was no difference in OS or 6-month cumulative incidence of relapse between the 2 GVHD prophylaxis groups.

The use of MMF-containing immunosuppressive regimens is common in SCT; however, there is a paucity of data on the pharmacokinetics and pharmacodynamics of MMF in this patient population [40-42]. There seem to be significant differences in the pharmacokinetics and pharmacodynamics of MMF in AlloSCT recipients compared with healthy adult volunteers and SOT recipients. Preliminary pharmacokinetic data suggest that systemic exposure to the pharmacologically active metabolite MPA is substantially less in AlloSCT recipients [40-42]. The second peak of MPA plasma concentration described in healthy volunteers and SOT recipients has not been observed in AlloSCT recipients, suggesting that enterohepatic recirculation may be impaired in patients after AlloSCT. The toxicity of the conditioning regimen leading to significant mucosal damage is the most likely explanation for this effect. The destruction of the bacterial flora of the gut by broad-spectrum antibacterial agents, selective gut decontamination, or both can also lead to reduced formation of glucuronidase, which is essential for conversion of MPAG back to MPA (the active metabolite). Furthermore, the half-life of MPA in AlloSCT recipients seems to be significantly shorter than reported in SOT recipients (1.5-3.6 versus 16-18 hours) [40]. This impaired deglucuronidation, lack of enterohepatic recirculation, shorter half-life, or a combination of these may explain in part the significantly lower trough concentrations of MPA in AlloSCT recipients observed in our study and others.

The importance of monitoring MPA levels is underscored by data from SOT recipients demonstrating that the steady-state MPA area under the curve (AUC) and, to a lesser extent, MPA trough concentrations predict the risk of acute organ rejection. Maintaining

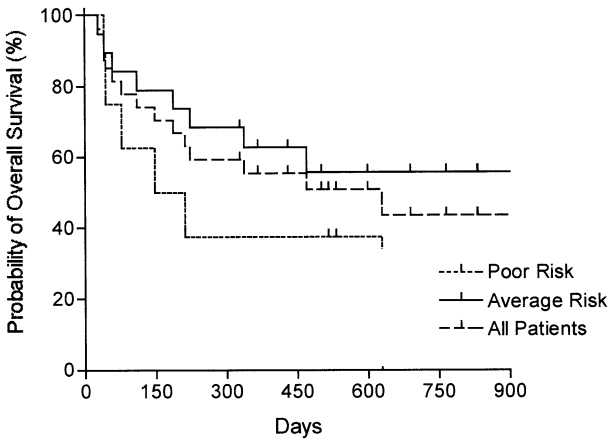

Figure 6. One-year overall survival. The probability of 1-year overall survival of 27 evaluable patients was determined by the Kaplan-Meier method in all patients or those who received either umbilical cord blood or matched family donor allogeneic stem cell transplants. All patients (----); average risk (— —); and poor risk (——) by International Bone Marrow Transplant Registry criteria.

MPA AUC at the target range of 30 to 60 μg.h/mL decreases the risk of acute rejection to <10% [43]. Currently, there is a paucity of data demonstrating a relationship between MPA concentrations and risk of aGVHD in AlloSCT recipients. It is clear, however, that MPA trough concentrations and AUC are significantly decreased in AlloSCT compared with SOT recipients [40-42]. Because there is no established therapeutic range for MPA trough concentrations in the AlloSCT setting, we have adopted the range used in the SOT setting (1-3.5 μg/mL). Our preliminary data in 14 pediatric AlloSCT patients who underwent steady-state MPA trough concentration monitoring demonstrated a striking relationship between MPA trough concentrations and the risk of aGVHD (Figure 4B). Patients who did not achieve MPA trough concentrations ≥1 μg/mL before day +30 had a significantly higher incidence of aGVHD (100% versus 16.7% ± 15.2%; $P = .02$).

There was a 21% incidence of graft failure in patients with hemoglobinopathies, hemophagocytic or metabolic disorders, MDS, and CML. These diseases are now known to be associated with a higher degree of graft failures after both reduced-intensity and myeloablative transplantation [44-46]. Furthermore, 7 of 8 patients with graft failure in our study had received UCB donor stem cells, and this is also known to be associated with a higher risk of failed engraftment. We therefore believe that underlying disease coupled with insufficient ablation in the preparative regimen, rather than the GVHD prophylactic regimen used, was responsible for the number of graft failures in our study. Our institutional protocols have since been revised to reflect the need for a more intensive preparative regimen.

