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Impact of Postgrafting Immunosuppressive Regimens on Nonrelapse Mortality and Survival after Nonmyeloablative Allogeneic Hematopoietic Stem Cell Transplant Using the Fludarabine and Low-Dose Total-Body Irradiation 200-cGy

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ABSTRACT

The development of nonmyeloablative (NM) hematopoietic cell transplantation (HCT) has extended the potential curative treatment option of allografting to patients in whom it was previously contraindicated because of advanced age or comorbidity. Acute and chronic graft versus host disease (GVHD) and its consequent nonrelapse mortality (NRM), remains the major limitation of NM HCT. In this report, we analyzed the outcome of 67 patients (median age, 45 years) with hematologic diseases receiving NM conditioning with fludarabine 90 mg/m^2 and total body irradiation (TBI) 200-cGy, followed by filgrastim-mobilized peripheral blood stem cell transplant from HLA identical (n = 61), 5/6 antigen-matched related (n = 1), 6/6 antigen-matched unrelated (n = 3), and 5/6 antigen-matched unrelated (n = 2) donors. The first cohort of 21 patients were given cyclosporine (CSP) and mycophenolate mofetil (MMF) as postgrafting immunosuppression, whereas the subsequent cohort was given additional methotrexate (MTX) and extended duration of CSP/MMF prophylaxis in an attempt to reduce graft-versus-host disease (GVHD). Sixty-four (95%) patients engrafted and 3 (5%) had secondary graft failure. Myelosuppression was moderate with neutrophil counts not declining below 500/μL in approximately 25% of patients, and with more than half of the patients not requiring any blood or platelet transfusion. The 2-year cumulative interval (CI) of grade II-IV, grade III-IV acute GVHD and chronic GVHD were 49%, 30%, and 34%, respectively. The 2-year probability of NRM, overall (OS), and progression-free (PFS) survival were 27%, 43%, and 28%, respectively. GVHD-related death accounted for 85% of NRM. Compared with patients receiving CSP/MMF, patients receiving extended duration of CSP/MMF with additional MTX in postgrafting immunosuppression had a significantly lower risk of grade III-IV acute GVHD (CI 20% versus 52%; $P = .009$) and NRM (CI at 2 years: 11% versus 62%; $P < .001$), without any significant adverse impact on the risk of relapse (CI at 2 years: 59% versus 33%; $P = .174$) Subgroup analysis of a cohort of patients given MTX/CSP/MMF showed that patients with "standard risk" diseases (n = 21) had a 3-year OS and PFS of 85% and 65%, respectively. This compares favorably to the 41% ($P = .02$) and 23% ($P = .03$) OS and PFS, respectively, in patients with "high-risk" diseases (n = 25). In conclusion, the addition of MTX onto the current postgrafting immunosuppression regimen with extended CSP/MMF prophylaxis duration provides more effective protection against severe GVHD, and is associated with more favorable

outcome in patients receiving NM fludarabine/TBI conditioning than in patients receiving fludarabine/TBI conditioning with CSP and MMF without MTX.

KEY WORDS
Nonmyleloablative • Allogeneic transplant • GVHD prophylaxis • Methotrexate • Fludarabine • Low-dose TBI

INTRODUCTION

Allogeneic hematopoietic cell transplantation (HCT) after myeloablative conditioning regimens has been an effective treatment for many patients with hematologic malignancies or inherited blood disorders. Unfortunately, such regimens have been associated with significant toxicities, limiting their use to otherwise healthy, relatively young patients. To extend allogeneic HCT to older patients and those with comorbid conditions, reduced-intensity or truly nonmyeloablative (NM) conditioning regimens lacking such toxicities [1-4] have been developed. These regimens have relied more on graft-versus-tumor effects than on chemoradiation therapy to facilitate engraftment and eradicate malignant cells. Although NM HCT has been associated with reduced regimen-related toxicities and has been curative for a number of patients with hematologic malignancies, challenges have remained with regard to graft-versus-host disease (GVHD), infections, and disease progression. Acute GVHD (aGVHD) (grade II or higher), which developed in 20% to 65% of patients in single or multicenter clinical trials [4-6], remains a major limitation to success of NM HCT. Furthermore, recent analysis suggests that aGVHD, particularly early-onset GVHD, is associated with increased transplant-related mortality (TRM) [7], but not with improved disease control, for which chronic GVHD (cGVHD) appears more important [8].

In an attempt to reduce GVHD-related death, various approaches have been employed. In vivo T cell depletion, such as incorporating alemtuzumab into the conditioning regimen, has been shown to reduce the incidence of GVHD [9-13]. However, this type of intervention, although reducing GVHD, may have an adverse impact on disease response. This is because of the inverse relationship between GVHD and relapse of malignancies [14-16] and the fact that NM HCTs exhibit their antitumor activity by relying on a graft-versus-malignancy effect [2,3,17-19]. In fact, several nonrandomized studies have demonstrated that such strategies have resulted in a reduction in risk for GVHD without any survival benefit [20-23]. Clearly, optimizing GVHD control without reducing graft-versus-malignancy effects after NM conditioning remains a critical research objective.

Different immunosuppressive drug combinations have also been evaluated in efforts to decrease the incidence and severity of GVHD [24-35]. However, the most effective combination and the optimal duration of immunosuppressive therapy to protect against GVHD have not been defined.

Here we report the results of a prospective pilot trial evaluating the feasibility and efficacy of allogeneic HCT after 2 Gy total body irradiation (TBI) and fludarabine NM conditioning developed in Seattle [3,4,36], followed by postgrafting immunosuppression with mycophenolate mofetil (MMF) and cyclosporine (CSP) in 67 patients with various hematologic diseases. In this study, a second patient cohort was accrued based on the modification of postgrafting immunosuppression, which was made following the observation of a considerably high incidence of severe GVHD in the first patient cohort. These 2 sequential patient cohorts, which differed only by GVHD prophylaxis regimen, allow us to compare the efficacy of 2 different immunosuppressive combination regimens on transplantation outcome.

PATIENTS, MATERIALS, AND METHODS

Patient Eligibility and Donors

Included in the study were results from 67 consecutive patients with hematologic diseases treated at 2 tertiary centers in Singapore between November 1999 and October 2005. Treatment protocols were approved by the ethics committee or institution review board at each institution. Informed consent was obtained from all patients and donors before treatment initiation. Patients with lymphoma, aplastic anemia, acute leukemia, myelodysplasia, multiple myeloma, chronic myelogenous leukemia (CML), and chronic lymphocytic leukemia, between ages 45 and 70 years were considered eligible. Patients were also eligible if they were younger than 45, but deemed poor candidates for conventional conditioning because of (1) medical comorbidities (eg, renal dysfunction, liver cirrhosis, existing fungal infections); (2) extensive prior therapy resulting in poor performance status; or (3) failed prior autologous transplantation. Exclusion criteria were cardiac ejection fraction $<35\%$; diffusion capacity of carbon monoxide $<35\%$ predicted; bilirubin >2 times and/or transaminase >4 times the upper limit of normal, and Karnofsky performance score <50.

HLA typing of patients and their donors were

Table 1. *Characteristics of 67 Patients with Nonmyeloablative Hematopoietic Cell Transplantation*

	All No. (%)	Non-MTX-Containing (CSP/MMF) No. (%)	MTX-Containing (CSP/MMF/MTX) No. (%)	Pψ
Patients studied	67	21	46	
Recipient sex				.8
Male	34 (51)	10 (48)	24 (52)	
Female	33 (49)	11 (52)	22 (48)	
Recipient age, years				.3
Median	45.5	48	43	
Range	16-63	19-59	16-63	
Donor sex				.4
Male	42 (63)	15 (71)	27 (59)	
Female	25 (37)	6 (29)	19 (41)	
Donor age, years				.33
Median	44	45	44	
Range	20-62	23-62	20-59	
Female donor/male recipient	17 (25)	5 (24)	12 (26)	.7
Age group				.1
≤30 y	11(16)	3 (14)	8 (17)	
31-40 y	9 (13)	0 (0)	9 (20)	
41-50 y	28 (42)	11 (53)	17 (37)	
51-60 y	16 (24)	7 (33)	9 (20)	
>60 y	3 (5)	0 (0)	3 (6)	
Diagnosis				.06
AML	15 (22)	8 (38)	7 (15)	
MDS/2° Leukemia	16 (24)	0 (0)	16 (35)	
CML	14 (21)	7 (33)	7 (15)	
ALL	1 (1.5)	1 (5)	0 (0)	
Myeloma	10 (15)	3 (14)	7 (15)	
Aplastic anemia	8 (12)	2 (10)	6 (13)	
CLL	1 (1.5)	0 (0)	1 (2)	
Hodgkin	1 (1.5)	0 (0)	1 (2)	
Myelofibrosis	1 (1.5)	0 (0)	1 (2)	
Disease status				.24
CR1†	8 (12)	4 (19)	4 (9)	
PR/relapse/refractory††	31 (46)	11 (52)	20 (43)	
Untreated‡	28 (42)	6 (29)	22 (48)	
Disease risk*				.3
Standard risk	34 (51)	13 (62)	21(46)	
High risk	33 (50)	8 (38)	25 (54)	
Prior autologous HCT	11 (16)	4 (19)	7 (15)	.5
CMV risk group**				.01
High risk	43 (64)	18 (86)	25 (54)	
Low/intermediate risk	24 (36)	3 (14)	21 (46)	
Number of prior regimens				.6
Median	1	1	1	
Range	0-4	0-3	0-4	
Donor				.4
Sibling				
HLA identical	61 (91)	19 (90)	42 (92)	
1 antigen HLA mismatched	1 (1)	0 (0)	1 (2)	
Unrelated				
HLA identical	3 (5)	2 (10)	1 (2)	
1 antigen HLA mismatched	2 (3)	0 (0)	2 (4)	
Cell dose, $\times 10^6$/kg recipient				
$CD34^+$ cells				
Median	6.04	5.53	6.31	.74
Range	1.12-22.89	1.12-21.63	1.68-22.89	
$CD3^+$ cells				.29
Median	2.97	3.23	2.85	
Range	1.34-5.40	1.38-5.09	1.34-5.40	

MDS indicates myelodysplastic syndromes; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; CR, complete remission; PR, partial remission; MTX, methotrexate; HCT, hematopoietic cell transplant; MMF, mycophenolate mofetil; CSP, cyclosporine.

