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Inferior Outcomes with Cyclosporine and Mycophenolate Mofetil after Myeloablative Allogeneic Hematopoietic Cell Transplantation

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A B S T R A C T

Combination therapy with a calcineurin inhibitor (CNI), such as cyclosporine (CSA) or tacrolimus (Tac), and methotrexate (MTX) or mycophenolate mofetil (MMF) is a widely used approach to graft-versus-host disease (GVHD) prevention. Data on the comparative effectiveness of MMF compared with MTX are limited and conflicting, however. We analyzed data from the Center for International Blood and Marrow Transplant Research for adult patients undergoing first myeloablative hematopoietic cell transplantation (HCT) from an HLA-identical matched related donor (MRD; n = 3979) or matched unrelated donor (URD; n = 4163) using CSA+MMF, CSA+MTX, Tac+MMF, or Tac +MTX for GVHD prevention between 2000 and 2013. Within the MRD cohort, 2252 patients received CSA+MTX, 1391 received Tac+MTX, 114 received CSA+MMF, and 222 received Tac+MMF. Recipients of CSA+MMF had a higher incidence of acute GVHD grade II-IV (hazard ratio [HR], 1.65; 95% confidence interval [CI], 1.24 to 2.20; $P < .001$) and grade III-IV (HR, 1.92; 95% CI, 1.31 to 2.83; $P < .001$) compared with Tac+MTX. The use of CSA+MMF was also associated with inferior overall survival (OS) (HR, 2.31; 95% CI, 1.73 to 3.09; $P < .001$) due to higher transplantation-related mortality (TRM) (HR, 4.03; 95% CI, 2.61 to 6.23; $P < .001$) compared with Tac+MTX. Within the URD cohort, 974 patients received CSA+MTX, 2697 received Tac+MTX, 68 received CSA+MMF, and 424 received Tac+MMF. CSA+MMF was again significantly associated with a higher incidence of grade III-IV acute GVHD (HR, 2.31; 95% CI, 1.57 to 3.42; $P <0001$), worse OS (HR, 2.36; 95% CI, 1.67 to 3.35; $P < .001$), and higher TRM (HR, 3.09; 95% CI, 2.00 to 4.77; $P < .001$), compared with Tac+MTX and other regimens. Thus, this large retrospective comparison of MMF versus MTX in combination with CSA or Tac demonstrates significantly worse GVHD and survival outcomes with CSA+MMF compared with Tac+MTX.

INTRODUCTION

Graft-versus-host disease (GVHD) remains a major cause of morbidity and mortality after allogeneic hematopoietic cell transplantation (HCT). The combination of a calcineurin inhibitor (CNI), such as tacrolimus (Tac) or cyclosporine (CSA), with methotrexate (MTX) has been a standard practice over the past several decades for GVHD prevention; however, this treatment is associated with several unfavorable toxicities, including mucositis [1], delayed engraftment [2], and hepatic toxicities, primarily due to MTX [3]. Mycophenolate mofetil (MMF) was initially shown to have synergy with CSA in preventing GVHD and improving survival in experimental models [4], and despite a limited number of prospective randomized trials comparing its efficacy to MTX, the combination of CNI and MMF is commonly used in both reduced-intensity conditioning (RIC) and myeloablative conditioning (MAC) HCT [5-10].

Some small prospective studies have suggested similar outcomes of MMF and MTX in MAC HCT with improved toxicity profiles [8-11], whereas others have demonstrated more severe acute GVHD, primarily in unrelated donor (URD) HCT [12,13]. Previous retrospective studies evaluating MMF have also confirmed improved toxicity but with similar GVHD and survival outcomes as MTX [14,15], whereas a more recent retrospective study of 414 patients undergoing MAC and RIC HCT demonstrated relatively high incidences of grade III-IV acute GVHD (22.3% in related donor HCT and 36.5% in URD HCT) and nonrelapse mortality (NRM) (33.3% in related donor HCT and 46.5% in URD HCT) [16]. In addition, a large Center for International Blood and Marrow Transplant Research (CIBMTR) analysis comparing bone marrow (BM) versus peripheral blood (PB) grafts in URD HCT demonstrated significantly worse outcomes in terms of overall survival (OS), NRM, and acute GVHD (aGVHD) and chronic GVHD (cGVHD) with MMF compared with MTX, further raising the question of MMF's efficacy compared with MTX in this setting [17].

Given these conflicting results, we sought to determine the rates of GVHD and OS in patients undergoing first myeloablative allogeneic HCT using MTX versus MMF in combination with CSA or Tac.

METHODS

Data Source

The CIBMTR is a combined research program of the Medical College of Wisconsin and the National Marrow Donor Program. The CIBMTR comprises a voluntary network of more than 420 transplantation centers worldwide that contribute data on consecutive allogeneic and autologous HCTs 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 used in the performance of such research is collected and maintained in the capacity of the CIBMTR as a public health authority under HIPPAA regulations. Additional details regarding the source have been reported previously [18].