The probability of developing grade ≥II and grade III to IV aGVHD with the use of FK506 and MMF in our study was 45.4% ± 9.7% and 33.8% ± 9.9%, respectively. The probability of developing grade II to IV aGVHD was higher (62.5% ± 17.1%) in patients who received related PBSC transplants. However, because of the small number of patients, it is difficult to deduce whether the stem cell source (PBSC) or low MPA trough concentrations, or both, were contributing factors. CsA and MTX have been widely used for aGVHD prophylaxis after AlloSCT with an MFD and have been associated with a 35% incidence of grade II to IV aGVHD and a 40% incidence of cGVHD [11,47]. After transplantation of unmodified marrow from HLA-matched unrelated donors receiving CsA and MTX GVHD prophylaxis, Hansen et al. [48] reported a 75% incidence of aGVHD. In a randomized multicenter trial that compared FK506/MTX with CsA/MTX for GVHD prophylaxis after AlloSCT with matched sibling donors, Ratanatharathorn et al. [22] reported a significantly lower incidence of grade II to IV aGVHD in patients who received FK506/MTX compared with patients in the CsA/MTX group (31.9% versus 44.4%, respectively; $P = .01$). The incidence of grade III to IV aGVHD was similar: 13.3% versus 17.1%. There was no difference in the rate of doubling of creatinine during the study period between the 2 groups (84% versus 80%; $P = .3$), whereas in the CsA group there was a higher incidence of hypertension that required medication ($P = .001$). Hiraoka [23] also reported an advantage of using FK506 over CsA for preventing aGVHD after AlloSCT: the incidence of grade II to IV aGVHD was lower in the FK506 group (17.5%) compared with the CsA group (48%; $P < .0001$). A third randomized study, by Nash et al. [21], compared CsA/MTX with FK506/MTX for aGVHD prevention after unrelated donor AlloSCT. This study demonstrated a decreased incidence of aGVHD in the FK506 group compared with the CsA group (56% versus 74%; $P = .0002$). There was a trend toward less severe GVHD in the FK506 group ($P = .005$) with less glucocorticoid use compared with the CsA group (65% versus 81%; $P = .019$). There was also no significant increase in infectious complications or other toxicities with the use of FK506 versus CsA.

We have found that the use of FK506/MMF has been associated with good tolerability and low overlapping toxicities, and it may be equivalent or superior to standard regimens in preventing aGVHD with the appropriate monitoring, aiming for steady-state MPA trough concentrations of 1.0 to 3.5 μg/mL. Because MMF and FK506 do not share overlapping toxicities, it is easy to monitor for side effects that can be attributed to either drug. It has become apparent that achievement of therapeutic levels of MMF via MPA trough monitoring and appropriate dose adjustments is an important component to the observed efficacy and tolerability of this regimen. Significantly higher MMF doses were necessary in our patients to achieve target MPA concentrations compared with the doses used in SOT recipients (≥900 mg/m^2 every 6 hours versus 600 mg/m^2 every 12 hours). The main limitations of this observation are the small number of patients available for analysis, the lack of information on the target MPA trough concentrations for GVHD prevention, and the lack of consensus on the necessity of monitoring MPA trough concentrations in either hematopoietic or SOT recipients. In addition, total MPA trough concentrations may not be the optimal monitoring parameter or predictor of the degree of immunosuppression after MMF administration. Free MPA concentration, AUC, and inosine monophosphate dehydrogenase measurements, alone or in combination, would offer more accurate information about the degree of immunosuppression and the risk of GVHD. Unfortunately, the tests are mainly used for research purposes and are not commercially available. The results obtained from MPA monitoring in

our patient cohort suggest the possibility of a relationship between the probability of developing aGVHD and the achievement of therapeutic MPA trough levels. These results are provocative and support the need for randomized controlled studies to further delineate this relationship and to determine the ideal dose schedule for MMF in pediatric and adolescent AlloSCT recipients when it is used in combination with FK506. These trials should be designed to compare the incidence of clinically significant aGVHD (grades II-IV) with FK506/MMF compared with FK506/MTX, CsA/MTX, or both in prospective randomized controlled studies.