*Standard risk was defined as AML/ALL/myeloma/lymphoma in first remission, chronic myelogenous leukemia in the first chronic phase, myelodysplastic syndrome-refractory anemia, aplastic anemia. All other diagnoses were classified as high risk.

**High risk CMV indicates patient CMV^+, low/intermediate risk, $patient^-$ and the $donor^-$ or $^{+48}$.

†This includes all leukemia, myeloma, lymphoma, or MDS in first complete remission following chemotherapy.

††This includes the patients who failed induction, relapsed diseases, failed to achieve complete remission with chemotherapy, and CML beyond first chronic phase.

‡This include patients with CML in the first chronic phase, aplastic anemia, MDS that proceeded to transplant without prior chemotherapy.

ψTwo sided *P*-values comparing "MTX-containing group" and "non-MTX-containing group" from Mann-Whitney *U* test for recipient age, donor age, number of prior regimens, $CD34^+$ cells, and $CD3^+$ cell count; χ^2 or Fischer's exact test for all other factors.

performed using the standard serologic techniques or low/intermediate resolution DNA techniques for HLA-A and -B antigens, and serologic level or high-resolution DNA techniques for HLA-DR antigens. For unrelated donors, high-resolution DNA techniques were performed for all HLA class I and II antigens. Donors included 61 HLA-identical siblings, 1 sibling with 1 class I antigen mismatch, 3 matched unrelated donors, and 2 unrelated with 1 class I antigen mismatch. Twenty-four cytomegalovirus (CMV)-seronegative patients had 17 seronegative and 7 seropositive donors. Among 43 seropositive patients, 31 had seropositive donors and 12 had seronegative donors.

Treatment and Evaluations

Patients were treated with 3 doses of fludarabine, 30 mg/m^2 per day, from days −4 to −2 and a single fraction of 2-Gy TBI delivered at 0.07 Gy/min from linear accelerators on day 0, followed by donor hematopoietic cell infusions. On the day of transplantation,

patients received unmanipulated allogeneic peripheral blood stem cell grafts mobilized with granulocyte colony-stimulating factor (G-CST) containing a median of 6.04 (range: 1.12-22.89) $\times 10^6$ $CD34^+$ cells/kg, and a median of 2.97 (range: 1.34-5.40) $\times 10^8$ $CD3^+$ cells/kg from their HLA-matched related donor (n = 62) or unrelated donor (n = 5). In the initial protocols, CSP was administered orally at 6.25 mg/kg twice daily. CSP levels were targeted to the individual institution's therapeutic range until day +35 and then tapered through day +56 for related recipients or until day +100 for unrelated recipients. MMF was given orally at 15 mg/kg twice a day or 10 mg/kg thrice a day starting from day 0 to day +27 for related recipients or until day 40 at full dose and then tapered through day +96 for unrelated recipients. In an attempt to decrease the observed incidence of GVHD, modification of postgrafting immunosuppression was made after July 2001 by adding standard course of methotrexate (MTX) at 15 mg/m^2 day +1, 10 mg/m^2 on day +3, day +6, and day +11. In addition, because of evolving treatment protocols, the duration of MMF and CSP was extended in the following manner: (1) CSP was given until day 80, and tapering was initiated in the absence of GVHD, until day 180; (2) MMF was given full dose until day 54, followed by a taper over 4 weeks. Accordingly, the first 21 patients received MMF and CSP (non-MTX-containing regimen) and the following 46 patients received extended MMF and CSP in addition to MTX (MTX-containing regimen) as postgrafting immunosuppression. Patients' characteristics were compared between those that were given the CSP/MMF 2-drug regimen versus those who were given the 3-drug regimen using MTX/CSP/MMF, as depicted in Table 1.

Antibacterial, antifungal, and antiviral prophylaxes were performed according to institutional protocols. These included trimethoprhim/sulfamethoxazole for *Pneumocystis carinii* prophylaxis, acyclovir for herpes simplex virus prophylaxis, and itraconazole or fluconazole for fungal prophylaxis. Patients were screened for CMV infection by PCR detection of viral DNA or by the viral pp65 antigenemia assay. Preemptive intravenous ganciclovir or foscarnet was given according to institutional guidelines. All blood products were irradiated (2500 cGy) and filtered before they were infused. Immunoglobulin was administered to all patients in a dose of 250 mg/kg weekly from day +7 to day +54.

Patients with neutropenic fever were treated with broad-spectrum antibiotics according to institutional protocols. Bone marrow aspiration was performed routinely 4 weeks after infusion and then 3 and 12 months later; aspirates were sent for morphologic evaluation, flow cytometry, cytogenetics, and chimerism evaluation. The levels of donor chimerism at granulocytes and mononuclear cells from peripheral blood or marrow were assessed at days 14, 28, 56, 84, 180, and 360 after HCT using FISH to detect X and Y chromosomes for recipients of grafts from sex-mismatched donors, and PCR-based amplification of variable numbers of tandem repeat sequences as previously described [37].

The primary endpoints of the study were to assess engraftment, regimen-related toxicity, nonrelapse mortality (NRM) and incidence of GVHD. The secondary endpoints included response rate, overall survival and progression-free survival (PFS). aGVHD and cGVHD were assessed according to the standard criteria [38,39]. With previous reports from Seattle that the median onset of GVHD was substantially delayed after NM versus myeloablative conditioning, clinical findings consistent with syndrome of aGVHD that appear after day 100 were also labeled as aGVHD (late-onset aGVHD) [46]. Toxicities were determined using the National Cancer Institute Common Toxicity Criteria, version 2 [40].

Statistical Analysis

In the evaluation of engraftment, patients who died before day +22 without engraftment were considered not evaluable and censored at time of death. Patients who died after day +22 without engraftment were considered as graft failures and censored at death or at day +42, whichever came first.

Data were analyzed according to previously published guidelines for assessment of outcomes after transplantation [41,42]. Time-to-event outcomes with competing risks (ie, NRM, relapse incidence, and GVHD) were estimated using cumulative-incidence curves by implementing the SAS macro as described by Tai et al. [43]. Time to NRM was defined from the date of transplantation until death from causes other than relapse, with relapse defined as a competitive risk. Similarly, NRM was defined as a competitive risk in analysis of relapse incidence, and death without GVHD as a competitive risk in GVHD analysis.

Progression free survival was defined only for patients who achieved complete remission (CR) and was measured from the date of CR until relapse or death, regardless of cause. Current PFS (CPFS) was calculated on the basis of disease status at last follow-up [41,44]. Patients who relapsed but responded to appropriate "salvage" therapy (second transplant, chemotherapy, or donor lymphocyte infusion) without subsequent progression at the time of analysis were censored at the last follow-up date in the analysis of CPFS. The time to event was defined as time from first transplant to time of hematologic relapse, death, or last contact in remission. Probabilities of overall survival (OS), PFS, and CPFS were calculated by the method of Kaplan and Meier [45] and differences in survival distributions between groups were compared

using the log-rank statistic [47]. In the analysis of OS and PFS, adjustment for prognostic factors was made using Cox regression, with aGVHD and cGVHD regarded as time-dependent covariates. All statistical analyses were performed using the SPSS version 13.0.

RESULTS

Patient Characteristics, Engraftment, and Donor Chimerism

Data were analyzed as of March 31, 2006. The median follow-up for surviving patients was 25 months (range: 6-72 months). Patient characteristics are summarized in Table 1. The median patient age was 45.5 years (range: 16-63 years), whereas the median donor age was 44 years (range: 20-62 years). Twenty-eight patients with age <45 years had NM transplant with the following primary indications: (1) failed prior autologous transplantation (N = 11); (2) medical comorbidities or organ dysfunction (N = 12); and (3) patients' decision because of the concern about regimen-related toxicity of myeloablative conditioning (N = 5). The median interval between diagnosis to HCT was 6 months (range: 1-60 months). Pretransplant treatment was heterogenous with a median of 1 (range: 0-4) prior chemotherapy regimen. At the time of HCT, 21 patients (30%) had chemotherapy-refractory disease. Eleven patients (16.4%) had relapsed from prior autologous HCT. Patients were classified as being at standard or high risk for disease progression after HCT as described in Table 1.

