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Comparative Analysis of Calcineurin Inhibitor–Based Methotrexate and Mycophenolate Mofetil–Containing Regimens for Prevention of Graft-versus-Host Disease after Reduced-Intensity Conditioning Allogeneic Transplantation

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A B S T R A C T

The combination of a calcineurin inhibitor (CNI) such as tacrolimus (TAC) or cyclosporine (CYSP) with methotrexate (MTX) or with mycophenolate mofetil (MMF) has been commonly used for graft-versus-host disease (GVHD) prophylaxis after reduced-intensity conditioning (RIC) allogeneic hematopoietic cell transplantation (alloHCT), but there are limited data comparing efficacy of the 2 regimens. We evaluated 1564 adult patients who underwent RIC alloHCT for acute myelogenous leukemia (AML) and acute lymphoblastic leukemia (ALL), chronic myelogenous leukemia (CML), and myelodysplastic syndrome (MDS) from 2000 to 2013 using HLA-identical sibling (matched related donor [MRD]) or unrelated donor (URD) peripheral blood graft and received CYSP or TAC with MTX or MMF for GVHD prophylaxis. Primary outcomes of the study were acute and chronic GVHD and overall survival (OS). The study divided the patient population into 4 cohorts based on regimen: MMF-TAC, MMF-CYSP, MTX-TAC, and MTX-CYSP. In the URD group, MMF-CYSP was associated with increased risk of grade II to IV acute GVHD (relative risk [RR], 1.78; $P < .001$) and grade III to IV acute GVHD (RR, 1.93; $P = .006$) compared with MTX-TAC. In the URD group, use of MMF-TAC (versus MTX-TAC) lead to higher nonrelapse mortality. (hazard ratio, 1.48; $P = .008$). In either group, no there was no difference in chronic GVHD, disease-free survival, and OS among the GVHD prophylaxis regimens. For RIC alloHCT using MRD, there are no differences in outcomes based on GVHD prophylaxis. However, with URD RIC alloHCT, MMF-CYSP was inferior to MTX-based regimens for acute GVHD prevention, but all the regimens were equivalent in terms of chronic GVHD and OS. Prospective studies, targeting URD recipients are needed to confirm these results.

INTRODUCTION

Although the development of reduced-intensity conditioning (RIC) or nonmyeloablative conditioning has allowed patients who are ineligible for myeloablative conditioning (MAC) allogeneic hematopoietic cell transplantation (alloHCT) to have access to this potentially curative therapy, nonrelapse mortality (NRM) remains a significant obstacle to its success [1]. The tight association between graft-versus-host disease (GVHD) and NRM has led to attempts to devise GVHD prevention strategies to decrease its incidence and severity [2]. For the past 3 decades, the regimen pioneered by the Seattle group combining a calcineurin inhibitor (CNI) with methotrexate (MTX) has been the most widely adopted for GVHD prevention [3]. Cyclosporine (CYSP) in combination with a short course of MTX has been widely used since the late 1980s [4,5]. In more recent years, tacrolimus (TAC) has emerged as an alternative to CYSP for GVHD prophylaxis [6]. CYSP and TAC share a final common pathway of inhibition of IL-2–mediated T cell expansion and cytotoxicity [7]. Randomized trials have shown that post-transplantation TAC-MTX is associated with decreased acute GVHD (aGVHD) compared with CYSP-MTX in patients with a matched sibling donor (matched related donor [MRD]) [8] or matched unrelated donor (URD) in the myeloablative setting [9]. There are, however, several caveats with the use of MTX, mainly delayed hematopoietic engraftment, increased oral mucositis, and gastrointestinal toxicity, as well as pulmonary and renal toxicity[2,10-12]. In patients undergoing RIC alloHCT, reducing procedure-related toxicities may be of critical importance, as these patients usually have greater comorbidities[1]. Therefore, to reduce MTX-associated toxicities, mycophenolate mofetil (MMF) has been investigated as a replacement for MTX in RIC regimens in recent years [13].

Currently, at most transplant centers, GVHD prophylaxis is largely based on CNI (CYSP or TAC) in combination with short-course MTX or MMF [14,15]. There is, however, significant variability among centers in GVHD prophylaxis regimens used. In RIC alloHCT, MMF is widely used instead of MTX in combination with a calcineurin inhibitor for GVHD prophylaxis[15], given the advantages of earlier engraftment and less mucositis. A recent Center for International Blood and Marrow Transplantation Research (CIBMTR) analysis [16] demonstrated significantly worse overall survival (OS), NRM, and aGVHD and chronic GVHD (cGVHD) with MMF compared with MTX after RIC alloHCT from unrelated donors (URD). With the 4 regimens of MTX-CYSP, MMF-CYSP, MTX-TAC and MMF-TAC being used frequently as the current standard to prevent GVHD after RIC alloHCT, an important and unanswered question is whether 1 of them is superior to others in preventing GVHD. We aimed to describe and evaluate the comparative efficacy of the 4 commonly used regimens in a large cohort of patients using the CIBMTR database.

METHODS

Data Sources

The CIBMTR is a combined research program of the Medical College of Wisconsin and the National Marrow Donor Program, which consists of a voluntary network of more than 450 transplantation centers worldwide that contribute detailed data on consecutive allogeneic and autologous transplantations to a centralized statistical center. Observational studies conducted by the CIBMTR are performed in compliance with all applicable federal regulations pertaining to the protection of human research participants. Protected health information issued in the performance of such research is collected and maintained in the CIBMTR's capacity as a Public Health Authority under the Health Insurance Portability and Accountability Act Privacy Rule.

Patients

The study population included adult patients with acute myelogenous leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myelogenous leukemia (CML), and myelodysplastic syndrome (MDS) who underwent a

first RIC alloHCT between 2000 and 2013 from an HLA-identical sibling or an 8/8- or 7/8-matched unrelated donor (HLA-A, -B, -C, -DRB1) [17] and received a combination of CNI (CYSP or TAC) and either MTX or MMF for prophylaxis against GVHD. Haploidentical related donor and cord blood transplants were excluded. All patients received a peripheral blood graft. Patients receiving ex vivo T cell depletion and bone marrow grafts were excluded given their small numbers.

Study Endpoints

The primary objective of the study was to evaluate and compare the risks of aGVHD, cGVHD, and overall mortality with each of the 4 GVHD prophylaxis regimens in RIC alloHCT patients receiving peripheral blood graft. The primary endpoints of the study, therefore, were grade II to IV and III to IV aGVHD, cGVHD, and OS. OS was defined as the time from alloHCT to death from any cause or until last follow-up. Death from any cause was considered an event. Surviving patients were censored at last follow-up. Secondary endpoints included absolute neutrophil count recovery, platelet recovery, disease-free survival (DFS), relapse and NRM. Patients were censored at subsequent transplant or date of last follow up. DFS was defined as time from alloHCT to either relapse/progression or death from any cause. Patients alive were censored at the time of relapse/progression or last follow-up, whichever came first. NRM was defined as death from any cause in continuous remission and was summarized by cumulative incidence estimate with relapse as competing risk. Relapse was defined as molecular, cytogenetic, or morphologic evidence of disease recurrence. Relapse was summarized by cumulative incidence estimate with NRM as the competing risk. For relapse and NRM, patients in continuous complete remission were censored at last follow-up. aGVHD and cGVHD were defined by the standard criteria[18,19]. For GVHD, death without the event was considered a competing risk. All patients received RIC/nonmyeloablative conditioning, which was defined as total body irradiation (TBI) ≤5 Gy (single dose) or ≤8 Gy (fractionated), or busulfan (Bu) ≤8 mg/kg (orally) or ≤6.4 mg/kg (intravenously) or melphalan <150 mg/m^2 [20].

Statistical Analysis

This is a retrospective cohort study describing and comparing outcomes after RIC alloHCT using MTX+CNI (TAC versus CYSP) versus MMF+CNI (TAC versus CYSP) as GVHD prophylaxis. To understand the impact of prophylaxis regimen on outcomes after alloHCT, the patient population was divided into 4 cohorts depending on CNI used and whether it was combined with MMF or MTX: MMF-TAC, MMF-CYSP, MTX-TAC, and MTX-CYSP. Furthermore, 2 separate analyses were performed: 1 for the group with MRD and the other for the URD group.