Patients

Patients age ≥18 years who underwent a first HLA-identical sibling or 8/8 or 7/8 HLA-matched URD HCT for acute myelogenous leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myelogenous leukemia (CML), or myelodysplastic syndrome (MDS) and received a CNI (CSA or Tac) in combination with MTX or MMF for GVHD prophylaxis reported to the CIBMTR between 2000 and 2013 were included. Patients receiving antithymocyte globulin (ATG) were included; however, those receiving ex vivo T cell-depleted grafts, alemtuzumab, or post-transplantation cyclophosphamide were excluded. Recipients of haploidentical, syngeneic, or cord blood transplants were also excluded.

Study Endpoints and Definitions

The primary study endpoints were the incidences of grade II-IV and III-IV aGVHD and cGVHD and OS. Secondary endpoints included relapse, transplantation-related mortality (TRM), and disease-free survival (DFS). The composite endpoint of GVHD-free, relapse-free survival (GRFS), including survival without grade III-IV aGVHD, cGVHD requiring systemic treatment, relapse, or death, was also evaluated. GVHD was graded according to historical consensus criteria [19,20]. Disease status was categorized as early, intermediate, or advanced [21]. Relapse was defined based on hematologic criteria of submitting centers, with NRM as a competing event. TRM was defined as death without evidence of disease recurrence, with relapse considered a competing event. DFS was defined as time to treatment failure (death or relapse). For relapse, TRM, and DFS, patients alive in continuous complete remission were censored at last follow-up. For GVHD, death without the event was considered a competing event. HLA matching was defined as described previously [22].

Statistical Analysis

Patient-, disease-, and transplantation-related variables for donor types were compared using chi-square statistics for categorical variables and the Kruskal-Wallis test for continuous variables. Probabilities of relapse, TRM, and GVHD were calculated using the cumulative incidence method to account for competing risks. Kaplan-Meier estimates were used to calculate the probability of DFS and OS. Multivariate Cox regression models were

constructed to evaluate hazard ratios (HRs) for endpoints of aGVHD, cGVHD, relapse, TRM, DFS, and OS for the various GVHD prophylaxis regimens (CSA +MTX, CSA+MMF, and Tac+MMF) compared with Tac+MTX as the reference. Pairwise comparisons were also made between each combination of Tac or CSA and MTX or MMF. Analyses were performed separately in MRD and URD HCT recipients, given the known differences in GVHD between these groups, as well as patterns of use of GVHD prophylaxis. Other variables in the multivariable model included age at transplantation, race, sex, Karnofsky performance status, Sorror HCT comorbidity index, disease diagnosis (AML, ALL, MDS, or CML), disease status (early, intermediate, or advanced), donor age (for URD), donor HLA match (7/8 versus 8/8, for URD), donor-recipient cytomegalovirus serostatus match, donor-recipient sex match, graft source (PB versus BM), use of ATG, conditioning regimen, use of total body irradiation, and year of transplantation. The assumption of proportional hazards for each factor in the Cox model was tested using time-dependent covariates. When the test indicated differential effects over time (non-proportional hazards), models were constructed breaking the post-transplant time course into 2 periods, using the maximized partial likelihood method to find the most appropriate breakpoint, which may have been different for different outcomes. Several outcomes, including cGVHD, TRM, and OS, demonstrated differential effects over time and are thus reported for different time points. A backward stepwise procedure was used to identify all significant risk factors and to develop models for each outcome, using a P value threshold of .05. Interactions between the main variable (GVHD prophylaxis) and adjusted covariates were tested at the significance level of .01.

RESULTS

Transplantation from an HLA-Identical Related Donor

Patient, Disease, and Transplant Characteristics

Patient characteristics are summarized in Table 1. In the MRD cohort, CSA+MTX was the most common GVHD prophylaxis regimen (n = 2252), followed by Tac+MTX (n = 1391), Tac +MMF (n = 222) and CSA+MMF (n = 114). Most patients receiving CSA+MTX (65%) underwent HCT before 2005, as reflected by a larger proportion of transplantations for CML (29%). ATG was used infrequently, but more commonly in the CSA+MMF (11%) and Tac+MMF (14%) groups compared with the CSA +MTX (5%) and Tac+MTX (4%) groups. The use of BM was more common in the CSA+MMF (28%) and CSA+MTX (36%) groups compared with the Tac+MTX (8%) and Tac+MMF (7%) groups.

Engraftment and GVHD

The median time to neutrophil engraftment was 16 days (range, 1 to 72 days) for CSA+MTX, 14 days (range, 7 to 29 days) for CSA+MMF, 13 days (range, 1 to 111 days) for Tac +MTX, and 12 days (range, 98 to 25 days) for Tac+MMF. Platelet recovery by day 28 was low with CSA+MTX (75%) and CSA +MMF (76%) compared with Tac+MTX (82%) and Tac+MMF (98%) (Tables 2 and 3).