Among the entire cohort of 67 patients, 2 patients with refractory leukemia died of pneumonia before day 14, leaving 65 patients evaluable for engraftment. Among these 65 patients, all but 1 engrafted. This patient with CML has prior secondary graft failure from previous allograft using a different reduced intensity conditioning regimen. She was treated with imatinib and was later enrolled into a phase II clinical trial using dasatinib and is currently in partial response 6 years after HCT.

Neutrophil counts $>0.5 \times 10^9$/L was achieved after a median of 17 days (range: 9-29 days), and platelet counts more than 20×10^9/L was achieved after a median of 14 days (range: 6-21 days). The median number of days in which absolute neutrophil count (ANC) was $<0.5 \times 10^9$/L was 6 days (range: 0-25 days) and 17 (26%) patients maintained ANC $>0.5 \times 10^9$/L after HCT. Thirty-nine patients (60%) maintained platelet count $>20 \times 10^9$/L after HCT. Thirty-six (54%) patients did not require any transfusions, 41 (61%) did not require platelet transfusions, and 38 (57%) did not require any red blood cell transfusions.

The median percentage of donor chimerism performed at days 14, 28, 54, 84, 180, and 365 were 50%, 95%, 95%, 95%, 100%, and 100%, respectively. At 6 months, 28 (42%) patients with diseases in complete remission had >95% donor chimerism. At 1 year, 19 (28%) patients were in remission and had complete donor cell engraftment.

Three matched-related recipients with myelofibrosis (n = 1), CML (n = 2), after their initial engraftment and attainment of mixed chimerism ranging between 75% and 95%, rejected their graft on day 83, day 130, and day 180, respectively. Graft rejection preceded disease progression or relapse in all these patients, and despite withdrawal of immunosuppressant followed by donor lymphocyte infusion (DLI), there was no response. One patient with CML was given imatinib and achieved complete remission. Another patient committed suicide because of depression on day 130 of HCT. A patient with myelofibrosis had disease recurrence with recurrent splenomegaly and marrow fibrosis after initial complete remission (CR). She is now in stable disease at 2 years after HCT with no therapy.

GVHD

With 2 early toxic deaths and 1 primary graft failure, only 64 patients were evaluable for aGVHD. aGVHD of grade II-IV developed in 52% of these 64 evaluable patients: grade II in 13 (20%), grade III in 19 (30%), and grade IV in 1 (2%). The median day of onset was 30 days (range: 11-182 days). Skin, liver, and gut were affected in 15, 18, and 19 patients, respectively. In most cases, aGVHD was treated with corticosteroids alone. In 4 patients, OKT3, rapamycin, or daclizumab were used because of inadequate response to corticosteroids. The 2-year cumulative incidence of grade II-IV and grade III-IV aGVHD were 49% (95% confidence interval [CI] 37%-62%) and 30% (95% CI 19%-41%), respectively (Figure 1A). Competing risk analysis demonstrated the use of MTX-containing regimen with extended duration of CSP and MMF as the only variable that was associated with a lower risk of developing grade III-IV aGVHD (20% versus 52%, difference in cumulative incidence, 32%; 95% CI 8%-58%; $P = .009$) (Figure 1B; Table 2). There was no significant difference in the incidence of grade II-IV GVHD between the 2 immunosuppressive regimens ($P = .512$).

There was no significant difference in the time to onset of aGVHD between the 2 immunosuppressive regimens. Acute grade II-IV GVHD occurred at the median time of 32 days (range: 17-182 days) and 26 days (range: 11-101 days), respectively, for patients receiving non-MTX-containing and MTX-containing immunosuppressive regimens ($P = .49$). Acute grade III-IV GVHD occurred at the median time of 35 days (range: 17-182 days) and 32 days (range: 11-86 days), respectively, for patients receiving non-MTX- con-

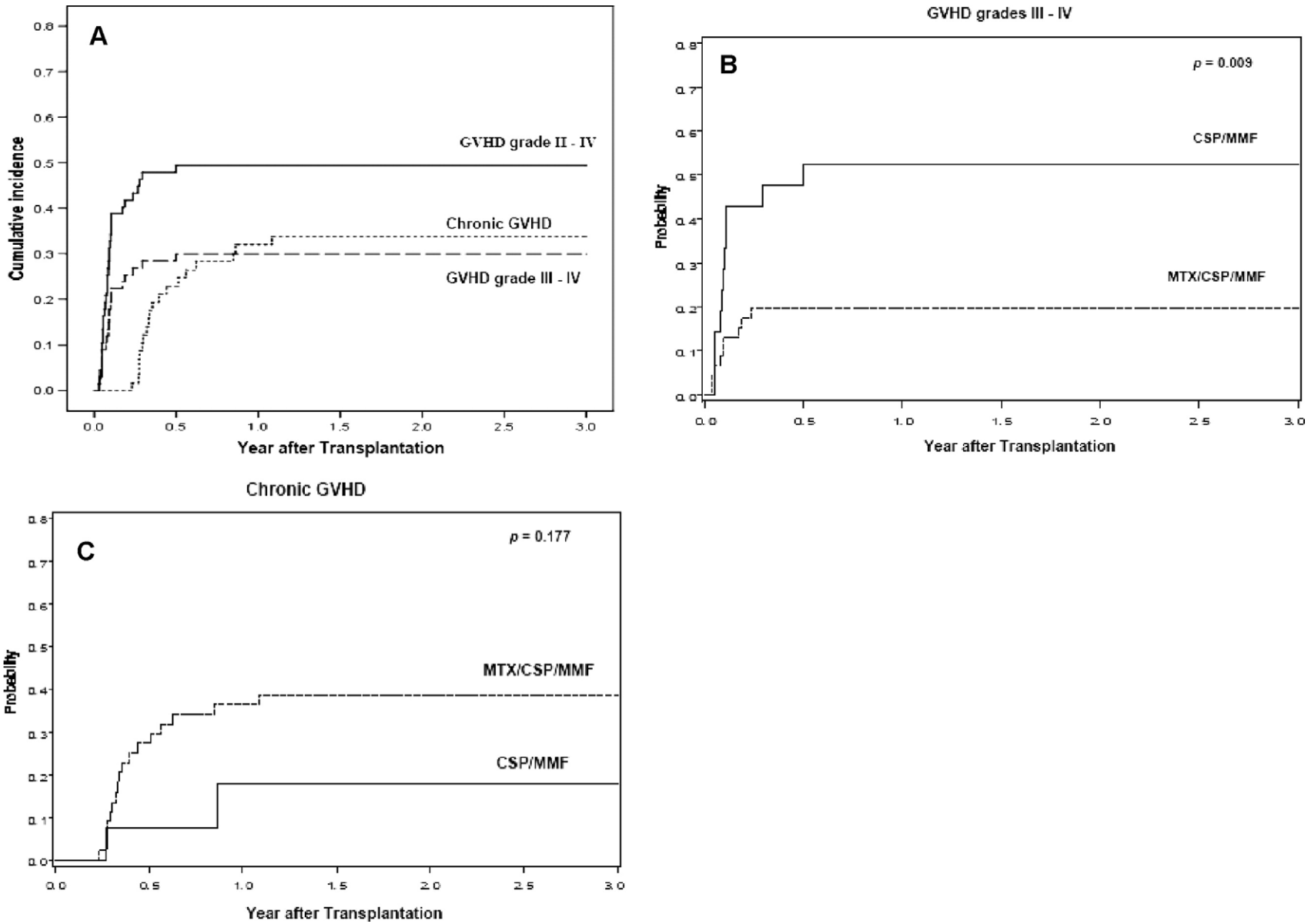

Figure 1. **A**, Cumulative incidence of aGVHD and cGVHD of 67 patients undergoing nonmyeloablative allogeneic transplantation. **B**, Grade III-IV GVHD. **C**, Chronic GVHD of 67 patients undergoing nonmyeloablative allogeneic transplantation stratified by postgrafting immunosuppression.

Table 2. *Two-Year Outcome Probabilities of all Patients and Stratified by Postgrafting Immunosuppression*

Outcomes	All Patients (N = 67) %	Postgrafting Immunosuppression CSA/MMF (N = 21) %	CSA/MMF/MTX (N = 46) %	Pψ
Nonrelapse mortality*	27	62	11	<.001
Relapse*	50	33	59	.174
Overall survival†	43	5	60	<.001
Progression-free survival†	28	0	42	<.001
Grade III-IV acute GVHD**	30	52	20	.009
Chronic GVHD*	34	18	39	.177

GVHD indicates graft-versus-host disease.
*Cumulative incidence.
**Six-month cumulative incidence.
†Kaplan-Meyer estimate.
ψ*P* values are based on comparisons of outcome between the 2 arms of postgrafting immunosuppression.

taining and MTX-containing immunosuppressive regimens ($P = .58$).

Given that patients on MTX-containing GVHD prophylaxis regimen were also on the extended CSP/MMF protocol, we asked the question of whether this could have any impact on the incidence and severity of aGVHD. However, with at least half of patients in the non-MTX-containing arm remaining on CSP/MMF longer than specified by protocols because of occurrence of aGVHD, the impact of extended CSP/MMF on the incidence or severity of aGVHD could not be adequately addressed in the current study.