The outcomes studied were aGVHD (grade II to IV, grade III to IV) and cGVHD, OS, DFS, relapse, and NRM. Categorical variables were summarized as frequency counts and percentages and compared among GVHD prophylaxis cohorts using the chi-square test. Continuous variables were summarized as the median and range and compared using the Mann-Whitney test. Probabilities of OS and DFS were calculated using Kaplan-Meier estimator and compared among the cohorts using the log-rank test. Probabilities of NRM, relapse and cGVHD were calculated by cumulative incidence function accounting for competing risks. Comparisons of cumulative incidence across time cohorts were performed via Gray's test. Multivariate Cox proportional hazards regression models for all the endpoints (aGVHD, cGVHD, OS, DFS, relapse, NRM, graft failure) were used to compare the treatment groups. The assumption of proportional hazards for each factor in the Cox model was tested using time-dependent covariates. There is no variable violating the proportional hazard assumption in this study. Stepwise selection was used to identify significant covariates that influenced outcomes to be included in the final model to get the adjusted treatment effects, the variables we considered in the variable selection included patient-related (age, sex, Karnofsky Performance Status [KPS], ethnicity, HCT-comorbidity index, disease-related [disease, disease status at alloHCT], donor-related [donor age (URD only), donor-recipient sex match and cytomegalovirus [CMV] match) and transplantation-related (year of transplant, in vivo T cell depletion using antithymocyte globulin [ATG] and use of TBI in the conditioning) variables. Statistical significance of the main effects was tested with the .01 level accounting for multiple comparisons across the endpoints. Potential interactions between the main effect (GVHD prophylaxis) and significant adjusting were tested, and there are no significant interactions at .01 level. Adjusted survival curves and cumulative incidence curves were generated stratified on the treatment groups and weighted averages of covariate values using the pooled sample proportion as the weight function. These adjusted curves represent likelihood of outcomes in populations with similar prognostic factors. The following power analyses were conducted for the main outcome grade II to IV aGVHD, given the current patient and event number in each GVHD prophylaxis cohort of the 2 groups. For the MRD group, to detect a hazard ratio (HR) of 1.5 in 1 of the 6 pairwise comparisons among treatments with significance level .05, the power ranges from 27% to 53% with Bonferroni adjustment used to adjust the multiple comparison problem. On the other hand, for the URD group, to detect a hazard ratio of 1.5 in 1 of the 6 pairwise comparisons among treatments with significance level .05, the power ranges from 51% to 90%.

RESULTS

Patient, Disease, and Transplantation Characteristics

In the MRD group (n = 690), patient, disease, and transplantation characteristics showed important differences (Table 1). The median age at alloHCT was as low as 53 years in MTX-CYSP and as high as 61 years in MMF-TAC cohort ($P < .001$). A significantly lower proportion of MTX-CYSP cohort patients had KPS of <90% (19%), whereas TAC-based cohorts had higher proportions of patients with KPS <90% (45% to 52%; $P < .001$). AML was the most common alloHCT indication (48% to 69%) in the MRD cohorts, followed by MDS (24% to 45%; $P < .001$). Donor-recipient CMV serostatus proportions were heterogeneous; for example, 11% of MTX-CYSP patients were donor-recipient seronegative, compared with 19% to 25% in the other 3 cohorts ($P < .001$). The combination of ATG with alkylator (Bu, melphalan [Mel]), nucleoside analog (fludarabine [Flu]), or TBI-based conditioning regimen was used in approximately a quarter of all 4 cohorts of MRD group. While in the 2000 to 2004 period, 43% of MTX-CYSP and 50% of MMF-CYSP patients received alloHCT, and in the most recent 2009 to 2013 period, 17% and 29% of the respective CYSP cohorts had alloHCT. In contrast, 13% of MTX-TAC and 19% of MMF-TAC received alloHCT in 2000 to 2004, and in the 2009 to 2013 period, 57% and 56% patients in the respective TAC cohorts underwent alloHCT ($P < .001$). The median follow-up of survivors ranged from 48 to 59 months in the MRD cohorts.

In the URD group (n = 874), pretransplant variables were similar among the 4 cohorts, with some exceptions (Table 2). AML (54% to 60%) and MDS (26% to 35%) were the 2 most common indications for alloHCT in the URD group ($P = .003$). In the URD group, 70% to 85% patients in the 4 cohorts were fully matched (8/8-) and 15% to 30% were matched at 7/8 loci with their donors ($P = .001$). ATG was used in 41% of each of the 2 MMF cohorts, but at a higher frequency in the MTX cohorts (62% of MTX-CYSP and 54% of MTX-TAC cohorts) of the URD group ($P < .001$). The median follow-up of survivors ranged from 49 to 61 months in the URD cohorts. In the earliest period of 2000 to 2004, 17% and 18% of MTX-CYSP and MMF-CYSP cohorts and 4% and 11% of MTX-TAC and MMF-TAC cohorts, respectively, received alloHCT ($P < .001$). The proportions of alloHCT recipients in the most recent 2009 to 2014 were 28% and 38% in the CYSP cohorts and 58% and 43% in the TAC cohorts, respectively.

Acute GVHD

Univariate analysis demonstrated that in the MRD group, the cumulative incidences of grade II to IV aGVHD at day 100 post-transplant were 27% (95% CI, 21% to 33%) in the MTX-CYSP cohort and 39% (95% CI, 30% to 48%) in the MMF-CYSP cohort (Table 3). In the MTX-TAC and MMF-TAC cohorts of the MRD group, however, the incidences were 21% (95% CI, 17% to 26%) and 29% (95% CI, 18% to 40%), respectively. Univariate analysis also showed that the cumulative incidences of grade III to IV aGVHD at day 100 were 8% (95% CI, 5% to 12%), 18% (95% CI, 12% to 26%), 8% (95% CI, 5% to 11%), and 14% (95% CI, 7% to 24%) in the MTX-CYSP, MMF-CYSP, MTX-TAC, and MMF-TAC cohorts of the MRD group, respectively (Table 3). Multivariate analysis did not show any significant difference in the

Table 1
Characteristics of adult patients receiving their first RIC alloHCT for AML, ALL, CML, and MDS with a peripheral blood stem cell graft from an MRD and treated with TAC/CYSP + MTX/MMF ± ATG from 2000 to 2013

	MTX-CYSP	MMF-CYSP	MTX-TAC	MMF-TAC	*P* Value
Patients	220	110	296	64	
Centers	49	30	58	23	
Patient related					
Age at transplant, yr	53 (20-71)	58 (20-76)	60 (19-77)	61 (19-73)	<.001*
Age at transplant					<.001*
18-29 yr	10 (5)	3 (3)	5 (2)	4 (6)	
30-39 yr	34 (15)	4 (4)	14 (5)	3 (5)	
40-49 yr	42 (19)	13 (12)	26 (9)	3 (5)	
50-64 yr	108 (49)	59 (54)	184 (62)	37 (58)	
65+ yr	26 (12)	31 (28)	67 (23)	17 (27)	
Ethnicity					<.001*
Caucasian	120 (54)	94 (85)	264 (89)	57 (89)	
Non-Caucasian	72 (33)	9 (8)	26 (9)	7 (11)	
Sorror comorbidity index					<.001*
before 2007	170 (77)	68 (61)	93 (31)	21 (33)	
0-1	25 (11)	17 (15)	87 (29)	10 (16)	
2+	20 (9)	24 (22)	113 (38)	33 (52)	
KPS					<.001*
<90	43 (19)	40 (36)	154 (52)	29 (45)	
≥90	171 (78)	69 (63)	138 (47)	35 (55)	
Disease related					
Disease					<.001*
AML	111 (50)	55 (50)	203 (69)	31 (48)	
ALL	15 (6)	10 (9)	8 (3)	3 (5)	
CML	43 (20)	8 (7)	11 (4)	1 (2)	
MDS	52 (24)	37 (34)	74 (25)	29 (45)	
Disease status at transplant*					<.001*
Early	109 (50)	62 (56)	189 (64)	37 (58)	
Intermediate	64 (29)	14 (13)	40 (14)	8 (13)	
Advanced	44 (20)	29 (26)	57 (19)	17 (27)	
Donor related					
Donor-recipient gender match					.11
M/M	70 (32)	35 (32)	92 (31)	28 (44)	
M/F	64 (29)	25 (22)	59 (20)	10 (16)	
F/M	42 (19)	28 (25)	85 (29)	15 (23)	
F/F	44 (20)	23 (21)	60 (20)	11 (17)	
Donor-recipient CMV status					<.001*
+/+	138 (63)	42 (38)	124 (42)	30 (47)	
+/−	25 (11)	13 (12)	30 (10)	5 (8)	
−/+	27 (12)	27 (25)	78 (26)	15 (23)	
−/−	24 (11)	28 (25)	56 (19)	14 (22)	
Transplant related					
Conditioning regimen and ATG					<.001*
Bu + Flu ± others	69 (31)	18 (16)	149 (50)	18 (28)	
Flu + Mel ± others	72 (33)	36 (33)	69 (23)	18 (28)	
TBI ± Cy ± Flu ± others	26 (12)	30 (27)	17 (6)	12 (19)	
ATG ± Bu ± Flu ± others	50 (23)	11 (10)	60 (20)	15 (23)	
ATG ± TBI ± others	3 (1)	15 (14)	1 (<1)	1 (2)	
Year of transplant					<.001*
2000-2004	110 (50)	47 (43)	39 (13)	12 (19)	
2005-2008	73 (33)	31 (28)	88 (30)	16 (25)	
2009-2013	37 (17)	32 (29)	169 (57)	36 (56)	
Follow-up of survivors, mo	54 (2-138)	59 (3-166)	56 (3-154)	48 (23-124)	

Values are n, median (range), or n (%).
M indicates male; F, female.
* statistical significance.

cumulative incidences of grade II to IV and grade III to IV aGVHD among the 4 cohorts in the MRD group (Table 4, Figure 1A).