The cumulative incidences of grade II-IV and III-IV aGVHD at day 100 are shown in Tables 2 and 3. In multivariable analysis (MVA), CSA+MMF (HR, 1.65; $P < .001$) and CSA+MTX (HR, 1.17; $P = .010$) were associated with worse grade II-IV aGVHD compared with reference Tac+MTX (Table 4, Figure 1A). CSA +MMF was also associated with an increased risk of grade III-IV aGVHD (HR, 1.92; $P < .001$) compared with Tac+MTX. Whereas our primary analysis focused on comparisons of GVHD prophylaxis regimens relative to Tac+MTX as the reference, pairwise comparisons between each regimen demonstrated a higher incidence of grade III-IV aGVHD with CSA +MMF relative to CSA+MTX (HR, 1.71; 95% confidence interval [CI], 1.17 to 2.50; $P = .006$). CSA+MMF also fared worse relative to CSA+MTX (HR, 1.41; 95% CI, 1.06 to 1.86; $P = .017$) and Tac +MMF (HR, 1.57; 95% CI, 1.10 to 2.22; $P = .012$) for grade II-IV aGVHD, but the differences did not reach our predefined level of significance ($P = .01$). Complete MVA results detailing additional patient, disease, and transplant characteristics are provided in the Supplementary Tables.

Given differential effects over time specific for this outcome, MVAs for cGVHD were divided into those experiencing cGVHD at <5 months post-transplantation and those doing so at ≥5 months post-transplantation (median time to cGVHD onset, 5.6 months). Relative to Tac+MTX, CSA+MTX was associated with higher rates of cGVHD at <5 months post-transplantation (HR, 1.41; $P < .001$), but a lower rate at ≥5 months post-transplantation (HR, .74; $P < .001$) (Table 4). Pairwise comparisons of each GVHD prophylaxis regimen relative to the others did not reveal any further significant associations (Supplementary Tables).

TRM

The incidence of TRM at 1 year post-transplantation was significantly higher for the CSA+MMF group (30%) compared with other GVHD regimens (Tables 2 and 3). In MVA, both CSA +MMF (HR, 4.03; $P < .001$) and CSA+MTX (HR, 2.29; $P < .001$) were significantly associated with higher TRM compared with Tac+MTX at <5 months post-transplantation but not after 5 months given time-varying effects (Table 4, Figure 2A). Pairwise comparisons between all GVHD prophylaxis regimens also demonstrated worse TRM at <5 months post-transplantation with CSA+MMF compared with CSA+MTX (HR, 1.76; 95% CI, 1.17 to 2.65; $P = .007$) and Tac+MMF (HR, 3.43; 95% CI, .77 to 3.03; $P < .001$) and with CSA+MTX relative to Tac+MMF (HR, 1.96; 95% CI, 1.19 to 3.22; $P = .0074$) (Supplementary Tables).

Relapse

The 1-year cumulative incidences of relapse for each regimen are shown in Tables 2 and 3. In MVA, there was no significant difference in relapse rate between any of the GVHD prophylaxis regimens and Tac+MTX. However, pairwise comparisons between all groups demonstrated a higher association of relapse with CSA+MMF relative to CSA+ MTX (HR, 1.67; 95% CI, 1.23 to 2.27, P = .001) (Supplementary Table).

DFS and OS

CSA+MMF recipients had poorer DFS and OS compared with all the other GVHD regimens (Tables 2 and 3). One-year probabilities of GRFS confirmed significantly worse outcomes with CSA+MMF (10%) compared with CSA+MTX (27%), Tac +MMF (20%), and Tac+MTX (20%).

In MVA, CSA+MMF was associated with poor DFS (HR, 1.64; $P < .001$) relative to Tac+MTX. Pairwise comparisons between the regimens also demonstrated inferior DFS with CSA+MMF compared with CSA+MTX (HR, 1.63; 95% CI, 1.29 to 2.05; $P < .001$) and Tac+MMF (HR, 1.74; 95% CI, 1.32 to 2.30; $P < .001$) (Supplementary Tables).

Two-year OS was 48% with CSA+MMF, compared with 67% with CSA+MTX, 69% with Tac+MMF, and 71% with Tac+MTX. Adjusted OS curves are shown in Figure 3A. In MVA, CSA+MMF was significantly associated with worse outcome (HR, 2.31; $P < .001$) compared with Tac +MTX at <5 months post-transplantation. CSA+MTX was also associated with worse survival (HR, 1.27; $P = .002$) compared with Tac+MTX within the first 5 months after HCT, but better survival at ≥5 months (HR, .74; $P = .003$) given nonproportional hazards over time (Table 4). Pairwise comparisons also demonstrated inferior survival of CSA+MMF relative to CSA+MTX (HR, 1.82; 95% CI, 1.36 to 2.42; $P < .001$) and Tac+MMF (HR, 2.17; 95% CI, 1.53 to 3.12; $P < .0001$) at <5 months post-transplantation (Supplementary index).