Fifty-three patients surviving beyond 100 days were evaluable for cGVHD. Overall, cGVHD developed in 19 patients at a median time of 123 days (range: 102-396 days) after transplantation. The 2-year cumulative incidence of cGVHD was 34 % (95% CI 21%-46%) (Figure 1A). Fourteen had limited disease and 5 had extensive disease. Fourteen (21%) patients died without relapse of their disease from complications arising from either aGVHD or cGVHD. The 2-year cumulative incidence of cGVHD was not statistically different between patients receiving the 2 postgrafting immunosuppression regimens, although there was a trend toward a higher incidence of cGVHD in patients given MTX-containing regimen (39% versus 18%; difference in cumulative incidence, 21%; 95% CI 9%-51%; $P = .177$) (Figure 1C; Table 2).

Toxicity and Nonrelapse Mortality (NRM)

Mild to moderate nausea caused by CSP/MMF was common. No patient experienced new onset of alopecia or veno-occlusive disease. Thirteen-patients (32%) did not have any regimen-related toxicity or infection during the first 100 days of transplant. In contrast, grade III-IV regimen-related toxicities were seen in 32 (47%) patients, causing deaths in 15 cases. The most frequent grade III and IV toxicities are shown in Table 3.

Within the first 30 days, an increase of serum creatinine and bilirubin of ≥1.5 times upper normal limit was documented in 15 (22%) and 24 (36%) patients, respectively. The majority of these cases were transient and reversible, and were ascribed to CSP, concomitant medications, or infections.

Eighteen (27%) died from nonrelapse causes, including GVHD (n = 14), pneumonia (n = 3), and pulmonary hemorrhage (n = 1). The cumulative incidences of NRM were 18% (95% CI 9%-27%) at day 100 and 27% (95% CI 16%-38%) at 1 year, respectively. Competing risk analysis identified the use of MTX-containing GVHD prophylaxis regimen as the only pretransplantation variable that was associated with lower risk of NRM (11% versus 62%; difference in cumulative incidence, 51%; 95% CI 26%-76%, $P < .001$) (Figure 2A; Table 2).

Overall, infection-related death occurred in 24 (36%) patients, with 11 (16%) of these occurring within the first 100 days of transplantation. Twenty-one (31%) of these cases were associated with aGVHD (N = 12) or underlying persistent residual diseases (N = 9). Thirteen (19%) patients developed pneumonia caused by fungi (n = 8), bacteria (n = 3), and of unknown etiologies (n = 2), leading to death in 12 cases. Nineteen (28%) patients developed neutropenic fever before engraftment. Bacteremia was documented in 5 (8%) patients, causing death in all the 5 cases. Proved or probable invasive aspergillosis occurred in 7 patients with grade III-IV GVHD receiving steroid prednisolone >1 mg/kg/day and 1 patient with refractory acute myelogenous leukemia (AML). Only 2 of these were successfully treated. The remaining 6 patients died either of refractory disease (n = 1) or of GVHD (n = 5).

Eighteen (42%) of the 43 patients at high risk of infection with CMV (ie, seropositive recipients) and 11 (46%) of the 24 low/intermediate-risk patients (seronegative recipients and seropositive or seronegative donors) developed CMV antigenemia [47]. All patients were treated successfully with either ganciclovir or foscarnet; none developed CMV disease. Although more patients in the group receiving CSP/MMF were at higher risk of CMV infections, no difference in the

Table 3. *Incidence of Grade III and IV Toxicities in 67 Patients Receiving Nonmyeloablative Hematopoietic Cell Transplant*

Grade	Renal	Hepatic	Pulmonary	GI	Mucositis	Hemorrhage
III	4	8	2	5	0	0
IV	0	0	13	0	1	2

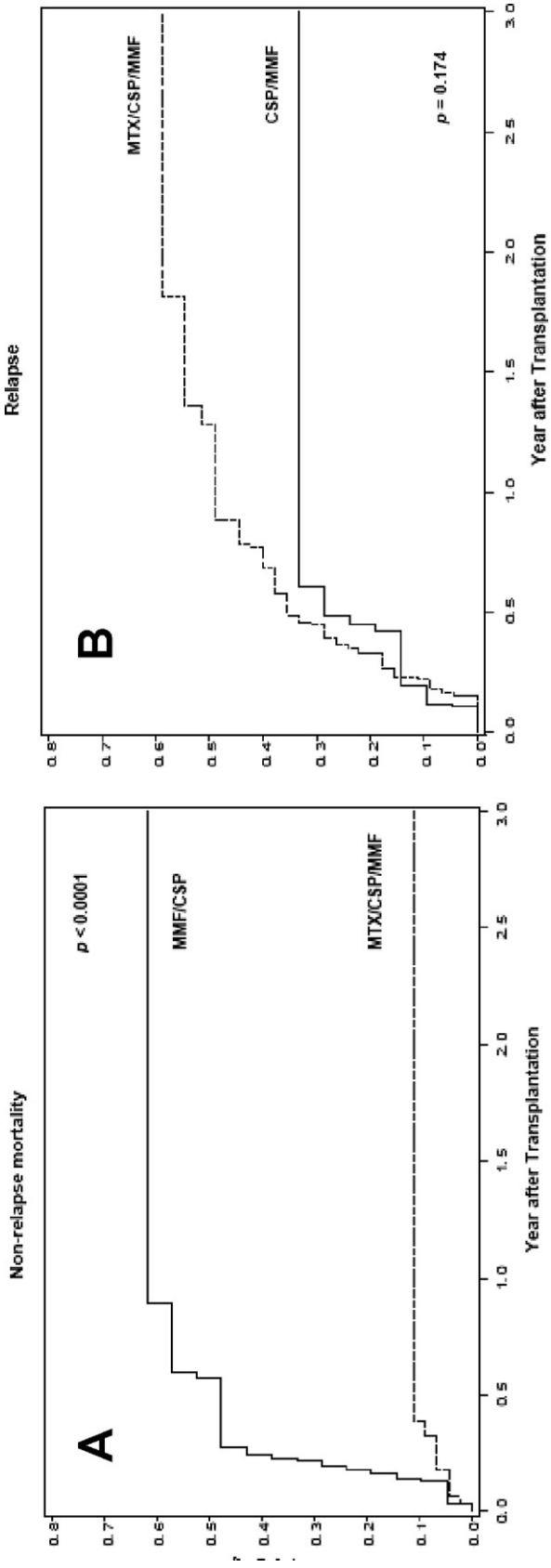

Figure 2. **A**, NRM and **B**, relapse rate of 67 patients undergoing nonmyelablative allogeneic transplantation stratified by postgrafting immunosuppression.

incidence of CMV antigenemia and disease in the 2 groups was noted.

The anticipated complications associated with the use of MTX were compared between the 2 cohorts of patients. The addition of MTX in the postgrafting immunosuppression was associated with a trend toward higher incidence of mucositis (9% versus 0%), longer duration of neutropenia (median duration 9.5 days versus 1 day) and thrombocytopenia (median duration 15 days versus 9 days), as well as a higher requirement for blood and platelet transfusion (52% versus 33%). However, because of the small number of patients studied, none of these observed differences reach statistical significance ($P > .05$).

Disease Response and Relapse

Sixty-four patients were evaluable for treatment response after transplantation. CR and PR were attained in 53 (83%) and 7 (11%) patients, respectively. Fifty-six evaluable patients had measurable disease before transplantation, and 45 (80%) achieved CR sometime after transplant. Four patients who had persistent disease after transplant had all succumbed to disease progression at a median of 235 days (range: 39-447 days) after transplantation.

Overall, 33 patients relapsed at the median time of 152 days (range: 33-663 days) after HCT, of whom 18 had died of disease progression. The cumulative incidence of relapse or disease progression at 2 years was 50% (95% CI 37%-63%). Of the remaining 15 patients who were alive at the last follow up, 4 had stable disease, 2 had progressive disease, 9 achieved CR following either with a second allogeneic transplant (N = 2), withdrawal of immunosuppressant and DLI (N = 4), salvage chemotherapy followed by DLI (N = 2), or imatinib (N = 1). Competing risks analysis did not reveal any pretransplantation variables to be significantly associated with the risk of relapse.

Donor Lymphocyte Infusion

Overall, 23 (34%) patients received DLI: 19 for persistent or progressive disease and 4 for declining or low level of donor chimerism. Median time to the first DLI from transplant was 129 days (range: 36-468 days). A median of 8×10^6 (range: 1×10^6 to 1.8×10^7) $CD3^+$ cells/kg was given with the first administration of DLI. In 11 patients, a second infusion of DLI with a median dose of 3×10^7 (range: 5.6×10^6 to 3.5×10^7) $CD3^+$ cells /kg was given at a median of 26 days (range: 3-72 days) following the first DLI. Three patients received a third dose ranging from 1.5-4.0 $\times 10^7$ $CD3^+$ cells/kg.

Seven patients responded to DLI, and these included 2 patients with CML with relapsed or persistent disease, 1 patient with relapsed Hodgkin's disease, 1 patient with MDS, and 3 patients with relapsed

Table 4. *Causes of Death in 67 Patients Receiving Nonmyeloablative Hematopoietic Cell Transplant*

Time	GVHD	Infection	Relapse/ PD	Others*	Death, N (%)	NRM, N (%)
<100 Days	8	3	1	1	13 (19)	12 (17)
>100 Days	6	0	16	1	23 (34)	7 (11)
Total	14	3	17	2	36 (53)	19 (28)

GVHD indicates graft-versus-host diesease; NRM, nonrelapse mortality; PD, progressive disease.
*Others include pulmonary hemorrhage (N = 1) and suicide (N = 1).

multiple myeloma. Two patients with relapsed AML were given chemotherapy to induce second CR before receiving DLI. Both patients remained alive and free of disease at the latest follow-up.