The URD cohorts had a higher cumulative incidence of grade II to IV aGVHD on day 100 post-alloHCT on univariate analysis: 32% (95%CI, 22% to 45%) and 53% (95% CI, 45% to 61%) in the MTX-CYSP and MMF-CYSP cohorts, respectively, and 37% (95% CI, 32% to 41%) and 47% (95% CI, 41% to 54%) using MTX-TAC and MMF-TAC, respectively (Table 5). The cumulative incidences of grade III to IV aGVHD at day 100 were 15% (95% CI, 8% to 25%), 21% (95% CI, 15% to 28%), 13% (95% CI, 10% to 17%), and 21% (95% CI, 16% to 27%) in the MTX-CYSP, MMF-CYSP, MTX-TAC, and MMF-TAC cohorts of the URD group on univariate analysis, respectively (Table 5). Multivariate analysis of the URD group demonstrated that MMF-CYSP resulted in increased incidence of grade II to IV aGVHD compared with MTX-TAC (HR, 1.78; $P < .001$) (Table 6, Figure 1B) and MTX-CYSP (HR, 2.23; $P < .001$) (not shown in Table 6). A significantly higher incidence of grade III to IV aGVHD was shown in the URD group with the GVHD prophylaxis of MMF-CYSP compared with MTX-TAC (HR, 1.93; $P = .006$) (Table 6).

Table 2
Characteristics of adult patients receiving their first RIC alloHCT for AML, ALL, CML, and MDS from a URD with a peripheral blood stem cell graft and treated with CNI (CYSP/TAC) + MTX/MMF + ATG from 2000 to 2013

	MTX-CYSP	MMF-CYSP	MTX-TAC	MMF-TAC	*P* Value
Patients	71	153	432	218	
Centers	26	35	68	39	
Patient related					
Age at transplant, yr	59 (23-74)	62 (21-76)	61 (18-76)	60 (20-79)	.08
Age at transplant					.16
18-29 yr	3 (4)	9 (6)	18 (4)	8 (3)	
30-39 yr	4 (6)	7 (5)	23 (5)	20 (9)	
40-49 yr	9 (13)	12 (8)	36 (8)	23 (11)	
50-64 yr	40 (56)	67 (44)	226 (52)	113 (52)	
65+ yr	15 (21)	58 (38)	131 (30)	54 (25)	
Ethnicity					<.001*
Caucasian	60 (85)	138 (90)	410 (95)	204 (93)	
Non-Caucasian	6 (8)	10 (7)	21 (5)	12 (6)	
Missing	5 (7)	5 (3)	1 (<1)	2 (<1)	
Sorror comorbidity index					<.001*
before 2007	46 (65)	75 (49)	129 (30)	98 (45)	
0-1	14 (20)	29 (19)	108 (25)	40 (18)	
2+	10 (14)	42 (27)	188 (43)	75 (34)	
Missing	1 (1)	7 (5)	7 (2)	5 (2)	
KPS at transplant					.07
<90	20 (28)	56 (37)	196 (45)	100 (46)	
≥90	47 (66)	93 (61)	223 (52)	112 (51)	
Missing	5 (6)	4 (3)	13 (3)	6 (3)	
Disease related					
Disease					.003*
AML	39 (55)	92 (60)	248 (57)	120 (55)	
ALL	3 (4)	12 (8)	9 (2)	15 (7)	
CML	9 (13)	9 (6)	23 (6)	7 (3)	
MDS	20 (28)	40 (26)	152 (35)	76 (35)	
Disease status at transplant*					.39
Early	40 (56)	95 (62)	255 (59)	125 (57)	
Intermediate	9 (13)	16 (10)	71 (16)	33 (14)	
Advanced	19 (26)	40 (26)	97 (22)	52 (24)	
Missing	3 (4)	2 (1)	9 (2)	10 (5)	
Donor-related					
Donor type					.001*
Unrelated, 8/8-matched	50 (70)	120 (78)	369 (85)	163 (75)	
Unrelated, 7/8-matched	21 (30)	33 (22)	63 (15)	55 (25)	
Unrelated donor age at transplant, yr	33 (20-60)	32 (20-56)	31 (18-61)	33 (18-60)	.25
Unrelated donor age at transplant					.27
18-29 yr	34 (49)	75 (49)	235 (54)	106 (49)	
30-49 yr	25 (35)	66 (43)	152 (35)	94 (43)	
50-60 yr	6 (8)	8 (5)	27 (6)	9 (4)	
Missing	6 (8)	4 (3)	18 (4)	9 (4)	
Donor-recipient sex match					.06
M/M	25 (35)	56 (37)	201 (47)	83 (38)	
M/F	25 (35)	39 (25)	121 (28)	58 (27)	
F/M	9 (13)	32 (21)	54 (13)	35 (16)	
F/F	12 (17)	26 (17)	56 (13)	42 (19)	
Donor-recipient CMV status					.82
+/+	12 (17)	42 (27)	102 (24)	50 (23)	
+/−	6 (8)	16 (10)	43 (10)	16 (7)	
−/+	30 (42)	52 (34)	175 (41)	89 (41)	
−/−	22 (31)	38 (25)	101 (23)	57 (26)	
Missing	1 (1)	5 (3)	12 (3)	6 (3)	
Transplant related					
Conditioning regimen and ATG					<.001*
Bu + Flu ± others	11 (15)	13 (9)	121 (28)	30 (14)	
Flu + Mel ± others	13 (18)	20 (13)	67 (16)	48 (22)	
TBI ± Cy ± Flu ± others	3 (4)	57 (37)	9 (2)	49 (22)	
ATG ± Bu ± Flu ± others	41 (58)	52 (34)	235 (54)	88 (40)	
ATG ± TBI ± others	3 (4)	11 (7)	0 (0)	3 (1)	
Year of transplant					<.001*
2000-2004	12 (17)	28 (18)	17 (4)	24 (11)	
2005-2008	39 (55)	67 (44)	164 (38)	100 (46)	
2009-2013	20 (28)	58 (38)	251 (58)	94 (43)	
Follow-up of survivors, mo	60 (4-120)	71 (16-191)	48 (5-126)	61 (12-146)	

Values are n, median (range), or n (%).
* statistical significance.

Chronic GVHD

The 1-year cumulative incidences of cGVHD with MTX-CYSP, MMF-CYSP, MTX-TAC, and MMF-TAC were 49% (95% CI, 42% to 56%), 39% (95% CI, 29% to 49%), 34% (95% CI, 29% to 40%), and 34% (95% CI, 23% to 46%), respectively, on univariate analysis of the MRD group (Table 3). Multivariate analysis did not reveal any significant difference in the incidence of cGVHD among the 4 cohorts in MRD group (Table 4, Figure 2A). In this group, the addition of ATG to Flu/Bu conditioning was associated with lower cGVHD incidence (HR, .55; $P = .001$).

Univariate analysis demonstrated 1-year cumulative incidences of cGVHD with MTX-CYSP, MMF-CYSP, MTX-TAC, and MMF-TAC of 36% (95% CI, 25% to 47%), 50% (95% CI, 42% to 58%), 40% (95% CI, 35% to 45%), and 44% (95% CI, 37% to 50%), respectively, in the URD group (Table 5). There was no significant difference in the incidence of cGVHD among the 4 URD cohorts on multivariate analysis (Table 6, Figure 2B).

Overall Survival

The MRD cohort exhibited 2-year OS of 59% (95% CI, 52% to 66%) in the MTX-CYSP cohort, 46% (95% CI, 37% to 56%) in the MMF-CYSP cohort, 48% (95% CI, 42% to 54%) in the MTX-TAC cohort and 47% (95% CI, 35% to 59%) in the MMF-TAC cohort, on univariate analysis (Table 3). Multivariate analysis was unrevealing for a statistically significant difference among the 4 MRD cohorts (Table 4, Figure 3A).

The unadjusted probabilities of 2-year OS were 40% (95% CI, 28% to 52%), 45% (95% CI, 37% to 53%), 47% (95% CI, 42% to 51%) and 41% (95% CI, 35% to 48%) in the MTX-CYSP, MMF-CYSP, MTX-TAC and MMF-TAC cohorts of the URD group,

Table 3
Univariate analyses (MRD)