Table 1
Characteristics of Patients Undergoinging Myeloablative HCT Treated with Tac/CSA In Combination with MTX or MMF

Characteristic	MRD					URD				
	CSA+MMF (N = 114)	CSA+MTX (N = 2252)	Tac+MMF (N = 222)	Tac+MTX (N = 1391)	*P* Value	CSA+MMF (N = 68)	CSA+MTX (N = 974)	Tac+MMF (N = 424)	Tac+MTX (N = 2697)	*P* Value
Age at HCT, yr, median (range)	48 (19-69)	48 (18-72)	49 (18-70)	48 (18-71)	<.001	42 (18-72)	40 (18-69)	46 (18-73)	45 (18-75)	<.001
Race										
Caucasian	95 (83)	1746 (78)	210 (95)	1142 (82)	<.001	59 (87)	904 (93)	386 (91)	2475 (92)	.05
Non-Caucasian	16 (14)	393 (17)	11(5)	166 (12)		7 (10)	39 (4)	30 (7)	160 (6)	
Missing	3 (3)	113 (5)	1(<1)	83 (6)		2 (3)	31 (3)	8 (2)	62 (2)	
Sorror HCT-CI										
Before 2007	57 (50)	2033 (90)	71 (32)	555 (40)	<.001	32 (47)	820 (84)	216 (51)	1354 (50)	<.001
0-1	30 (26)	155 (7)	68 (31)	412 (30)		16 (24)	84 (9)	104 (25)	669 (25)	
2+	25 (22)	52 (2)	82 (37)	419 (30)		13 (19)	53 (5)	103 (24)	657 (24)	
Missing	2(2)	12 (<1)	1(<1)	5 (<1)		7 (10)	17 (2)	1 (<1)	17 (<1)	
KPS score before HCT										
<90	36 (32)	441 (20)	71 (32)	455 (33)	<.001	23 (38)	232 (24)	168 (40)	838 (31)	<.001
≥90	67 (59)	1763 (78)	68 (31)	886 (64)		42 (62)	669 (69)	245 (58)	1666 (62)	
Missing	11 (10)	48 (2)	82 (37)	50 (4)		0	73 (7)	11 (3)	193 (7)	
Disease										
AML	62 (54)	879 (43)	133 (60)	741 (53)	<.001	41 (60)	451 (46)	245 (58)	1519 (56)	<.001
ALL	12 (11)	490 (22)	42 (19)	271 (19)		12 (18)	226 (23)	70 (17)	479 (18)	
CML	24 (21)	648 (29)	15 (7)	166 (12)		8 (12)	199 (20)	51 (12)	291 (11)	
MDS	16 (14)	136 (6)	32 (14)	213 (15)		7 (10)	98 (10)	58 (14)	408 (15)	
Disease status at HCT*										
Early	46 (40)	977 (43)	124 (56)	741 (53)	<.001	27 (40)	470 (48)	195 (46)	1334 (49)	.002
Intermediate	35 (31)	881 (39)	34 (15)	323 (23)		19 (28)	278 (29)	90 (21)	635 (24)	
Advanced	29 (25)	377 (17)	60 (27)	309 (22)		21 (31)	216 (22)	136 (32)	691 (26)	
Missing	4(4)	17 (<1)	4 (2)	18 (1)		1 (1)	10 (1)	3 (<1)	37 (1)	
Donor type										
HLA-identical sibling	114	2252	222	1391		0	0	0	0	<.001
Unrelated, 8/8-matched	0	0	0	0		46 (68)	686 (70)	316 (75)	2130 (79)	
Unrelated, 7/8- matched	0	0	0	0		22 (32)	288 (30)	108 (25)	567 (21)	
Donor-recipient sex match										
Male/male	40 (35)	750 (33)	78 (35)	429 (31)	.96	25 (37)	341 (35)	174 (41)	1032 (38)	.34
Male/female	28 (25)	500 (22)	48 (22)	326 (23)		17 (25)	275 (28)	125 (29)	775 (29)	
Female/male	24 (21)	545 (24)	51 (23)	342 (25)		11 (16)	185 (19)	54 (13)	447 (17)	
Female/female	22 (19)	456 (20)	45 (20)	294 (21)		15 (22)	173 (18)	71 (17)	442 (16)	
Missing	0	1 (<1)	0	0		0	0	0	1 (<1)	

(*continued*)

Table 1 (*Continued*)