ACKNOWLEDGMENTS

We thank Linda Rahl and Erin Morris, RN, for their expert editorial assistance and all the nurses and allied health professionals who participated in the superb care of our AlloSCT recipients. We would also like to thank Les Shaw, PhD, at the University of Pennsylvania for his helpful suggestions on MPA monitoring and MMF dose adjustments. Presented in part at the American Society of Hematology, December 2002, Philadelphia, PA, and the American Society of Blood and Marrow Transplantation, February 2003, Keystone, CO. Supported in part by grants from the Pediatric Cancer Research Foundation, the Pediatric Cancer Foundation, and the Swim Across America Foundation.

REFERENCES

1. Chao N. *Graft-versus-Host Disease*. Second Edition. Austin, TX: R.G. Landes Co; 1999.
2. Goker H, Haznedaroglu IC, Chao NJ. Acute graft-vs-host disease: pathobiology and management. *Exp Hematol.* 2001;29: 259-277.
3. Nademanee A, Schmidt GM, Parker P, et al. The outcome of matched unrelated donor bone marrow transplantation in patients with hematologic malignancies using molecular typing for donor selection and graft-versus-host disease prophylaxis regimen of cyclosporine, methotrexate, and prednisone. *Blood.* 1995;86:1228-1234.
4. Ruutu T, Niederwieser D, Gratwohl A, Apperley JF. A survey of the prophylaxis and treatment of acute GVHD in Europe: a report of the European Group for Blood and Marrow, Transplantation (EBMT). Chronic Leukaemia Working Party of the EBMT. *Bone Marrow Transplant.* 1997;19:759-764.
5. Gluckman E, Rocha V, Boyer-Chammard A, et al. Outcome of cord-blood transplantation from related and unrelated donors. Eurocord Transplant Group and the European Blood and Marrow Transplantation Group. *N Engl J Med.* 1997;337:373-381.
6. Cairo MS, Wagner JE. Placental and/or umbilical cord blood: an alternative source of hematopoietic stem cells for transplantation. *Blood.* 1997;90:4665-4678.
7. Laughlin MJ. Umbilical cord blood for allogeneic transplantation in children and adults. *Bone Marrow Transplant.* 2001;27: 1-6.
8. Laughlin MJ, Barker J, Bambach B, et al. Hematopoietic engraftment and survival in adult recipients of umbilical-cord blood from unrelated donors. *N Engl J Med.* 2001;344:1815-1822.
9. Locatelli F, Rocha V, Chastang C, et al. Factors associated with outcome after cord blood transplantation in children with acute leukemia. Eurocord-Cord Blood Transplant Group. *Blood.* 1999;93:3662-3671.
10. Mrsic M, Labar B, Bogdanic V, et al. Combination of cyclosporin and methotrexate for prophylaxis of acute graft-versus-host disease after allogeneic bone marrow transplantation for leukemia. *Bone Marrow Transplant.* 1990;6:137-141.
11. Storb R, Deeg HJ, Whitehead J, et al. Methotrexate and cyclosporine compared with cyclosporine alone for prophylaxis of acute graft versus host disease after marrow transplantation for leukemia. *N Engl J Med.* 1986;314:729-735.
12. Jensen CW, Flechner SM, Van Buren CT, et al. Exacerbation of cyclosporine toxicity by concomitant administration of erythromycin. *Transplantation.* 1987;43:263-270.
13. Loughran TP Jr, Deeg HJ, Dahlberg S, Kennedy MS, Storb R, Thomas ED. Incidence of hypertension after marrow transplantation among 112 patients randomized to either cyclosporine or methotrexate as graft-versus-host disease prophylaxis. *Br J Haematol.* 1985;59:547-553.
14. Thompson CB, June CH, Sullivan KM, Thomas ED. Association between cyclosporin neurotoxicity and hypomagnesaemia. *Lancet.* 1984;2:1116-1120.
15. Kahan BD, Flechner SM, Lorber MI, Jensen C, Golden D, Van Buren CT. Complications of cyclosporin therapy. *World J Surg.* 1986;10:348-360.
16. Lazarus HM, Coccia PF, Herzig RH, et al. Incidence of acute graft-versus-host disease with and without methotrexate prophylaxis in allogeneic bone marrow transplant patients. *Blood.* 1984;64:215-220.
17. Shepherd JD, Shore TB, Reece DE, et al. Cyclosporine and methylprednisolone for prophylaxis of acute graft- versus-host disease. *Bone Marrow Transplant.* 1988;3:553-558.
18. Pietryga D. Prevention and treatment of acute graft-vs-host disease. *Am J Pediatr Hematol Oncol.* 1993;15:28-48.
19. Peters DH, Fitton A, Plosker GL, Faulds D. Tacrolimus. A review of its pharmacology, and therapeutic potential in hepatic and renal transplantation. *Drugs.* 1993;46:746-794.
20. Lin CS, Boltz RC, Siekierka JJ, Sigal NH. FK-506 and cyclosporin A inhibit highly similar signal transduction pathways in human T lymphocytes. *Cell Immunol.* 1991;133:269-284.
21. Nash RA, Antin JH, Karanes C, et al. Phase 3 study comparing methotrexate and tacrolimus with methotrexate and cyclosporine for prophylaxis of acute graft-versus-host disease after marrow transplantation from unrelated donors. *Blood.* 2000;96: 2062-2068.
22. Ratanatharathorn V, Nash RA, Przepiorka D, et al. Phase III study comparing methotrexate and tacrolimus (prograf, FK506) with methotrexate and cyclosporine for graft-versus-host disease prophylaxis after HLA-identical sibling bone marrow transplantation. *Blood.* 1998;92:2303-2314.
23. Hiraoka A. Results of phase III study on prophylactic use of FK506 for acute GVHD compared with cyclosporin in allogeneic bone marrow transplantation. *Blood.* 1997;90:2500 (abstr.).
24. Allison AC, Eugui EM. Purine metabolism and immunosup-