Five patients developed GVHD (4 of whom were treated for progressive disease and 1 for decreasing donor chimerism), with acute presentation in 3 patients (2 grade II and 1 grade III) and chronic limited presentation in 2 patients.

Survival Analyses

At the time of last follow-up, 31 (46%) patients were alive, at a median follow-up of 22 months (range: 6-72 months) after HCT. Of these, 25 (81%) achieved and remained in CR, 4 had stable disease, and 2 had progressed or relapsed. The causes of death are listed in Table 4. Thirteen (19%) deaths occurred during the first 100 days after transplantation with majority of the patients (77%) not receiving MTX as part of GVHD prophylaxis: 1 died of refractory AML, 8 died of aGVHD, 1 died of pulmonary hemorrhage, and 3 died of pneumonia.

The 5-year probabilities of OS and PFS were 43% (95% CI 31%-55%) and 28% (95% CI 16%-40%), respectively (Figure 3A). We determined the current PFS [41,44], based on disease status at the latest assessment. In this analysis, we assumed that patients who relapsed but reentered and remained in remission after appropriate "salvage" therapy (eg, second transplant, chemotherapy, or DLI) were disease-free. The estimated 5-year current PFS was 36% (95% CI 24%-48%) (Figure 3A).

Univariate and multivariate analysis demonstrated that GVHD prophylaxis regimen and grade III-IV aGVHD were the only 2 factors that had significant impact on both OS and PFS. Patients given the MTX-containing regimen had significantly superior OS (adjusted hazard ratio [HR] 0.12, 95% CI 0.06-0.27, $P < .001$) and PFS (adjusted HR 0.19, 95% CI 0.10-0.38, $P < .001$) compared to those who received only MMF/CSP (Figure 3B and C; Table 2). Patients who developed grade III-IV aGVHD had significantly poorer OS (adjusted HR 0.28, 95% CI 0.14-0.57, $P < .001$) and PFS (adjusted HR 0.43, 95% CI 0.23-0.83, $P < .001$) compared to those who did not.

Table 5 summarizes the outcome of all patients stratified by disease categories (AML/myelodysplastic syndromes [MDS] versus others), disease risk (standard versus high risk), and postgrafting immunosuppression (MTX versus no MTX). As shown in Figure 3D and E, patients with diagnosis other than AML/MDS receiving extended MTX-containing postimmunosuppression had a more favorable OS (adjusted HR 0.33, 95% CI 0.12-0.97, $P = .04$), but there was no statistical difference in their current PFS (adjusted HR 0.66, 95% CI 0.27-1.60, $P = .35$) when compared to patients with AML/MDS receiving similar postgrafting immunosuppression. The difference in OS was attributed to higher incidence of NRM (GVHD and infection) seen among the AML/MDS group (22% versus 0%; $P = .02$). The table also clearly demonstrates that patients given non-MTX containing regimens had a dismal outcome with none surviving in disease-free status at 3 years. This high mortality may be attributed to both transplant-related complications and relapsed disease. Despite demonstrating a trend toward a higher cumulative incidence of relapse (50% versus 33%; $P = .174$), patients receiving MTX had a much superior disease-free survival (DFS) (14 of 23 patients in CR during the last follow-up). This suggests the possible protective effect of MTX against lethal GVHD and its associated complications, resulting in much lower NRM seen among patients receiving MTX (Figure 2 and Table 2).

Subgroup analysis was performed on 21 of the 67 patients with "standard risk" diseases (hematologic malignancies in first remission, CML in the first chronic phase, MDS-refractory anemia subtype, and aplastic anemia) and were given the MTX-containing regimen as postgrafting immunosuppression. The median age of this subgroup of patients was 43 years (range: 25-62 years) with 6 (29%) patients exceeding 60 years of age. The 3-year OS and PFS for this subgroup were 85% (95% CI, 70-100%) and 65% (95% CI, 44-86%) (Figure 3F). This compares favorably with 25 "high-risk" patients given similar postgrafting immunosuppression, who demonstrated a 41% (95% CI, 19%-63%) 3 year OS ($P = .02$), and a 23% (95% CI, 15%-31%) 3 year PFS ($P = .03$). Larger studies with more standard risk patients are needed to confirm these encouraging results.

DISCUSSION

Considerable clinical evidence has established that NM conditioning, which relies on optimizing pre- and posttransplant immunosuppression to overcome host-versus-graft rejection facilitates prompt and stable engraftment, whereas enabling eradication of tumors via its powerful immune-mediated graft-versus-tumor effect. More importantly, the procedure was

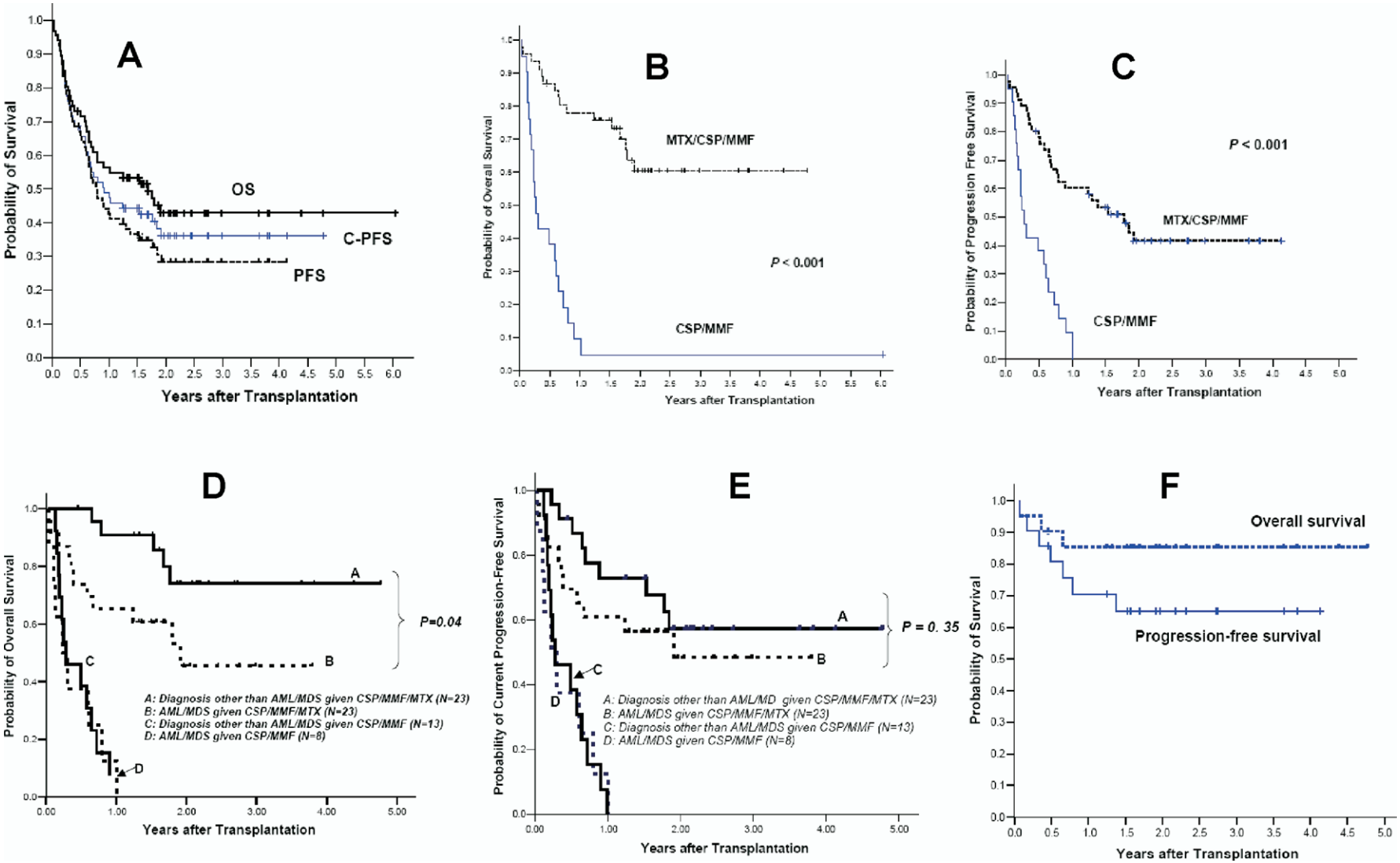

Figure 3. **A**, OS, current PFS (CPFS), and PFS of 67 patients undergoing nonmyeloablative allogeneic transplantation. **B**, OS, and **C**, PFS of 67 patients undergoing nonmyeloablative allogeneic transplantation stratified by postgrafting immunosuppression. **D**, Overall survival. **E**, current PFS of 67 patients stratified by disease categories and post-grafting immunosuppression. **F**, OS and PFS of a subgroup of 21 patients with "standard-risk" disease given MTX/MMF/CSP as postgrafting immunousppression.