Characteristic	MRD					URD				
	CSA+MMF (N = 114)	CSA+MTX (N = 2252)	Tac+MMF (N = 222)	Tac+MTX (N = 1391)	*P* Value	CSA+MMF (N = 68)	CSA+MTX (N = 974)	Tac+MMF (N = 424)	Tac+MTX (N = 2697)	*P* Value
Donor-recipient CMV status										
Negative/negative	44 (39)	1247 (55)	77 (35)	558 (40)	<.001	19 (28)	164 (17)	108 (25)	567 (21)	.003
Negative/positive	16 (14)	208 (9)	25 (11)	142 (10)		9 (13)	102 (10)	44 (10)	263 (10)	
Positive/negative	19 (17)	273 (12)	63 (28)	331 (24)		24 (35)	339 (35)	138 (33)	948 (35)	
Positive/positive	32 (28)	430 (19)	54 (24)	337 (24)		15 (22)	306 (31)	122 (29)	801 (30)	
Missing	3 (3)	94 (4)	3 (1)	23 (2)		1 (1)	64 (7)	12 (3)	118 (4)	
Graft type										
Bone marrow	41 (36)	633 (28)	15 (7)	114 (8)	<.001	17 (25)	479 (49)	53 (13)	813 (30)	<.001
Peripheral blood	73 (54)	1619 (72)	207 (93)	1277 (92)		51 (75)	500 (51)	371 (88)	1884 (70)	
ATG use										
Yes	13 (11)	118 (5)	30 (14)	55 (4)	<.001	23 (34)	146 (15)	144 (34)	718 (27)	<.001
No	101 (89)	2134 (95)	192 (86)	1336 (96)		45 (66)	828 (85)	280 (66)	1979 (73)	
TBI										
Yes	30 (26)	834 (37)	99 (45)	600 (43)	<.001	24 (35)	614 (63)	141 (33)	1128 (42)	<.001
No	84 (74)	1418 (63)	123 (55)	791 (57)		44 (65)	360 (37)	283 (67)	1569 (58)	
Conditioning regimen										
Bu + Cy ± others	54 (47)	1215 (54)	32 (14)	461 (33)	<.001	25 (37)	284 (29)	64 (15)	688 (26)	<.001
ATG+ Bu ± Cy ± Flu ± others	11 (10)	88 (4)	8 (4)	49 (4)		15 (22)	60 (6)	101 (24)	533 (20)	
Bu + Flu ± others	9 (8)	47 (2)	72 (32)	248 (18)		2 (3)	11 (1)	98 (23)	288 (11)	
TBI ± Cy ± others	25 (22)	749 (33)	72 (32)	494 (36)		16 (24)	516 (53)	88 (21)	897 (33)	
ATG + TBI ± Cy ± others	1 (<1)	29 (1)	22 (10)	5 (<1)		6 (9)	85 (9)	41 (10)	177 (7)	
TBI + etoposide ± others	4 (4)	65 (3)	5 (2)	101 (7)		2 (3)	13 (1)	12 (3)	54 (2)	
Others	10 (9)	68 (3)	11 (5)	33 (2)		2 (3)	5 (<1)	20 (5)	60 (2)	
Year of HCT										
2000-2004	41 (36)	1460 (65)	33 (15)	248 (18)	<.001	11 (16)	560 (57)	49 (12)	506 (19)	<.001
2005-2008	29 (25)	635 (28)	82 (37)	464 (33)		34 (50)	321 (33)	219 (52)	1148 (43)	
2009-2013	44 (39)	157 (7)	107 (48)	679 (49)		23 (34)	93 (10)	156 (37)	1043 (39)	
Follow-up of survivors, mo, median (range)	70 (3-168)	61 (1-194)	65 (3-150)	66 (3-174)	<.001	76 (37-169)	97 (6-193)	72 (25-191)	72 (5-172)	<.001

HCT-CI indicates HCT comorbidity index; KPS, Karnofsky Performance Status; CMV, cytomegalovirus; TBI, total body irradiation; Bu, busulfan; Cy, cyclophosphamide; Flu, fludarabine; side.

Table 2
Univariate Outcomes in Myeloablative Related and Unrelated Donor Transplant

	Matched Related Donor (MRD)								
	N eval	CSA+MMF Prob (95% CI)	N eval	CSA+MTX Prob (95% CI)	N eval	Tac+MMF Prob (95% CI)	N eval	Tac+MTX Prob (95% CI)	*P*-Value
Acute GVHD, II-IV									
Day 100	114	44 (35-53%)	2218	34 (32-36%)	220	35 (28-41%)	1369	32 (20-35%)	.07
Acute GVHD, III-IV									
Day 100	114	26 (18-34%)	2203	18 (16-19%)	220	18 (13-23%)	1369	14 (12-16%)	.005
Chronic GVHD									
6 months	113	20 (13-28%)	2194	27 (25-29%)	219	28 (23-35%)	1372	26 (24-28%)	.35
1 year		30 (21-39%)		41 (39-43%)		45 (39-52%)		46 (44-99%)	<.001
Relapse									
1 year	112	37 (28-46%)	2212	21 (19-23%)	219	28 (23-35%)	1368	30 (28-33%)	<.001
2 years		40 (31-49%)		27 (25-29%)		45 (39-52%)		36 (34-39%)	<.001
Transplant-related mortality									
1 year	112	30 (22-39%)	2212	18 (16-19%)	219	14 (10-19%)	1368	13 (11-15%)	<.001
2 years		35 (26-44%)		20 (18-21%)		17 (12-22%)		17 (15-19%)	<.001
Disease-free survival									
1 y ear	112	33 (25-42%)	2212	61 (59-63%)	219	54 (47-60%)	1368	57 (54-60%)	<.001
2 years		25 (18-34%)		54 (52-56%)		45 (39-52%)		47 (44-49%)	<.001
Overall Survival									
1 year	114	46 (37-55%)	2252	69 (67-71%)	222	66 (59-72%)	1391	68 (66-71%)	<.001
2 years		35 (26-44%)		61 (59-64%)		52 (45-59%)		55 (53-58%)	<.001
GVHD-Relapse-free									
Survival (GRFS)	114		2214		221		1377		
1 year		16 (10-23%)		27 (25-29%)		20 (15-25%)		20 (18-22%)	<.001
ANC recovery									
14 days	113	46 (37-55%)	2228	30 (28-32%)	220	66 (60-72%)	1379	50 (48-53%)	<.001
Platelet recovery									
28 days	105	76 (68-84%)	2159	75 (74-77%)	201	89 (84-93%)	1349	82 (80-84%)	<.001