	MTX-CYSP (n = 220)	MMF-CYSP (n = 110)	MTX-TAC (n = 296)	MMF-TAC (n = 64)	
Outcomes	**Probability (95% CI) (%)**	**Probability (95% CI) (%)**	**Probability (95% CI) (%)**	**Probability (95% CI) (%)**	***P* Value**
aGVHD II-IV					
1 mo	14 (9-19)	20 (13-28)	7 (4-10)	21 (12-32)	<.001
2 mo	21 (15-26)	33 (25-42)	15 (11-20)	24 (14-35)	.003
100 d	27 (21-33)	39 (30-48)	21 (17-26)	29 (18-40)	.01
aGVHD III-IV					
1 mo	2 (1-5)	11 (6-18)	2 (1-4)	14 (7-24)	.02
2 mo	6 (3-9)	16 (9-23)	6 (4-9)	14 (7-24)	.03
100 d	8 (5-12)	18 (12-26)	8 (5-11)	14 (7-24)	.04
cGVHD					
100 d	6 (3-10)	3 (1-7)	2 (1-4)	3 (0-9)	.24
6 mo	30 (24-37)	26 (18-35)	18 (13-22)	18 (9-28)	.01
1 yr	49 (42-56)	39 (29-49)	34 (29-40)	34 (23-46)	.01
OS					
100 d	91 (87-94)	87 (80-93)	93 (90-96)	83 (73-91)	.08
6 mo	80 (75-85)	75 (67-83)	78 (73-83)	69 (57-79)	.31
1 yr	67 (61-74)	57 (48-66)	60 (54-66)	55 (42-67)	.13
2 yr	59 (52-66)	46 (37-56)	48 (42-54)	47 (35-59)	.05
DFS					
100-d	82 (76-87)	76 (67-83)	76 (71-81)	71 (60-82)	.22
6 mo	68 (61-74)	61 (51-70)	61 (55-66)	54 (42-66)	.17
1 yr	57 (50-64)	46 (37-56)	50 (45-56)	48 (35-60)	.22
2 yr	50 (43-57)	41 (32-50)	41 (36-47)	44 (32-57)	.21
Relapse					
100 d	10 (6-14)	17 (10-24)	21 (16-25)	14 (7-24)	.005
6 mo	18 (13-23)	26 (18-35)	32 (27-38)	32 (21-44)	<.001
1 yr	24 (19-30)	34 (25-43)	37 (31-43)	33 (22-46)	.007
2 yr	28 (22-35)	36 (27-46)	43 (37-48)	33 (22-46)	.01
NRM					
100-d	9 (5-13)	7 (3-13)	3 (2-6)	14 (7-24)	.04
6 mo	15 (10-20)	13 (7-20)	7 (4-10)	14 (7-24)	.02
1 yr	19 (14-24)	20 (13-28)	13 (9-17)	19 (10-30)	.15
2 yr	21 (16-27)	23 (15-32)	16 (12-21)	22 (13-33)	.29
GRFS					
100 d	71 (64-77)	61 (52-70)	69 (63-74)	64 (52-75)	.36
6 mo	39 (32-46)	35 (26-44)	42 (37-48)	39 (27-51)	.53
1 yr	16 (11-22)	16 (9-24)	22 (18-27)	23 (13-34)	.23
2 yr	13 (9-18)	13 (7-20)	15 (11-19)	13 (6-22)	.94
CRFS					
100 d	79 (73-84)	75 (67-83)	74 (69-79)	70 (59-81)	.49
6 mo	56 (49-62)	49 (39-58)	53 (47-58)	50 (37-62)	.67
1 yr	40 (33-47)	30 (22-40)	39 (34-45)	40 (28-52)	.35
2 yr	35 (29-42)	27 (19-36)	31 (25-36)	34 (23-46)	.52
Neutrophil recovery					
14 d	36 (30-43)	38 (29-47)	35 (30-41)	57 (45-69)	.02
28 d	93 (89-96)	91 (84-96)	96 (94-98)	98 (92-100)	.07
Platelet recovery					
14 d	14 (9-19)	29 (20-39)	20 (15-25)	22 (13-34)	.02
28 d	78 (71-83)	84 (75-91)	82 (77-87)	93 (85-98)	.01

CRFS indicates chronic GVHD- and relapse-free survival.
*statistical significance.

Table 4
Multivariate analyses in MRD RIC alloHCT

	n (%)	Estimate (95% CI)	*P* Value
Grade II-IV aGVHD			
GVHD prophylaxis			.14
MTX-TAC (reference)	290 (43)	1.0	
MMF-CYSP	109 (16)	1.6 (1.1-2.4)	.02
MTX-CYSP	213 (32)	1.3 (.9-1.8)	.17
MMF-TAC	63 (9)	1.2 (.7-2.0)	.44
Conditioning regimen / ATG			<.001*
BU+FLU ± others (reference)	248 (37)	1.00	
ATG ± BU ± FLU ± others	134 (20)	.9 (.4-1.1)	.10
FLU+LPAM ± others	193 (29)	1.7 (1.2-2.4)	.001*
TBI ± CY ± FLU ± others	100 (15)	1.2 (.8-1.9)	.31
KPS			.04*
<90 (reference)	258 (38)	1.0	
90-100	404 (60)	.7 (.5-.9)	.01
Missing	13 (2)	.8 (.2-2.5)	.69
Grade III-IV aGVHD			
GVHD prophylaxis			.26
MTX-TAC (reference)	291 (43)	1.0	
MMF-CYSP	109 (16)	1.6 (.9-2.8)	.15
MTX-CYSP	213 (31)	.9 (.5-1.6)	.76
MMF-TAC	63 (9)	1.5 (.8-3.1)	.23
Conditioning regimen / ATG			.03*
Bu+Flu ± others (reference)	248 (37)	1.0	
ATG ± Bu ± Flu ± others	134 (20)	.6 (.3-1.3)	.25
Flu+Mel ± others	193 (29)	1.7 (1.0-2.8)	.04
TBI ± Cy ± Flu ± others	101 (15)	1.2 (.6-2.4)	.53
cGVHD			
GVHD prophylaxis			.42
MTX-TAC (reference)	287 (43)	1.0	
MMF-CYSP	103 (15)	1.1 (.7-1.7)	.58
MTX-CYSP	210 (32)	1.3 (.9-1.8)	.13
MMF-TAC	63 (9)	1.3 (.8-2.0)	.25
Conditioning regimen / ATG			.006*
Bu+Flu ± others (reference)	246 (37)	1.0	
ATG ± Bu ± Flu ± others	134 (20)	.5 (.4-.8)	.001*
Flu+Mel ± others	186 (28)	1.0 (.7-1.4)	.95
TBI ± Cy ± Flu ± others	97 (15)	1.1 (.7-1.7)	.66
Disease status			.04*
Early (reference)	385 (58)	1.0	
Advanced	142 (21)	1.1 (.8-1.4)	.74
Intermediate	117 (18)	1.2 (.8-1.6)	.37
Missing	19 (3)	2.5 (1.3-4.7)	.005*
Donor-recipient CMV match			.02*
−/− (reference)	113 (17)	1.0	
+/+	321 (48)	1.1 (.7-1.5)	.74
+/−	71 (11)	.6 (.4-1.0)	.05
−/+	144 (22)	.7 (.5-1.1)	.11
Missing	14 (2)	1.8 (.7-4.5)	.22
Donor-recipient sex match			.01*
M-M (reference)	219 (33)	1.0	
F-F	131 (20)	1.4 (1.0-2.0)	.06
F-M	161 (24)	1.7 (1.2-2.4)	.002*
M-F	152 (23)	1.5 (1.1-2.0)	.02
Ethnicity			.005*
Caucasian (reference)	512 (77)	1.0	
Missing	43 (6)	.9 (.5-1.6)	.78
Non-Caucasian	108 (16)	1.7 (1.2-2.4)	.001*
OS			
GVHD prophylaxis			.69
MTX-TAC (reference)	296 (43)	1.0	
MMF-CYSP	110 (16)	1.2 (.9-1.5)	.26
MTX-CYSP	220 (32)	1.0 (.8-1.3)	.77
MMF-TAC	64 (9)	1.1 (.8-1.6)	.52
Age at transplant			.002*
18-29 yr (reference)	22 (3)	1.0	
30-39 yr	55 (8)	1.6 (.6-4.0)	.35
40-49 yr	84 (12)	1.9 (.8-4.7)	.13
50-64 yr	388 (56)	2.8 (1.2-6.4)	.01
65+ yr	141 (20)	3.3 (1.4-7.7)	.006*
Disease			.008*
AML (reference)	400 (58)	1.0	
ALL	35 (5)	1.3 (.8-2.0)	.28
CML	63 (9)	.6 (.3-1.0)	.05
MDS	192 (28)	.7 (.6-.9)	.02
Disease status			.01*
Early (reference)	397 (57)	1.0	
Advanced	147 (21)	1.4 (1.1-1.8)	.004*
Intermediate	126 (18)	.9 (.6-1.2)	.50
Missing	20 (3)	1.5 (.8-2.7)	.16
KPS			.006*
<90 (reference)	264 (38)	1.0	
90-100	413 (60)	.7 (.6-.8)	.002*
Missing	13 (2)	1.1 (.6-2.2)	.78
DFS			
GVHD prophylaxis			.22
MTX-TAC (reference)	287 (43)	1.0	
MMF-CYSP	108 (16)	1.1 (.9-1.5)	.29
MTX-CYSP	212 (32)	.8 (.7-1.1)	.19
MMF-TAC	63 (9)	1.1 (.8-1.5)	.71
Disease status			.003*
Early (reference)	390 (58)	1.0	
Advanced	146 (22)	1.4 (1.1-1.8)	.002*
Intermediate	116 (17)	.9 (.7-1.2)	.55
Missing	18 (3)	1.6 (.9-2.7)	.08
Donor-recipient CMV match			.006*
−/− (reference)	118 (18)	1.0	
+/+	325 (48)	1.1 (.9-1.5)	.38
+/−	69 (10)	1.3 (.9-1.8)	.20
−/+	144 (21)	1.2 (.9-1.7)	.20
Missing	14 (2)	3.1 (1.7-5.6)	<.001*
KPS			.01*
<90 (reference)	259 (39)	1.0	
90-100	399 (60)	.7 (.6-.9)	.004*
Missing	12 (2)	1.0 (.5-2.1)	.97
NRM			
GVHD prophylaxis			.54
MTX-TAC (reference)	287 (43)	1.0	
MMF-CYSP	108 (16)	1.3 (.8-2.1)	.23
MTX-CYSP	212 (32)	1.3 (.9-2.0)	.19
MMF-TAC	63 (9)	1.2 (.7-2.1)	.51
Conditioning regimen / ATG			.02*
Bu+Flu ± others (reference)	246 (37)	1.0	
ATG ± Bu ± Flu ± others	131 (20)	1.2 (.7-2.0)	.47
Flu+Mel ± others	188 (28)	1.9 (1.3-2.8)	.002*
TBI ± Cy ± Flu ± others	105 (16)	1.5 (.9-2.6)	.10
Disease status			.03*
Early (reference)	390 (58)	1.0	
Advanced	146 (22)	1.2 (.8-1.7)	.32
Intermediate	116 (17)	.6 (.4-1.0)	.07
Missing	18 (3)	2.2 (1.0-4.7)	.04
Donor-recipient sex match			.002*
M-M (reference)	219 (33)	1.0	
F-F	135 (20)	.9 (.6-1.5)	.77
F-M	164 (24)	1.8 (1.2-2.7)	.003*
M-F	152 (23)	1.0 (.6-1.6)	.98
Sorror comorbidity index			.03*
0-1 (reference)	134 (20)	1.0	
≥2	187 (28)	2.3 (1.3-4.2)	.003*
Missing	10 (1)	2.4 (.7-8.3)	.18
N/A before 2007	339 (51)	2.0 (1.2-3.5)	.01
Relapse			
GVHD prophylaxis			.22
MTX-TAC (reference)	287 (43)	1.0	
MMF-CYSP	108 (16)	1.1 (.7-1.6)	.70
MTX-CYSP	212 (32)	.7 (.5-1.0)	.07
MMF-TAC	63 (9)	.9 (.6-1.5)	.80
Conditioning regimen / ATG			<.001*
Bu+Flu ± others (reference)	246 (37)	1.0	
ATG ± Bu ± Flu ± others	131 (20)	1.1 (.8-1.5)	.65
Flu+Mel ± others	188 (28)	.4 (.3-.6)	<.001*
TBI ± Cy ± Flu ± others	105 (16)	.9 (.6-1.3)	.55
Disease			.03*