pressive effects of mycophenolate mofetil (MMF). *Clin Transplant.* 1996;10:77-84.
25. Basara N, Blau WI, Romer E, et al. Mycophenolate mofetil for the treatment of acute and chronic GVHD in bone marrow transplant patients. *Bone Marrow Transplant.* 1998;22:61-65.
26. Bornhauser M, Schuler U, Porksen G, et al. Mycophenolate mofetil and cyclosporine as graft-versus-host disease prophylaxis after allogeneic blood stem cell transplantation. *Transplantation.* 1999;67:499-504.
27. Mookerjee B, Altomonte V, Vogelsang G. Salvage therapy for refractory chronic graft-versus-host disease with mycophenolate mofetil and tacrolimus. *Bone Marrow Transplant.* 1999;24: 517-520.
28. Ahsan N, Johnson C, Gonwa T, et al. Randomized trial of tacrolimus plus mycophenolate mofetil or azathioprine versus cyclosporine oral solution (modified) plus mycophenolate mofetil after cadaveric kidney transplantation: results at 2 years. *Transplantation.* 2001;72:245-250.
29. Grenier FC, Luczkiw J, Bergmann M, et al. A whole blood FK 506 assay for the IMx analyzer. *Transplant Proc.* 1991;23:2748-2749.
30. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. *Transplantation.* 1974;18: 295-304.
31. Renner UD, Thiede C, Bornhauser M, Ehninger G, Thiede HM. Determination of mycophenolic acid and mycophenolate mofetil by high-performance liquid chromatography using postcolumn derivatization. *Anal Chem.* 2001;73:41-46.
32. Osunkwo I, Cooney E, Bessmertny O, DiNatale J, Garvin J, Cairo MS. Liposomal amphotericin B (AMB) prophylaxis is safe and effective in preventing invasive fungal infections (IFI) in related and unrelated allogeneic stem cell transplantation (ASCT). *Biol Blood Marrow Transplant.* 2003;9:134 (abstr.).
33. Shereck EB, Bessmertny O, Cooney EM, et al. Cytomegalovirus (CMV) prophylaxis with alternate day ganciclovir/foscarnet in at risk pediatric allogeneic stem cell transplant (AlloSCT) recipients is 100% effective in preventing CMV infections and reducing ganciclovir hematopoietic toxicity. *Blood.* 2002;100: 627a (abstr.).
34. Bearman SI, Anderson GL, Mori M, Hinds MS, Shulman HM, McDonald GB. Venoocclusive disease of the liver: development of a model for predicting fatal outcome after marrow transplantation. *J Clin Oncol.* 1993;11:1729-1736.
35. Pirsch JD, Miller J, Deierhoi MH, Vincenti F, Filo RS. A comparison of tacrolimus (FK506) and cyclosporine for immunosuppression after cadaveric renal transplantation. FK506 Kidney Transplant Study Group. *Transplantation.* 1997;63:977-983.
36. Mayer AD, Dmitrewski J, Squifflet JP, et al. Multicenter randomized trial comparing tacrolimus (FK506) and cyclosporine in the prevention of renal allograft rejection: a report of the European Tacrolimus Multicenter Renal Study Group. *Transplantation.* 1997;64:436-443.