	Unrelated donor (URD)								
	N eval	CSA+MMF Prob (95% CI)	N eval	CSA+MTX Prob (95% CI)	N eval	Tac+MMF Prob (95% CI)	N eval	Tac+MTX Prob (95% CI)	
Acute GVHD, II-IV									
Day 100	68	57 (46-69%)	964	50 (47-53%)	419	55 (50-59%)	2678	49 (47-51%)	.11
Acute GVHD, III-IV									
Day 100	68	40 (28-52%)	965	23 (20-25%)	420	25 (21-30%)	2673	20 (19-22%)	.001
Chronic GVHD									
6 months	66	21 (12-32%)	962	35 (32-38%)	417	35 (30-40%)	2673	28 (27-30%)	<.001
1 year		NE		48 (44-51%)		51 (46-56%)		45 (43-47%)	.01
Relapse									
1 year	64	23 (14-25%)	954	23 (20-25%)	415	28 (24-32%)	2652	27 (25-29%)	.03
2 years		25 (15-36%)		27 (24-30%)		32 (28-37%)		31 (30-33%)	.03
Transplant-related mortality									
1 year	64	41 (29-53%)	954	26 (24-29%)	415	24 (20-28%)	2652	20 (19-22%)	<.001
2 years		44 (32-56%)		30 (27-33%)		29 (25-34%)		24 (22-26%)	<.001
Disease-free survival									
1 y ear	64	36 (25-48%)	954	51 (48-54%)	415	48 (43-53%)	2652	53 (51-54%)	.02
2 years		31 (21-43%)		43 (40-47%)		39 (34-43%)		45 (43-47%)	.02
Overall Survival									
1 year	68	40 (28-52%)	974	57 (54-60%)	424	54 (49-59%)	2697	60 (58-62%)	.001
2 years		34 (23-45%)		49 (46-52%)		44 (39-49%)		50 (48-52%)	.005
GVHD-Relapse-free									
Survival (GRFS)	67		968		422		2691		
1 year		10 (4-19%)		16 (13-18%)		10 (7-13%)		17 (15-18%)	<.001
ANC recovery									
14 days	67	43 (32-55%)	970	26 (23-28%)	424	70 (65-74%)	2686	41 (39-43%)	<.001
Platelet recovery									
28 days	67	64 (52-75%)	960	59 (56-62%)	402	83 (79-86%)	2646	68 (67-70%)	<.001

CSA- cyclosporine; MMF- mycophenolate mofetil; MTX- methotrexate; Tac- tacrolimus; GVHD- graft-versus-host diseae; ANC- absolute neutrophil count; IPS- idiopathic pneumonia syndrome; SN- subsequent neoplasm.

Table 3
Cause of Death in Myeloablative Related and Unrelated Donor Transplant

Cause of Death	Matched Related Donor (MRD)							
	N eval	CSA+MMF	N eval	CSA+MTX	N eval	Tac+MMF	N eval	Tac+MTX
		N (%)		N (%)		N (%)		N (%)
	80		992		132		780	
Primary disease		33 (41)		384 (39)		71 (54)		404 (52)
Graft failure		2 (3)		8 (<1)		1 (<1)		1 (<1)
GVHD		24 (30)		222 (22)		28 (21)		158 (20)
IPN		5 (6)		102 (10)		10 (8)		70 (9)
Infection		3 (4)		57 (6)		4 (3)		20 (3)
Organ failure		8 (10)		91 (9)		7 (5)		59 (8)
SN		0		14 (1)		2 (2)		10 (1)
Other		4 (5)		86 (9)		7 (5)		40 (5)
Missing		1 (1)		28 (3)		2 (2)		18 (2)