(*continued*)

(*continued*)

AML (reference)	393 (59)	1.0	
ALL	34 (5)	.9 (.5-1.8)	.85
CML	59 (9)	1.0 (.6-1.7)	.96
MDS	184 (27)	.6 (.5-.8)	.003*
Disease status			<.001*
Early (reference)	390 (58)	1.0	
Advanced	146 (22)	2.1 (1.5-2.8)	<.001*
Intermediate	116 (17)	1.2 (.8-1.9)	.28
Missing	18 (3)	1.9 (.9-4.2)	.11
Donor-recipient CMV match			.02*
−/− (reference)	118 (18)	1.0	
+/+	325 (48)	1.2 (.9-1.8)	.23
+/−	69 (10)	1.4 (.9-2.2)	.15
−/+	144 (21)	1.3 (.9-1.9)	.21
Missing	14 (2)	3.3 (1.6-6.7)	<.001*
Sorror comorbidity index			.003*
0-1 (reference)	134 (20)	1.0	
≥2	187 (28)	1.0 (.7-1.4)	.94
Missing	10 (1)	.4 (.1-1.4)	.18
N/A before 2007	339 (51)	.6 (.4-.8)	.001*

* statistical significance.

respectively (Table 5). Multivariate analysis did not show any significant difference among the cohorts of URD group (Table 6, Figure 3B).

Disease-Free Survival

Univariate analysis demonstrated 2-year DFS of in the MRD group of 50% (95% CI, 43% to 57%) in the MTX-CYSP cohort, 41% (95% CI, 32% to 50%) in the MMF-CYSP cohort, 41% (95% CI, 36% to 47%) with MTX-TAC, and 44% (95% CI, 32% to 57%) with MMF-TAC (Table 3). There was no significant difference in DFS among any of the GVHD cohorts in the MRD group on multivariate analysis (Table 4).

In the URD group, DFS at 2 years was 36% (95% CI, 25% to 48%) in the MTX-CYSP cohort, 41% (95% CI, 33% to 49%) in the MMF-CYSP cohort, 38% (95% CI, 33% to 42%) in the MTX-TAC cohort, and 33% (95% CI, 27% to 40%) in the MMF-TAC cohort (Table 5). Multivariate analysis did not show any significant difference in DFS among any of the URD cohorts (Table 6).

Relapse

Univariate analysis revealed that in the MRD group, the cumulative incidences of relapse at 2 years were 28% (95% CI, 22% to 35%) and 36% (95% CI, 27% to 46%) in the MTX-CYSP and MMF-CYSP cohorts, respectively, and 43% (95% CI, 37% to 48%) and 33% (95% CI, 22% to 46%) in the MTX-TAC and MMF-TAC cohorts, respectively (Table 3). The risk of relapse was not shown to be significantly different among any of the 4 cohorts in the MRD group on multivariate analysis (Table 4).

In the URD group, the 2-year cumulative incidences of relapse were 34% (95% CI, 23% to 46%), 27% (95% CI, 21% to 35%), 40% (95% CI, 35% to 44%), and 31% (95% CI, 25% to 37%) in the MTX-CYSP, MMF-CYSP, MTX-TAC, and MMF-TAC cohorts, respectively, on univariate analysis (Table 5). On multivariate analysis, URD patients receiving MMF-CYSP had a significantly lower risk of relapse, compared with those receiving MTX-TAC (HR, .53; $P < .001$) (Table 6).

Nonrelapse Mortality

On univariate analysis, the cumulative incidences of NRM at 2 years post-alloHCT in the MRD group were 21% (95% CI, 16% to 27%), 23% (95% CI, 15% to 32%), 16% (95% CI, 12% to 21%), and 22% (95% CI, 13% to 33%) in the MTX-CYSP, MMF-CYSP, MTX-TAC, and MMF-TAC cohorts, respectively (Table 3). Multivariate analysis did not show any significant difference in NRM among the 4 GVHD cohorts of MRD group (Table 4).

The cumulative incidences of NRM at 2 years in the URD group were 31% (95% CI, 20% to 43%), 31% (95% CI, 24% to 39%), 23% (95% CI, 19% to 27%), and 37% (95% CI, 31% to 43%) in the MTX-CYSP, MMF-CYSP, MTX-TAC, and MMF-TAC cohorts, respectively (Table 5). Multivariate analysis of the URD group demonstrated that compared with MTX-TAC, MMF-TAC was associated with increased risk of NRM (HR, 1.48; $P = .008$) (Table 6), notwithstanding the faster neutrophil recovery observed with MMF-TAC compared with other regimens ($P < .001$) (Table 5).

DISCUSSION

The combinations of CNI with MTX or MMF for prevention of aGVHD and cGVHD after alloHCT have been accepted as the current standard,[6,8,9,18] although conflicting reports of outcomes with MMF- and MTX-containing CNI-based regimens have been noted. Despite the lack of prospective comparative data between MMF- and MTX-based regimens in the RIC

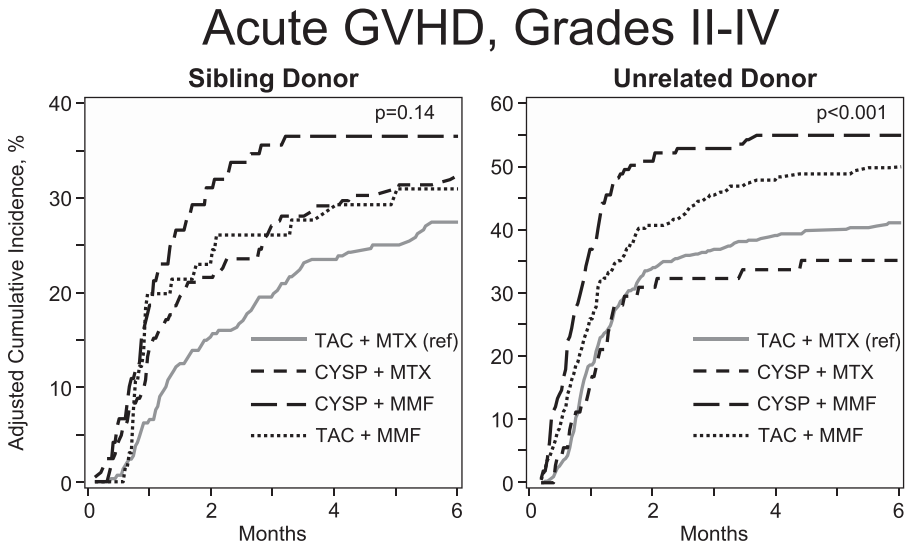

Figure 1. (A) Adjusted curves for cumulative incidence of grade II-IV acute GVHD in MRD recipients of RIC alloHCT using 1 of the 4 GVHD prophylaxis regimens: TAC-MTX, CYSP-MTX, CYSP-MMF, and TAC-MMF. (B) Adjusted curves for cumulative incidence of grade II-IV aGVHD in URD RIC alloHCT patients on 1 of the 4 GVHD prophylaxis regimens: TAC-MTX, CYSP-MTX, CYSP-MMF, and TAC-MMF.