37. Mueller AR, Platz KP, Bechstein WO, et al. Neurotoxicity after orthotopic liver transplantation. A comparison between cyclosporine and FK506. *Transplantation.* 1994;58:155-170.
38. Wijdicks EF, Wiesner RH, Dahlke LJ, Krom RA. FK506-induced neurotoxicity in liver transplantation. *Ann Neurol.* 1994;35:498-501.
39. Pavletic SZ, Abhynakar S, Foran J, et al. Tacrolimus and mycophenolate mofetil are superior to cyclosporine and methotrexate for GVHD prevention after HLA-matched related donor hematopoietic cell transplantation. *Blood.* 2002;100:5352 (abstr.).
40. Maris MB, Neiderweiser D, Sandmaier BM, et al. Nonmyeloablative hematopoietic stem cell transplants (HSCT) using 10/10 HLA antigen matched unrelated donors (URDs) for patients with advanced hematologic malignancies ineligible for conventional HSCT. *Blood.* 2001;98:858a (abstr.).
41. Jacobson PA, Mifek J, Rogosheske J, et al. Highly variable mycophenolate pharmacokinetics in hematopoietic stem cell transplantation (HSCT): potential need for clinical drug monitoring. *Blood.* 2002;100:1595 (abstr.).
42. Bessmertny O, Harrison L, Osunkwo I, et al. Significant alterations of mycophenolate mofetil (MMF) pharmacokinetics in pediatric allogeneic stem cell transplant (AlloSCT) recipients: alternate dosing is required in AlloSCT compared to solid organ allograft recipients. *Blood.* 2002;100:418a (abstr.).
43. Shaw LM, Korecka M, Brayman KL. Pharmacokinetic-pharmacodynamic relationship for mycophenolic acid in renal transplant patients. *Graft.* 1999;2:196-200.
44. Iannone R, Casella JF, Fuchs EJ, et al. Results of minimally toxic nonmyeloablative transplantation in patients with sickle cell anemia and beta-thalassemia. *Biol Blood Marrow Transplant.* 2003;9:519-528.
45. Grewal SS, Krivit W, Defor TE, et al. Outcome of second hematopoietic cell transplantation in Hurler syndrome. *Bone Marrow Transplant.* 2002;29:491-496.
46. Hale GA, Bowman LC, Woodard JP, et al. Allogeneic bone marrow transplantation for children with histiocytic disorders: use of TBI and omission of etoposide in the conditioning regimen. *Bone Marrow Transplant.* 2003;31:981-986.
47. Storb R, Deeg HJ, Pepe M, et al. Methotrexate and cyclosporine versus cyclosporine alone for prophylaxis of graft-versus-host disease in patients given HLA-identical marrow grafts for leukemia: long-term follow-up of a controlled trial. *Blood.* 1989; 73:1729-1734.
48. Hansen JA, Gooley TA, Martin PJ, et al. Bone marrow transplants from unrelated donors for patients with chronic myeloid leukemia. *N Engl J Med.* 1998;338:962-968.