	Unrelated donor (URD)							
	N eval	CSA+MMF	N eval	CSA+MTX	N eval	Tac+MMF	N eval	Tac+MTX
		N (%)		N (%)		N (%)		N (%)
	48		628		293		1659	
Primary disease		13 (27)		209 (33)		111 (38)		686 (41)
Graft failure		0		9 (1)		1(<1)		12 (<1)
GVHD		16 (33)		167 (27)		90 (31)		393 (24)
IPS		7 (15)		73 (12)		30 (10)		170 (10)
Infection		4 (8)		35 (6)		11(4)		66 (4)
Organ failure		5 (10)		69 (11)		22 (8)		169 (10)
SN		0		2 (<1)		3 (1)		15 (<1)
Other		3 (6)		58 (9)		17 (6)		120 (7)
Missing		0		6 (<1)		8 (3)		29 (2)

Table 4
Multivariate Outcomes in Myeloablative Related and URD HCT

Outcome	Tac+MTX	CSA+MMF	CSA+MTX	Tac+MMF
MRD, HR (95% CI), *P* value				
aGVHD grade II-IV		**1.65 (1.24-2.20) *P* < .001**	**1.17 (1.04-1.33) *P* = .010**	1.05 (.83-1.34) *P* = .661
aGVHD grade III-IV		**1.92 (1.31-2.83) *P* < .001**	1.13 (.92-1.37) *P* = .283	1.19 (.85-1.66) *P* = .319
cGVHD* <5 mo		1.34 (.83-2.17) *P* = .233	**1.41 (1.20-1.67) *P* < .001**	1.28 (.94-.74) *P* = .119
cGVHD* ≥5 mo		.56 (.34-.95) *P* = .030	**.74 (.65-.85) *P* < .001**	.92 (.71-1.19) *P* = .512
TRM* <5 mo	Ref (1.00)	**4.03 (2.61-6.23) *P* < .001**	**2.29 (1.78-2.95) *P* < .001**	1.17 (.71-1.94) *P* = .537
TRM* ≥5 mo		1.38 (.78-2.44) *P* = .275	.83 (.67-1.03) *P* = .095	.90 (.59-1.36) *P* = .609
Relapse		1.43 (1.05-1.93) *P* = .022	.86 (.74-.98) *P* = .029	.92 (.73-1.14) *P* = .444
OS* <5 mo		**2.31 (1.73-3.09) *P* < .001**	**1.27 (1.10-1.48) *P* = .002**	1.05 (.81-1.37) *P* = .704
OS* ≥5 mo		.94 (.62-1.43) *P* = .767	**.74 (.64-.87) *P* < .001**	.92 (.70-1.20) *P* = .530
URD, HR (95% CI), *P* value				
aGVHD, grade II-IV		1.49 (1.08-2.07) *P* = .016	1.00 (.90-1.12) *P* = .961	1.14 (.99-1.32) *P* = .066
aGVHD, grade III-IV		**2.31 (1.57-3.42) *P* < .001**	1.02 (.87-1.20) *P* = .806	1.26 (1.02-1.56) *P* = .030
cGVHD* <4 mo		1.54 (.79-2.98) *P* = .203	**1.62 (1.35-1.93) *P* < .001**	1.34 (1.04-1.73) *P* = .022
cGVHD* ≥4 mo		.92 (.52-1.63) *P* = .779	.85 (.74-.98) *P* = .025	**1.47 (1.24-1.75) *P* < .001**
TRM* <4 mo	Ref (1.00)	**3.09 (2.00-4.77) *P* < .001**	1.24 (1.02-1.51) *P* = .030	1.02 (.77-1.36) *P* = .896
TRM* ≥4 mo		.89 (.42-1.90) *P* = .763	1.00 (.84-1.20) *P* = .979	**1.45 (1.16-1.81) *P* = .001**
Relapse		.81 (.50-1.32) *P* = .398	.97 (.85-1.12) *P* = .699	.93 (.78-1.12) *P* = .449
OS* <4 mo		**2.36 (1.67-3.35) *P* < .001**	**1.23 (1.08-1.41) *P* = .002**	1.20 (1.01-1.44) *P* = .044
OS* ≥4 mo		1.10 (.64-1.89) *P* = .730	.91 (.79-1.05) *P* = .221	**1.34 (1.12-1.61) *P* = .001**

Bolded values represent statistically significant associations.

* Given differential effects over time (nonproportional hazards), models were constructed breaking the post-transplantation time course into 2 periods, using the maximized partial likelihood method to find the most appropriate breakpoint.