Table 5
Univariate analyses (URD)

	MTX-CYSP (n = 71)	MMF-CYSP (n = 153)	MTX-TAC (n = 432)	MMF-TAC (n = 218)	
Outcomes	**Probability (95% CI) (%)**	**Probability (95%CI) (%)**	**Probability (95% CI) (%)**	**Probability (95% CI) (%)**	*P* Value
aGVHD II-IV					
1 mo	15 (8-25)	38 (30-45)	18 (15-22)	26 (21-32)	<.001
2 mo	31 (21-42)	51 (43-59)	33 (29-37)	41 (35-48)	<.001
100 d	32 (22-44)	53 (45-61)	37 (32-41)	47 (41-54)	<.001
aGVHD III-IV					
1 mo	8 (3-16)	16 (10-22)	8 (6-11)	14 (10-19)	.03
2 mo	15 (8-25)	21 (15-28)	12 (9-16)	19 (14-25)	.02
100 d	15 (8-25)	21 (15-28)	13 (10-17)	21 (16-27)	.06
cGVHD					
100 d	3 (0-8)	10 (6-15)	4 (3-6)	2 (1-5)	.03
6 mo	19 (10-29)	36 (28-43)	22 (18-26)	26 (21-33)	.01
1 yr	36 (25-47)	50 (42-58)	40 (35-45)	44 (37-50)	.11
OS					
100 d	77 (67-86)	83 (77-89)	87 (83-90)	81 (75-86)	.12
6 mo	66 (54-76)	69 (62-76)	78 (74-82)	67 (61-73)	.006
1 yr	54 (42-66)	57 (49-65)	60 (55-65)	49 (42-56)	.06
2 yr	40 (28-52)	45 (37-53)	47 (42-51)	41 (35-48)	.49
DFS					
100 d	72 (60-82)	73 (66-80)	74 (69-78)	70 (64-76)	.78
6 mo	59 (47-71)	60 (52-68)	61 (57-66)	56 (49-62)	.59
1 yr	50 (38-62)	50 (42-58)	48 (43-53)	41 (34-47)	.21
2 yr	36 (25-48)	41 (33-49)	38 (33-42)	33 (27-40)	.47
Relapse					
100 d	16 (9-26)	15 (10-21)	17 (14-21)	15 (11-20)	.85
6 mo	26 (16-37)	19 (13-26)	26 (22-31)	24 (18-30)	.29
1 yr	30 (20-42)	23 (17-30)	35 (30-39)	28 (22-34)	.03
2 yr	34 (23-46)	27 (21-35)	40 (35-44)	31 (25-37)	.02
NRM					
100 d	12 (5-21)	12 (7-17)	9 (6-12)	15 (10-20)	.16
6 mo	15 (7-25)	21 (15-28)	13 (10-16)	21 (16-27)	.02
1 yr	20 (11-30)	27 (20-34)	17 (14-21)	31 (25-37)	<.001
2 yr	30 (19-42)	31 (24-39)	23 (19-27)	36 (29-42)	.005
GRFS					
100 d	63 (52-74)	52 (44-60)	63 (58-67)	58 (51-65)	.12
6 mo	41 (30-52)	31 (24-38)	38 (34-43)	33 (26-39)	.20
1 yr	24 (15-34)	14 (9-21)	16 (13-20)	11 (8-16)	.10
2 yr	13 (6-22)	10 (5-15)	11 (8-14)	7 (4-11)	.29
CRFS					
100 d	66 (55-77)	67 (60-75)	73 (68-77)	70 (63-76)	.52
6 mo	49 (37-61)	47 (40-55)	55 (50-59)	47 (41-54)	.22
1 yr	37 (27-49)	35 (27-42)	37 (32-42)	31 (25-38)	.50
2 yr	21 (12-32)	26 (20-34)	28 (24-33)	22 (17-28)	.31
Neutrophil recovery					
14 days	46 (35-58)	32 (25-40)	35 (31-40)	69 (62-75)	<.001
28 days	97 (92-100)	96 (92-99)	96 (94-98)	95 (92-98)	.91
Platelet recovery					
14 days	17 (9-27)	19 (13-27)	19 (13-27)	18 (13-24)	.61
28 days	73 (62-84)	82 (75-89)	83 (79-87)	80 (74-85)	.38

*statistical significance.

setting, MMF-CNI has been an established regimen after RIC alloHCT. While some studies have compared the CNIs (TAC versus CYSP) and others have compared MTX- and MMF-containing GVHD prophylaxis, no study to date has investigated all 4 regimens concomitantly to compare the outcomes after RIC alloHCT, and, therefore, there has been no convincing evidence to date supporting the use of a particular regimen in the RIC setting.

We have made several important observations in this analysis. No single GVHD prevention regimen is superior, the limited power notwithstanding, to detect differences in aGVHD and survival outcomes in the MRD group. In those with URD, however, MTX-TAC performed better than MMF-CYSP and resulted in 44% risk reduction in grade II to IV and 48% risk reduction in grade III to IV aGVHD. Furthermore, MTX-CYSP resulted in 48% reduction in the incidence of grade II to IV aGVHD relative to MMF-CYSP, but did not show statistically significant difference in the grade III to IV aGVHD risk. All 4 regimens resulted in similar cGVHD incidence after URD alloHCT. MTX-TAC, in addition, was associated with 32% lower NRM risk compared with MMF-TAC. Furthermore, MTX-TAC was associated with 88% increase in relapse risk relative to MMF-CYSP but did not meet statistical significance when compared with MMF-TAC. Higher relapse risk observed with MTX-TAC did not translate into worse DFS and OS as the analysis revealed no significant difference among any of the cohorts in the URD group. No significant interaction was found between GVHD prophylaxis and the conditioning, but patients receiving Flu/Mel conditioning had significantly higher risk of grade II to IV (HR, 1.75; $P < .001$) and grade III to IV (HR, 2.71; $P < .001$) aGVHD compared with the Flu/Bu regimen (Table 6). The risks of cGVHD and NRM with Flu/Mel conditioning were also increased but did not meet statistical significance ($P = .03$ and .05, respectively).