Table 5. *Summary of Outcome of Patients Stratified by Disease Categories, Risk Categories, and Postgrafting Immunosuppression*

GVHD Prophylaxis Regimen	Total	Alive (Disease Status at Last Follow Up) (N)			Death (Cause of Death) (N)			
		CR	PR/SD	PD	GVHD	Infection	Relapse/PD	Others
Diagnosis								
AML/MDS								
No MTX	8	0	0	0	2	2	4	0
With MTX	23	11	0	1	3	1	5	2
Diagnosis other than AML/MDS								
No MTX	13	0	1	0	9	0	3	0
With MTX	23	14	3	1	0	0	5	0
Disease risk								
Standard risk								
No MTX	13	0	1	0	9	0	3	0
With MTX	21	17	1	0	0	1	1	1
High risk								
No MTX	8	0	0	0	2	2	4	0
With MTX	25	8	2	2	3	0	9	1

CR indicates complete remission; PR, partial remission; SD, stable disease; PD, progressive disease; AML, acute myelogenous leukemia; MDS, myelodysplastic syndrome; MTX, methotrexate; GVHD, graft-versus-host disease.

remarkably well tolerated, thereby extending the age range of potential recipients suitable for transplantation and included those less medically fit [3,4,36]. Despite the considerably lower regimen-related toxicities and early mortality compared to conventional transplantation, NM HCT is frequently associated with severe complications such as aGVHD and cGVHD, resulting in significant transplant-related morbidity and mortality. Even though GVH-reactions ensuing after NM HCT are, in theory, needed for achieving stable engraftment and for eradication of underlying malignant diseases, the prevention of severe aGVHD and its documented associated morbidity and mortality have remained important objectives [14,48,49].

Here, we report our results in a cohort of 67 patients with various hematologic diseases treated with 2 similar GVHD prophylaxis regimens. The present study confirms results from Seattle that this NM regimen was well tolerated and resulted in a high rate of engraftment in patients who are otherwise excluded from conventional HCT because of age or comorbidities. The hematologic toxicities of this NM regimen were moderate with ANCs in approximately 25% of patients not declining below 500/μL and more than half of the patients not requiring any blood or platelet transfusion.

In this study, however, an unexpectedly high cumulative incidence of NRM (62% at 1 year) and grade III-IV aGVHD (52%) were observed among the first cohort of patients receiving CSP/MMF as postgrafting immunosuppression. The 1-year probabilities of OS and PFS on this regimen were 43% and 5%, respectively. Main causes of NRM were GVHD (85%) and infection (15%). The NRM and severe GVHD reported here are in excess of that previously reported. In an analysis of the first 451 patients from the Fred Hutchinson Cancer Research Center consortium using similar conditioning and postgrafting immunosuppression, grade III-IV aGVHD occurred in 14% of patients, and the 2-year probabilities of OS and NRM were 51% and 22%, respectively [36]. Similar to most studies on NM HCT, GVHD, and infections were the main causes of NRM. This study has shown a relatively higher incidence of severe aGVHD. Previous studies have suggested that this may be attributable to genetic factors, such as ethnic heterogeneity, and diversity of major and minor histocompatibility frequencies or cytokine gene polymorphism [50,51].

The use of MTX used in conjunction with CSP has been regarded as the gold standard for GVHD prophylaxis [25-27]. We therefore hypothesized that the addition of MTX onto the CSP/MMF combination would enhance GVHD protection and reduce transplant-related morbidity and mortality. With this approach, the reduction of NRM and improvement in survival was evident in our patient population. This reduction of NRM, hypothesized to result from the addition of MTX, could have resulted from the unequivocally lower incidence of grade III-IV aGVHD (20% versus 52%). Despite demonstrating a trend towards higher probability of relapse in patients given MTX/CSP/MMF (59% versus 33%; $P = .174$) compared with those given CSP/MMF, addition of MTX was associated with lower NRM and more favorable OS and PFS. A number of other studies have reported similar negative impact of grade III-IV aGVHD on PFS in patients given HCT after reduced intensity or NM conditioning [8,52]. A recent study among recipients of HLA-matched related and unrelated NM transplants from Seattle has demonstrated that grades

III–IV aGVHD resulted in significantly increased NRM without measurable protective effects against recurrent malignancies. The protective effects against disease recurrence and the consequent superior PFS were exclusively associated with extensive cGVHD [8]. In our study, the overall cumulative incidence of cGVHD was 34%, with a trend toward higher incidence observed in patients receiving MTX-containing postgrafting immunosuppression. This higher risk of cGVHD, however, did not compromise the OS and PFS. In light of these results, the prevention of severe aGVHD appeared to be more desirable than the prevention of cGVHD.

Although the reduced intensity of the conditioning regimen has resulted in reduced NRM in patients with hematologic malignancies, relapse remains another critical barrier that limits the eventual success of the procedure [53]. The overall cumulative incidence of relapse of 50% in this series appears higher than most other series of allogeneic transplant recipients using nonmyeloblative or reduced-intensity conditioning, which reported relapse rates ranging between 30% and 46% [1,53-57]. Differences in patient selection and disease stage/type, may to some extent, account for the disparity of our results and other series. "High risk" patients consisted of 50% of the subjects enrolled in our current series. "High risk" in our definition and also by others [57], were patients who did not fulfill one of the following criteria: AML/ALL/myeloma/lymphoma in the first remission, CML in the first chronic phase, MDS-refractory anemia, aplastic anemia. These "high-risk" patients were deemed to be at higher risk of disease progression or relapse after NM conditioning. Notably, of the 33 patients with relapse, 22 (66.7%) were in the poor risk category. With our series consisting of a significant proportion of patients with unfavorable pretransplant disease status (ie, not in first remission) or disease entities with higher risk of relapse, our relapse rate is comparable with some of the published series using non-T cell depletion regimens [54,56,58].

In the current study, subgroup analysis of a cohort of patients given MTX/CSP/MMF showed that patients with "standard-risk diseases" had a 3-year OS and PFS of 85% and 65%, respectively. This compares favorably to the 41% and 23% OS and PFS, respectively, in patients with poor-risk diseases ($P < .05$) (data not shown). The difference is attributed to higher death rate from relapse among the "high risk" group (36% versus 5%; $P = .02$) (Table 5). Our findings, together with the other published results, have highlighted several important points: (1) the intensity of the conditioning regimen does affect the rate of relapse with increased risk of relapse noted after NM transplantation, although some of the published data may be confounded by including a high proportion of subjects from "high risk" categories. (2) The importance of patient selection and pretransplantation disease status in NM SCT, with significantly longer survival in patients with indolent or chemotherapy-sensitive malignancies [59,60]. (3) There is a continuing need to investigate the dose intensity of conditioning regimen for allogeneic transplant of diseases with a higher risk of relapse.

The ability to promote durable engraftment and eliminate severe aGVHD with MMF/CSP/MTX after fludarabine/low dose TBI conditioning has potential implications for NM allografting of nonmalignant disease, in which GVHD is especially counterproductive. We have recently reported encouraging results in a subgroup of 8 multiply-transfused patients with aplastic anemia and paroxysmal nocturnal hemoglobulinemia, who received NM allografting using this approach [61]. Our observations suggested that this approach allowed prompt and stable engraftment in all patients. Furthermore, addition of MTX into the postgrafting immunosuppression effectively avoids fatal aGVHD and resulted in 100% DFS in all 6 patients at a median follow-up of 24 months.

Although the combination of CSP and MTX has been the gold standard for GVHD prophylaxis in myeloablative transplant for decades [25-27], toxicities from MTX, even at low doses, may result in significant morbidity and mortality. As an antiproliferative agent, MTX inhibits GVHD primarily via killing of antigen-activated T cells. It also causes tissue damage and can activate the initial phase of the GVHD response [62,63]. The use of MTX in the GVHD prophylaxis has been associated with oral mucositis, delay in the time to neutrophil engraftment [25,35], and pulmonary toxicity [64], which may adversely affect transplantation outcome. Two previous comparative studies on patients receiving myeloablative HCT have demonstrated that, when a non-MTX-containing GVHD prophylaxis regimen was used, a faster rate of hematopoietic engraftment, a decrease in incidence and severity of mucositis, and mucositis-related morbidities such as total parenteral nutrition (TPN) use, narcotic use, and hospitalization duration were observed. Importantly, there was no compromise in GVHD control and survival was similar [65,66]. In the current analysis, the use of an MTX-containing regimen was associated with a trend toward higher incidence of mucositis and more severe hematologic toxicities. These differences did not reach statistical difference, and this was probably because of the small sample size in our study.

Despite the impressive decrease in grade III-IV aGVHD among MTX-treated patients, the incidence of cGVHD was not statistically different between the 2 postgrafting immunosuppression arms. There was, however, a trend toward higher incidence of cGVHD in patients receiving MTX-containing postgrafting immunosuppression. This can be explained in part by

the observation that those patients given a non-MTX-containing regimen who would have been at highest risk of developing cGVHD died earlier of complications from aGVHD, whereas comparable MTX-containing regimen-treated patients survived long enough to be at risk for cGVHD. Previous studies have shown that MTX-containing GVHD prophylaxis regimens were associated with a lower incidence of cGVHD after peripheral blood stem cell (PBSC) or marrow transplant using myeloablative transplant conditioning [67,68], although this relationship was not confirmed by others [69]. It remains unclear whether the use of MTX has any impact on the risk of cGVHD in patients receiving NM transplant, in which the use of PBSC has been recognized to be a significant compounding factor for cGVHD [70].

In previous studies from Seattle, the lack of stable mixed chimerism in most patients together with development of GVHD in some patients after discontinuation of CSP on day 35 led to the extension of CSP administration to day +56 for GVHD control [3,71]. A recent retrospective analysis showed that longer CSP duration decreased the risk of grade III-IV aGVHD and increased likelihood of discontinuing all systemic immunosuppression when compared to shorter CSP regimens [71]. In the current study, patients given MTX also received an extended duration of MMF and CSP. It is possible that the difference in the risk of aGVHD resulted from the protective effect of extended MMF/CSP rather than the effect of MTX alone. However, the impact of extended CSP/MMF on the severity or severity of aGVHD could not be adequately addressed in the current study because many patients receiving the non-MTX-containing postgrafting immunosuppressants were still on CSP/MMF prophylaxis at the time acute GVHD developed. Also, our evaluation is further hindered by the fact that many patients remained on CSP/MMF longer than specified by protocols because of occurrence of aGVHD. In our study, we did not find significant differences in the time to onset of GVHD nor in the cumulative incidence of grades II-IV aGVHD between the 2 immunosuppressive regimens. This suggested that the addition of MTX did not affect the time of onset or overall incidence of grade II-IV aGVHD but rather prevented progression of this complication to grade III-IV aGVHD.

The primary limitation of this study is that this finding was based on a relatively small number of patients. In addition, the effect of the 2 immunosuppressive regimens was not studied within the context of randomized controlled trial. Hence, it is possible that outcome was influenced by latent covariates that, although unknown, were both unevenly distributed. Also, it should be noted that other differences in practice over time beside alteration of immunosuppressive regimen could have influenced the outcome in the present study. Nevertheless, the encouraging results seen in this small cohort of patients provides rationale to assess the feasibility of this approach in larger number of patients.

In conclusion, we have shown that the addition of MTX onto the current postgrafting immunosuppression regimen with extended CSP/MMF prophylaxis duration offers the possibility of further optimization of GVHD control in patients receiving fludarabine/low-dose TBI NM conditioning. This immunosuppressive regimen decreased the risk of grade III-IV aGVHD, resulting in lower NRM and improved survival. Protection against severe aGVHD did not affect risks of cGVHD or relapse. Future prospective studies are needed to determine whether substituting alternative calcineurin inhibitors, such as tacrolimus, for CSP [72], might be more effective in preventing severe or therapy-refractory GVHD without compromising engraftment and control of the underlying malignancies.

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REFERENCES

1. Giralt S, Thall PF, Khouri I, et al. Melphalan and purine analog-containing preparative regimens: reduced-intensity conditioning for patients with hematologic malignancies undergoing allogeneic progenitor cell transplantation. *Blood*. 2001;97:631-637.
2. Slavin S, Nagler A, Naparstek E, et al. Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. *Blood*. 1998;91:756-763.
3. McSweeney PA, Niederwieser D, Shizuru JA, et al. Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects. *Blood*. 2001;97:3390-3400.
4. Niederwieser D, Maris M, Shizuru JA, et al. Low-dose total body irradiation (TBI) and fludarabine followed by hematopoietic cell transplantation (HCT) from HLA-matched or mismatched unrelated donors and postgrafting immunosuppression with cyclosporine and mycophenolate mofetil (MMF) can induce durable complete chimerism and sustained remissions in

patients with hematological diseases. *Blood*. 2003;101:1620-1629.
5. Maris MB, Sandmaier BM, Storer BE, et al. Allogeneic hematopoietic cell transplantation after fludarabine and 2 Gy total body irradiation for relapsed and refractory mantle cell lymphoma. *Blood*. 2004;104:3535-3542.
6. Mielcarek M, Martin PJ, Leisenring W, et al. Graft-versus-host disease after nonmyeloablative versus conventional hematopoietic stem cell transplantation. *Blood*. 2003;102:756-762.
7. Mielcarek M, Burroughs L, Leisenring W, et al. Prognostic relevance of "early-onset" graft-versus-host disease following non-myeloablative haematopoietic cell transplantation. *Br J Haematol*. 2005;129:381-391.
8. Baron F, Maris MB, Sandmaier BM, et al. Graft-versus-tumor effects after allogeneic hematopoietic cell transplantation with nonmyeloablative conditioning. *J Clin Oncol*. 2005;23:1993-2003.
9. Kottaridis PD, Milligan D, Chopra R, et al. In vivo CAMPATH-IH prevents graft-versus-host disease following nonmyeloablative stem cell transplantation. *Blood*. 2000;96:2419-2425.
10. Morris E, Thomson K, Craddock C, et al. Outcomes after alemtuzumab-containing reduced-intensity allogeneic transplantation regimen for relapsed and refractory non-Hodgkin lymphoma. *Blood*. 2004;104:3865-3871.
11. Ho AY, Pagliuca A, Kenyon M, et al. Reduced intensity allogeneic hematopoietic stem cell transplantation for myelodysplastic syndrome and acute myeloid leukemia with multilineage dysplasia using fludarabine, busulphan, and alemtuzumab (FBC) conditioning. *Blood*. 2004;104:1616-1623.
12. Peggs KS, Hunter A, Chopra R, et al. Clinical evidence of a graft versus Hodgkin's lymphoma effect after reduced intensity allogeneic transplantation. *Lancet*. 2005;365:1934-1941.
13. Faulkner R, Craddock C, Byrne J, et al. BEAM-alemtuzumab reduced intensity allogeneic stem cell transplantation for lymphoproliferative diseases: GVHD, toxicity, and survival in 65 patients. *Blood*. 2004;103:428-434.
14. Weiden PL, Flournoy N, Thomas ED, et al. Antileukemic effect of graft-versus-host disease in human recipients of allogeneic-marrow grafts. *N Engl J Med*. 1979;300:1068-1073.
15. Horowitz MM, Gale RP, Sondel PM, et al. Graft-versus-leukemia reactions after bone marrow transplantation. *Blood*. 1990; 75:555-562.
16. Weiden PL, Sullivan KM, Flournoy N, Storb R, Thomas ED. Antileukemic effect of chronic graft-versus-host disease: contribution to improved survival after allogeneic marrow transplantation. *N Engl J Med*. 1981;304:1529-1533.
17. Slavin S, Weiss L, Morecki S, Weigensberg M. Eradication of murine leukemia with histoincompatible marrow grafts in mice conditioned with total lymphoid irradiation. *Cancer Immunother*. 1981;11:155-158.
18. Storb R, Yu C, Wagner JL, et al. Stable mixed hematopoietic chimerism in DLA-identical littermate dogs given sublethal total body irradiation before and pharmacological immunosuppression after marrow transplantation. *Blood*. 1997;89:3048-3054.
19. Giralt S, Estey E, Albitar M, et al. Engraftment of allogeneic hematopoietic progenitor cells with purine analog-containing chemotherapy: harnessing graft versus leukemia without myeloablative therapy. *Blood*. 1997;89:4531-4536.
20. Kroger N, Shaw B, Iacobelli S, et al. Comparison between antithymocyte globulin and alemtuzumab and the possible impact of KIR-ligand mismatch after dose reduced conditioning and unrelated stem cell transplantation in patients with multiple myeloma. *Br J Haematol*. 2005;129:631-643.
21. Crawley C, Lalancette M, Szydlo R, et al. Outcomes for reduced intensity allogeneic transplantation for multiple myeloma; an analysis of prognostic factors from the Chronic Leukemia Working Party of the EBMT. *Blood*. 2005;105:4532-4539.
22. Perez-Simon JA, Kottaridis PD, Martino R, et al. Nonmyeloablative transplantation with or without alemtuzumab: comparison between 2 prospective studies in patients with lymphoproliferative disorders. *Blood*. 2002;100:3121-3127.
23. Crawley C, Szydlo R, Lalancette M, et al. Outcomes of reduced-intensity transplantation for chronic myeloid leukemia: an analysis of prognostic factors from the Chronic Leukemia Working Party of the EBMT. *Blood*. 2005;106:2969-2976.
24. Ramsay NKC, Kersey JH, Robison LL, et al. A randomized study of the prevention of acute graft-versus-host disease. *N Engl J Med*. 1982;306:392-397.
25. 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.
26. Storb R, Deeg HJ, Pepe M, et al. Graft-versus-host disease prevention by methotrexate combined with cyclosporin compared to methotrexate alone in patients given marrow grafts for severe aplastic anaemia: long-term follow-up of a controlled trial. *Br J Haematol*. 1989;72:567-572.
27. 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;20:314:729-735.
28. Chao NJ, Schmidt GM, Niland JC, et al. Cyclosporine, methotrexate, and prednisone compared with cyclosporine and prednisone for prophylaxis of acute graft-versus-host disease. *N Engl J Med*. 1993;329:1225-1230.
29. Deeg HJ, Lin D, Leisenring W, et al. Cyclosporine or cyclosporine plus methylprednisolone for prophylaxis of graft-versus-host disease: a prospective, randomized trial. *Blood*. 1997; 89:3880-3887.
30. 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.
31. Ruutu T, Volin L, Parkkali T, et al. Cyclosporine, methotrexate, and methylprednisolone compared with cyclosporine and methotrexate for the prevention of graft-versus-host disease in bone marrow transplantation from HLA-identical sibling donor: a prospective randomized study. *Blood*. 2000;96:2391-2398.
32. Locatelli F, Bruno B, Zecca M, et al. Cyclosporin A and short-term methotrexate versus cyclosporin A as graft versus host disease prophylaxis in patients with severe aplastic anemia given allogeneic bone marrow transplantation from an HLA-identical sibling: results of a GITMO/EBMT randomized trial. *Blood*. 2000;96:1690-1697.
33. 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.
34. Srinivasan R, Geller N, Chakrabarti S, et al. Evaluation of three different cyclosporine-based graft versus host disease (GVHD) prophylaxis regimens following nonmyeloablative hematopoi-