Table 6
Multivariate analyses in URD RIC alloHCT

	n (%)	Estimate (95% CI)	*P* Value
Grade II-IV aGVHD			
GVHD prophylaxis			<.001*
MTX-TAC (reference)	429 (49)	1.0	
MMF-CYSP	152 (17)	1.8 (1.3-2.4)	<.001*
MTX-CYSP	71 (8)	.8 (.5-1.2)	.29
MMF-TAC	216 (25)	1.3 (1.0-1.7)	.03
Conditioning regimen / ATG			.002*
Bu+Flu ± others (reference)	173 (20)	1.0	
ATG ± Bu ± Flu ± others	414 (48)	1.1 (.8-1.4)	.61
Flu+Mel ± others	147 (17)	1.7 (1.3-2.4)	<.001*
TBI ± Cy ± Flu ± others	134 (15)	1.2 (.8-1.7)	.37
Disease status			.01*
Early (reference)	512 (59)	1.0	
Advanced	206 (24)	1.1 (.9-1.5)	.25
Intermediate	127 (15)	.7 (.5-1.0)	.03
Missing	23 (3)	1.6 (.9-2.7)	.08
Donor type			
7/8-matched URD (reference)	172 (20)	1.0	
8/8-matched URD	696 (80)	.7 (.5-.9)	.002*
Grade III-IV aGVHD			
GVHD prophylaxis			.01*
MTX-TAC (reference)	428 (49)	1.0	
MMF-CYSP	152 (18)	1.9 (1.2-3.1)	.006*
MTX-CYSP	71 (8)	.8 (.4-1.6)	.53
MMF-TAC	214 (257)	1.5 (1.0-2.3)	.03
Conditioning regimen / ATG			<.001*
Bu+Flu ± others (reference)	173 (20)	1.0	
ATG ± Bu ± Flu ± others	412 (48)	1.1 (.7-1.7)	.78
Flu+Mel ± others	146 (17)	2.7 (1.6-4.4)	<.001*
TBI ± Cy ± Flu ± others	134 (15)	.9 (.5-1.6)	.66
Disease			.02*
AML (reference)	495 (57)	1.0	
ALL	39 (4)	.4 (.1-1.2)	.10
CML	48 (5)	2.1 (1.1-4.0)	.03
MDS	283 (33)	1.4 (1.0-2.0)	.08
Disease status			.04*
Early (reference)	511 (59)	1.0	
Advanced	204 (24)	1.2 (.8-1.7)	.46
Intermediate	127 (15)	.7 (.4-1.3)	.25
Missing	23 (3)	2.3 (1.2-4.5)	.01
Donor type			
7/8-matched URD (reference)	172 (20)	1.0	
8/8-matched URD	693 (80)	.5 (.4-.7)	<.001*
Chronic GVHD			
GVHD prophylaxis			.35
MTX-TAC (reference)	428 (49)	1.0	
MMF-CYSP	152 (17)	1.3 (.9-1.7)	.10
MTX-CYSP	70 (8)	1.0 (.7-1.5)	.96
MMF-TAC	217 (25)	1.2 (.9-1.5)	.18
Conditioning regimen / ATG			<.001*
Bu+Flu ± others (reference)	174 (20)	1.0	
ATG ± Bu ± Flu ± others	413 (48)	.7 (.6-1.0)	.03
Flu+Mel ± others	146 (17)	1.4 (1.0-1.9)	.03
TBI ± Cy ± Flu ± others	134 (15)	1.4 (1.0-2.0)	.04
Year of transplant			.03*
2000-2004 (reference)	80 (9)	1.0	
2005-2008	366 (42)	.7 (.5-1.0)	.03
2009-2013	421 (49)	.6 (.4-.9)	.007*
OS			
GVHD prophylaxis			.82
MTX-TAC (reference)	432 (49)	1.0	
MMF-CYSP	153 (17)	1.0 (.8-1.2)	.97
MTX-CYSP	71 (8)	1.1 (.8-1.5)	.49
MMF-TAC	218 (25)	1.1 (.9-1.3)	.47
Age at transplant			.008*
18-29 yr (reference)	38 (4)	1.0	
30-39 yr	54 (6)	.6 (.3-1.0)	.03
40-49 yr	80 (9)	.8 (.5-1.3)	.37
50-64 yr	444 (51)	.9 (.6-1.4)	.67
65+ yr	258 (29)	1.1 (.7-1.7)	.55
Disease status			.004*
Early (reference)	515 (59)	1.0	
Advanced	208 (24)	1.2 (1.0-1.5)	.02
Intermediate	127 (14)	1.0 (.8-1.3)	.82
Missing	24 (3)	2.0 (1.3-3.1)	.002*
Donor type			
7/8-matched URD (reference)	172 (20)	1.0	
8/8-matched URD	702 (80)	.7 (.6-.8)	<.001*
KPS			.002*
<90 (reference)	372 (43)	1.0	
90-100	475 (54)	.8 (.6-.9)	.001*
Missing	27 (3)	1.1 (.7-1.6)	.71
Sorror comorbidity index			.05*
0-1 (reference)	191 (22)	1.0	
≥2	315 (36)	1.1 (.9-1.4)	.43
Missing	20 (2)	.5 (.3-1.1)	.09
N/A before 2007	348 (40)	1.2 (1.0-1.5)	.08
DFS			
GVHD prophylaxis			.68
MTX-TAC (reference)	423 (49)	1.0	
MMF-CYSP	153 (18)	.9 (.7-1.1)	.28
MTX-CYSP	67 (8)	1.0 (.7-1.4)	.94
MMF-TAC	216 (25)	1.0 (.8-1.2)	.82
Age at transplant			.04*
18-29 yr (reference)	38 (4)	1.0	
30-39 yr	52 (6)	.5 (.3-.9)	.02
40-49 yr	78 (9)	.8 (.5-1.2)	.24
50-64 yr	439 (51)	.9 (.6-1.3)	.59
65+ yr	252 (29)	1.0 (.6-1.4)	.88
Disease status			.003*
Early (reference)	511 (59)	1.0	
Advanced	204 (24)	1.4 (1.1-1.6)	<.001*
Intermediate	121 (14)	1.1 (.9-1.4)	.46
Missing	23 (3)	1.7 (1.0-2.6)	.03
Donor type			
7/8-matched URD (reference)	170 (20)	1.0	
8/8-matched URD	689 (80)	.8 (.6-.9)	.006*
KPS			.005*
<90 (reference)	361 (42)	1.0	
90-100	471 (55)	.8 (.7-.9)	.002*
Missing	27 (3)	1.1 (.7-1.6)	.70
NRM			
GVHD prophylaxis			.05
MTX-TAC (reference)	423 (49)	1.0	
MMF-CYSP	153 (18)	1.4 (1.0-1.9)	.06
MTX-CYSP	67 (8)	1.3 (.8-2.0)	.26
MMF-TAC	216 (25)	1.5 (1.1-2.0)	.008*
Age at transplant			.003*
18-29 yr (reference)	38 (4)	1.0	
30-39 yr	52 (6)	.6 (.3-1.3)	.20
40-49 yr	78 (9)	.8 (.4-1.7)	.60
50-64 yr	439 (51)	1.1 (.6-2.2)	.69
65+ yr	252 (29)	1.5 (.8-3.0)	.20
Conditioning regimen / ATG			.008*
Bu+Flu ± others (reference)	172 (20)	1.0	
ATG ± Bu ± Flu ± others	410 (48)	.9 (.6-1.2)	.43
Flu+Mel ± others	143 (17)	1.4 (1.0-2.1)	.05
TBI ± Cy ± Flu ± others	134 (16)	.8 (.5-1.2)	.35
Disease status			.02*
Early (reference)	511 (59)	1.0	
Advanced	204 (24)	.9 (.7-1.2)	.68
Intermediate	121 (14)	.8 (.6-1.2)	.34
Missing	23 (3)	2.2 (1.3-3.9)	.006*
Donor type			<.001*
7/8-matched URD (reference)	170 (20)	1.0	
8/8-matched URD	689 (80)	.5 (.4-.7)	<.001*
Donor-recipient CMV match			.04*
−/− (reference)	215 (25)	1.0	
+/+	201 (23)	1.2 (.8-1.7)	.30
+/−	80 (9)	1.7 (1.1-2.6)	.01
−/+	340 (40)	1.3 (1.0-1.8)	.05
Missing	23 (3)	2.2 (1.1-4.2)	.02
KPS			.007*
<90 (reference)	361 (42)	1.0	

(*continued*)

(*continued*)

90-100	471 (55)	.7 (.5-.9)	.002*
Missing	27 (3)	.9 (.5-1.7)	.82
Relapse			
GVHD prophylaxis			.006*
MTX-TAC (reference)	423 (49)	1.0	
MMF-CYSP	153 (18)	.5 (.4-.7)	<.001*
MTX-CYSP	67 (8)	.8 (.5-1.2)	.35
MMF-TAC	216 (25)	.7 (.5-1.0)	.03
Conditioning regimen / ATG			.003*
Bu+Flu ± others (reference)	172 (20)	1.0	
ATG ± Bu ± Flu ± others	410 (48)	.8 (.6-1.1)	.26
Flu+Mel ± others	143 (17)	.5 (.3-.7)	<.001*
TBI ± Cy ± Flu ± others	134 (16)	1.0 (.7-1.5)	.99
Disease			.006*
AML (reference)	492 (57)	1.0	
ALL	37 (4)	1.1 (.7-1.9)	.66
CML	48 (6)	.6 (.3-1.1)	.09
MDS	282 (33)	.6 (.5-.9)	.002*
Disease status			<.001*
Early (reference)	511 (59)	1.0	
Advanced	204 (24)	1.8 (1.4-2.3)	<.001*
Intermediate	121 (14)	1.2 (.9-1.7)	.17
Missing	23 (3)	1.2 (.5-2.8)	.64
Sex			.03
Male (reference)	487 (57)	1.0	
Female	372 (43)	1.3 (1.0-1.6)	.03

* statistical significance.

Previously, 3 randomized studies had compared outcomes of alloHCT after CYSP [21,22] or TAC [23] combined with MTX or MMF in the myeloablative setting. One study enrolled alloHCT from URD [21], another study from MRD [22], and a third study included both [23]. None of the studies showed a statistically significant difference in the cumulative incidence of aGVHD among the regimens. Bolwell et al. [22] reported the randomized study comparing MMF-CYSP and MTX-CYSP (n = 40) after marrow transplantation using MRD []. No difference was observed in the incidence of GVHD or survival. Perkins et al. [23] reported the results of a randomized phase II study comparing MMF-TAC and MTX-TAC after alloHCT from MRD and URD (n = 89) []. Patients in the MMF cohort were less likely to experience severe mucositis, and the cumulative incidence of grade II to IV aGVHD was similar. However, the cumulative incidence of grade III to IV aGVHD was higher in the MMF arm (19% versus 4%; P = .03), predominantly in MAC alloHCT using URD. A meta-analysis of the previously mentioned 3 randomized trials by the Cochrane Collaboration found no differences in the rates of aGVHD and cGVHD among the different regimens [24]. There was no evidence for a significant difference between MMF and MTX for the incidence of aGVHD and cGVHD, neutrophil engraftment, incidence of relapse, NRM, and OS. The results are also in accord with those of a meta-analysis of 11 studies [2] including 1076 patients (a mix of MAC and RIC alloHCT recipients) that determined greater incidence of grade III to IV aGVHD in MMF recipients (HR, 1.6; 95% CI, 1.2 to 2.3). The increased risk of severe aGVHD with MMF was limited to the patients with URD and was not evident after MRD alloHCT. The 3 prospective trials had relatively small sample sizes and only included patients receiving MAC and therefore, their findings cannot be applied to RIC patients. It is noteworthy that none of the previously mentioned studies including the meta-analyses demonstrated any significant differences in the relapse risk between MTX- and MMF-based GVHD regimens, unlike reported by our study. We can only speculate that there are unknown variables and confounders, in addition to the competing risk of low NRM that contributed to the increased relapse risk and neutralized any possible survival advantage MTX-based regimens could have had in URD group.