etic stem cell transplantation (NST) [abstract 1236]. *Blood*. 2004;104(Supp 1):349a.
35. Chao NJ, Snyder DS, Jain M, et al. Equivalence of 2 effective graft-versus-host disease prophylaxis regimens: results of a prospective double-blind randomized trial. *Biol Blood Marrow Transplant*. 2000;6:254-261.
36. Sandmaier BM, Maris M, Maloney DG, et al. Low-dose total body irradiation (TBI)conditioning for hematopoietic cell transplantation (HCT) from HLA-matched related (MRD) and unrelated (URD) donors for patients with hematologic malignancies: a 5 year experience [abstract 264]. *Blood*. 2003; 102(Suppl 1):78a-79a.
37. Ugozzoli L, Yam P, Petz LD, et al. Amplification by the polymerase chain reaction of hypervariable regions of the human genome for evaluation of chimerism after bone marrow transplantation. *Blood*. 1991;77:1607-1615.
38. Przepiorka D, Weisdorf D, Martin P, et al. Consensus conference on acute GVHD grading. *Bone Marrow Transplant*. 1995; 15:825-828.
39. Sullivan KM, Agura E, Anasetti C, et al. Chronic graft-versus-host disease and other late complications of bone marrow transplantation. *Semin Hematol*. 1991;28:250-259.
40. Common terminology criteria for adverse events. Available at: http://ctep.cancer.gov/reporting/ctc.html.
41. Klein JP, Rizzo JD, Zhang MJ, Keiding N. Statistical methods for the analysis and presentation of the results of bone marrow transplants. Part I: unadjusted analysis. *Bone Marrow Transplant*. 2001;28:909-915.
42. Klein JP, Rizzo JD, Zhang MJ, Keiding N. Statistical methods for the analysis and presentation of the results of bone marrow transplants. Part 2: Regression modeling. *Bone Marrow Transplant*. 2001;28:1001-1011.
43. Tai BC, Machin D, White I, Gebski V, on behalf of the European Osteosarcoma Intergroup. Competing risks analysis of patients with osteosarcoma: a comparison of four different approaches. *Stat Med*. 2001;20:661-684.
44. Craddock C, Szydlo RM, Klein JP, et al. Estimating leukemia-free survival after allografting for chronic myeloid leukemia: a new method that takes into account patients who relapse and are restored to complete remission. *Blood*. 2000;96:86-90.
45. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc*. 1958;53:457-481.
46. Mantel N. Evaluation of survival data and two new rank order statistics arising in its consideration. *Cancer Chemother Rep*. 1966;50:163-170.
47. Junghanss C, Boeckh M, Carter RA, et al. Incidence and outcome of cytomegalovirus infections following nonmyeloablative compared with myeloablative allogeneic stem cell transplantation, a matched control study. *Blood*. 2002;99:1978-1985.
48. Nash RA, Pepe MS, Storb R, et al. Acute graft-versus-host disease: analysis of risk factors after allogeneic marrow transplantation and prophylaxis with cyclosporine and methotrexate. *Blood*. 1992;80:1838-1845.
49. Storb R, Prentice RL, Buckner CD, et al. Graft-versus-host disease and survival in patients with aplastic anemia treated by marrow grafts from HLA-identical siblings. Beneficial effect of a protective environment. *N Engl J Med*. 1983;308:302-307.
50. Oh H, Loberiza FR Jr, Zhang MJ, et al. Comparison of graft-versus-host-disease and survival after HLA-identical sibling bone marrow transplantation in ethnic populations. *Blood*. 2005; 105:1408-1416.
51. Lin MT, Storer B, Martin PJ, et al. Relation of an interleukin-10 promoter polymorphism to graft-versus-host disease and survival after hematopoietic-cell transplantation. *N Engl J Med*. 2003;349:2201-2210.
52. de Lima M, Anagnostopoulos A, Munsell M, et al. Nonablative versus reduced-intensity conditioning regimens in the treatment of acute myeloid leukemia and high-risk myelodysplastic syndrome: dose is relevant for long-term disease control after allogeneic hematopoietic stem cell transplantation. *Blood*. 2004; 104:865-872.
53. Hegenbart U, Niederwieser D, Sandmaier BM, et al. Treatment for acute myelogenous leukemia by low-dose, total-body, irradiation-based conditioning and hematopoietic cell transplantation from related and unrelated donors. *J Clin Oncol*. 2006;24:444-453.
54. Bethge WA, Storer BE, Maris MB, et al. Relapse or progression after hematopoietic cell transplantation using nonmyeloablative conditioning: effect of interventions on outcome. *Exp Hematol*. 2003;31:974-980.
55. van Besien K, Artz A, Smith S, et al. Fludarabine, melphalan, and alemtuzumab conditioning in adults with standard-risk advanced acute myeloid leukemia and myelodysplastic syndrome. *J Clin Oncol*. 2005;23:5728-5738.
56. Alyea EP, Kim HT, Ho V, et al. Comparative outcome of nonmyeloablative and myeloablative allogeneic hematopoietic cell transplantation for patients older than 50 years of age. *Blood*. 2005;105:1810-1814.
57. Maris MB, Niederwieser D, Sandmaier BM, et al. HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative conditioning for patients with hematologic malignancies. *Blood*. 2003;102:2021-2030.
58. Sloand E, Childs RW, Solomon S, et al. The graft-versus-leukemia effect of nonmyeloablative stem cell allografts may not be sufficient to cure chronic myelogenous leukemia. *Bone Marrow Transplant*. 2003;32:897-901.
59. Michallet M, Bilger K, Garban F, et al. Allogeneic hematopoietic stem-cell transplantation after nonmyeloablative preparative regimens: impact of pretransplantation and posttransplantation factors on outcome. *J Clin Oncol*. 2001;19:3340-3349.
60. Robinson SP, Goldstone AH, Mackinnon S, et al. Chemoresistant or aggressive lymphoma predicts for a poor outcome following reduced-intensity allogeneic progenitor cell transplantation: an analysis from the Lymphoma Working Party of the European Group for Blood and Bone Marrow Transplantation. *Blood*. 2002;100:4310-4316.
61. Koh LP, Koh MB, Ng HY, et al. Allogeneic hematopoietic stem cell transplantation for patients with severe aplastic anemia following nonmyeloablative conditioning using 200-cGy total body irradiation and fludarabine. *Biol Blood Marrow Transplant*. 2006;12:887-890.
62. Ferrara JL, Reddy P. Pathophysiology of graft-versus-host disease. *Semin Hematol*. 2006;43:3-10.
63. Hill GR, Ferrara JL. The primacy of the gastrointestinal tract as a target organ of acute graft-versus-host disease: rationale for the use of cytokine shields in allogeneic bone marrow transplantation. *Blood*. 2000;95:2754-2759.
64. Ho VT, Weller E, Lee SJ, Alyea EP, Antin JH, Soiffer RJ. Prognostic factors for early severe pulmonary complications after hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2001;7:223-229.
65. Cutler C, Kim HT, Hochberg E, et al. Sirolimus and tacrolimus without methotrexate as graft-versus-host disease prophy-

laxis after matched related donor peripheral blood stem cell transplantation. *Biol Blood Marrow Transplant*. 2004;10:328-336.
66. Bolwell B, Sobecks R, Pohlman B, et al. A prospective randomized trial comparing cyclosporine and short course methotrexate with cyclosporine and mycophenolate mofetil for GVHD prophylaxis in myeloablative allogeneic bone marrow transplantation. *Bone Marrow Transplant*. 2004;34:621-625.
67. Copelan EA, Penza SL, Theil KS, et al. The influence of early transplantation, age, GVHD prevention regimen, and other factors on outcome of allogeneic transplantation for CML following BuCy. *Bone Marrow Transplant*. 2000;26:1037-1043.
68. Kumar S, Chen MG, Gastineau DA, et al. Prophylaxis of graft-versus-host disease with cyclosporine-prednisone is associated with increased risk of chronic graft-versus-host disease. *Bone Marrow Transplant*. 2001;27:1133-1140.
69. Ross M, Schmidt GM, Niland JC, et al. Cyclosporine, methotrexate, and prednisone compared with cyclosporine and prednisone for prevention of acute graft-vs-host disease: effect on chronic graft-vs.-host disease and long-term survival. *Biol Blood Marrow Transplant*. 1999;5:285-291.
70. Stem Cell Trialists' Collaborative Group. Allogeneic peripheral blood stem-cell compared with bone marrow transplantation in the management of hematologic malignancies: an individual patient data meta-analysis of nine randomized trials. *J Clin Oncol.* 2005;23:5074-5087.
71. Burroughs L, Mielcarek M, Leisenring W, et al. Extending postgrafting cyclosporine decreases the risk of severe graft-versus-host disease after nonmyeloablative hematopoietic cell transplantation. *Transplantation*. 2006;81:818-825.
72. Nieto Y, Patton N, Hawkins T, et al. Tacrolimus and mycophenolate mofetil after nonmyeloablative matched-sibling donor allogeneic stem-cell transplantations conditioned with fludarabine and low-dose total body irradiation. *Biol Blood Marrow Transplant*. 2006;12:217-225.