Eapen et al. [16] compared outcomes between bone marrow and peripheral blood grafts for RIC alloHCT for patients with AML, MDS and non-Hodgkin lymphoma using URD in 88 US transplant centers (2000 to 2008) and reported no differences in outcomes between the 2 graft sources [], but patients receiving MMF (versus MTX) had an increased risk of grade II to IV and III to IV aGVHD, cGVHD, NRM, and worse OS. Patients with ALL and those receiving TBI were excluded in this study. Despite the differences in the primary objectives and patient populations between the 2 studies, the results of our analysis are in concordance with Eapen et al.'s [16] study as we show MMF-containing regimens are associated with worse intermediate outcomes without impact on OS in the URD setting. This is an important study because it examines not only the efficacy of MTX or MMF, but also the added impact of TAC and CYSP in ensuring post-alloHCT outcomes. To compare only MTX and

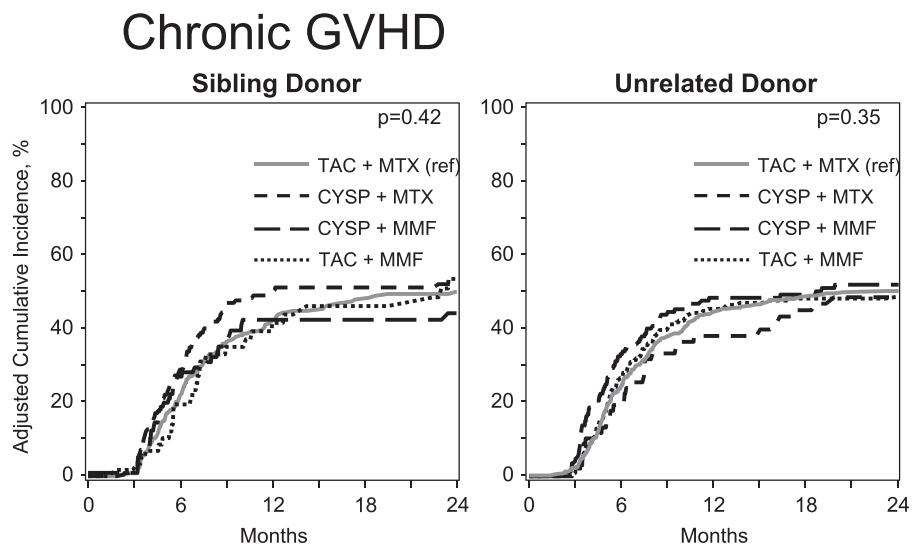

Figure 2. (A). Adjusted curves for cumulative incidence of aGVHD in MRD RIC alloHCT patients on 1 of the 4 GVHD prophylaxis regimens: TAC-MTX, CYSP-MTX, CYSP-MMF, and TAC-MMF. (B) Adjusted curves for cumulative incidence of aGVHD in URD RIC alloHCT patients receiving 1 of the 4 GVHD prophylaxis regimens: TAC-MTX, CYSP-MTX, CYSP-MMF, and TAC-MMF.

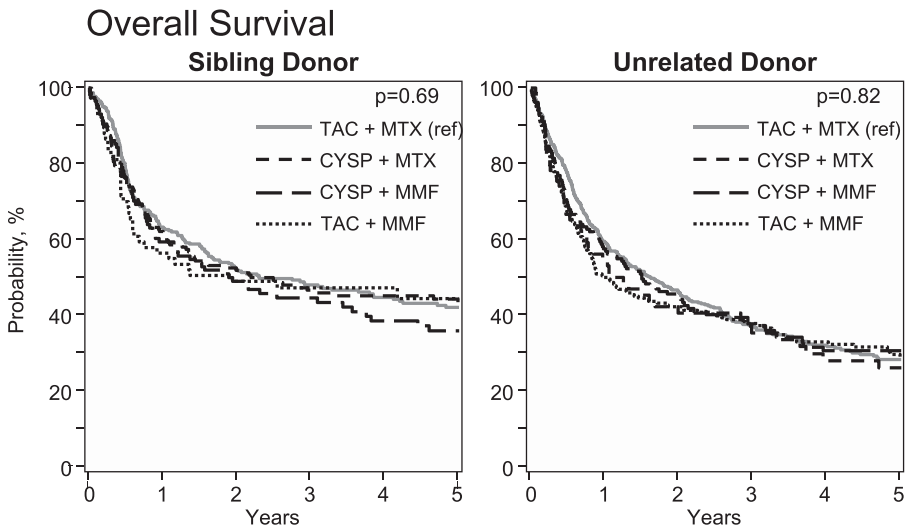

Figure 3. (A). Adjusted curves for OS in MRD RIC alloHCT patients receiving 1 of the 4 GVHD prophylaxis regimens: TAC-MTX, CYSP-MTX, CYSP-MMF, and TAC-MMF. (B) Adjusted curves for OS in URD RIC alloHCT patients receiving 1 of the 4 GVHD prophylaxis regimens: TAC-MTX, CYSP-MTX, CYSP-MMF, and TAC-MMF.

MMF would be assuming that the 2 CNIs, TAC and CYSP, have no difference in efficacy and can be used interchangeably and the study findings do not support this assumption.

Owing to the retrospective nature, the findings of the study need to be interpreted with caution. We acknowledge the differences in patient, disease, and transplant characteristics among the cohorts in both donor groups, especially the small sample size in certain cohorts (MMF-TAC cohort in the MRD group and MTX-CYSP in the URD group), differences in the proportions of ATG recipients in the cohorts of the URD group and the fact that the inclusion of ATG in the conditioning makes for a heterogeneous study population. These differences were addressed by performing a controlled analysis that accounted for all the characteristics and any center effects. We also examined the study population for differences in the outcomes of grade II to IV and grade III to IV aGVHD and cGVHD after excluding ATG recipients: univariate analysis showed cumulative incidence of grade II to IV aGVHD was highest with MMF-CYSP and lowest with MTX-TAC in both the MRD and URD groups (Supplementary Tables 1A and 1B), but no significant differences in the incidence of grade III to IV aGVHD were observed in either group. cGVHD was observed more frequently with CYSP regimens than TAC in the MRD group, and similarly, in the URD group, MMF-CYSP had higher incidence of cGVHD compared with TAC-based regimens.

Despite carefully considering multiple potentially significant variables, the effect of unrecognized biases and residual confounding in the analysis cannot be ruled out. For instance, the dose of MTX and the dose and schedule of MMF in the regimens are variable among the transplant centers. Different dosing protocols for short-course MTX and different doses and duration of MMF adopted by the transplant centers were not captured in the database. It has been demonstrated that higher trough levels of MMF attributed to intensified dosing are correlated with a decreased incidence of severe GVHD after umbilical cord blood transplantation [25,26]. Moreover, the proportion of patients that did not receive all 4 doses of MTX due to severe oropharyngeal mucositis is not known. We cannot confirm that oral (and not intravenous) formulations of TAC and CYSP were used for all RIC alloHCT in the study. We also recognize the limitation in having variable therapeutic target blood level ranges for TAC and CYSP at different centers. Furthermore, we examined the cumulative incidences of cGVHD of any grade reported to the database and did not specifically evaluate the risk of moderate-to-severe or organ-specific cGVHD in the cohorts.

It is also important to note in the study the trade-off between low NRM and higher relapse risk with MTX-TAC compared with MMF-CYSP, resulting in no difference in OS. For this reason, it would be worth considering a future prospective study in the URD patient population using the composite endpoint such as GVHD- and relapse-free survival (GRFS) that assesses all significant and relevant endpoints [27]. The events for GRFS include grade III to IV aGVHD, systemic therapy-requiring cGVHD, relapse, or death. A similar composite endpoint that has been in vogue is cGVHD- and relapse-free survival, which includes survival without development of cGVHD, disease relapse or progression and death [28]. Interestingly, the analysis for both GRFS and cGVHD- and relapse-free survival did not reveal any significant differences among the MRD and URD cohorts (Tables 3 and 5).

In summary, in this observational study, we described the outcomes after RIC alloHCT using the 4 CNI-based regimens. This differentiates the study in that we considered the 2 drugs of each prophylactic regimen as a unique combination, which enabled comparisons among the 4 regimens. This analysis demonstrated equivalent outcomes in those with MRD using either of the 4 CNI-based combinations and inferior efficacy of MMF-based approach with regard to grade II to IV and III to IV aGVHD and NRM in those with URD. Moreover, the analysis did not suggest using a particular regimen in URD alloHCT recipients using RIC and peripheral blood graft, based on the lack of significant differences in OS, even though aGVHD risk was significantly improved with MTX-CNI regimens and there may be a trend for improved 1-year GRFS in the URD group with MTX-CNI than with MMF-CNI. Finally, a prospective randomized controlled trial of URD RIC alloHCT recipients is needed to evaluate these GVHD prophylaxis regimens with uniform dosing schedules and target pharmacokinetic ranges and using novel endpoints such as GRFS to confirm the findings of this study. The results of such a trial may also inform the ideal partner for GVHD prevention strategies such as

post-transplant cyclophosphamide and other novel agents in the future clinical trials.

SUPPLEMENTARY DATA

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bbmt.2018.08.018.

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