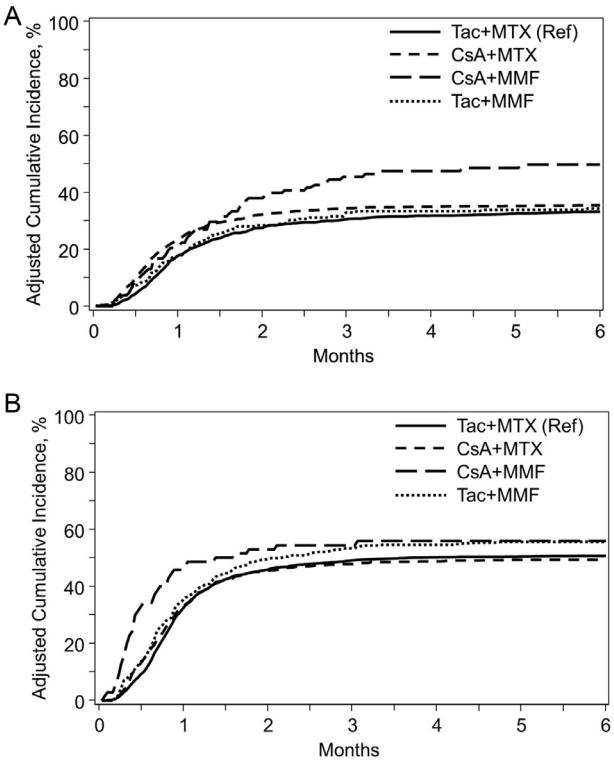

Figure 1. Incidence of grade II-IV aGVHD by GVHD prophylaxis regimen in myeloablative MRD HCT (A) and myeloablative URD HCT (B).

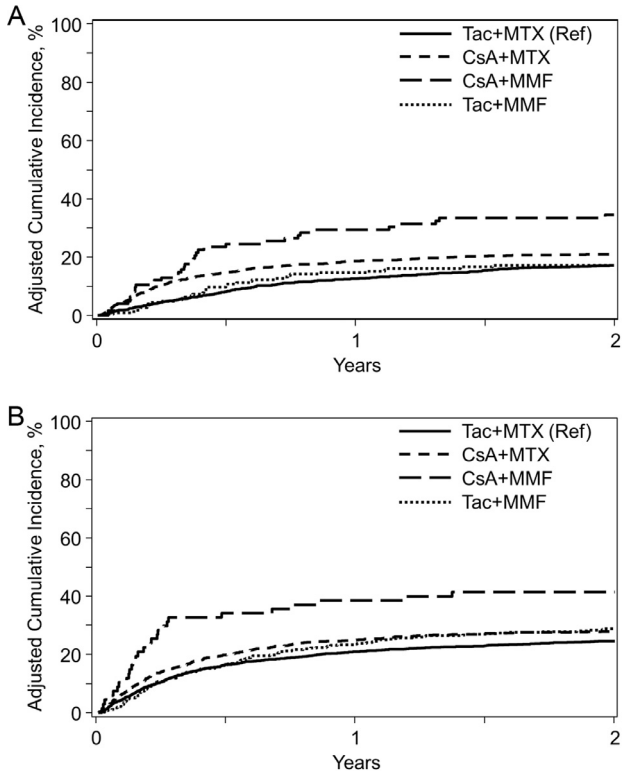

Figure 2. Incidence of TRM by GVHD prophylaxis regimen in myeloablative MRD HCT (A) and myeloablative URD HCT.

Transplantation from a URD

Patient, Disease, and Transplantation Characteristics

In the URD cohort, Tac+MTX was the predominant GVHD prophylaxis regimen (n = 2697), followed by CSA+MTX (n = 974), Tac +MMF (n = 424), and CSA+MMF (n = 68) (Table 1). Similar to MRD, there were more patients in the CSA+MTX (57%) cohort transplanted before 2005. The use of BM as a cell source was also more common in the CSA+MTX group (49%) compared with other regimens: 25% with CSA+MMF, 13% with Tac+MMF, and 30% with Tac +MTX. ATG was used less commonly with CSA+MTX (15%) relative to CSA+MMF (34%), Tac+MMF (34%), and Tac+MTX (27%).

Engraftment and GVHD

The median time to neutrophil engraftment with CSA+MTX was 17 days (range, 6 to 42 days), followed by 14 days for both CSA+MMF (range, 9 to 27 days) and Tac+MTX (range, 2 to 205 days) and 12 days (range, 5 to 48 days) for Tac+MMF. Platelet recovery by day 28 was also low in CSA+MTX recipients (59%), compared with 64% for CSA+MMF, 68% for Tac+MTX, and 83% for Tac+MMF (Tables 2 and 3).

The cumulative incidence of grade II-IV aGVHD at day 100 was highest in the CSA+MMF group (57%) compared with other regimens (Tables 2 and 3). Day 100 grade III-IV aGVHD was also highest for CSA+MMF (40%) compared with CSA+MTX (23%), Tac+MMF (25%), and Tac+MTX (20%). In MVA, CSA+MMF was significantly associated with grade III-IV aGVHD (HR, 2.31; $P < .001$), as well as worse grade II-IV aGVHD (HR ,1.49; $P = .016$) compared with Tac+MTX, although this did not reach the predefined significance level (Table 4, Figure 1B). Pairwise comparisons also demonstrated similar higher risks of grade III-IV aGVHD with CSA+MMF compared with CSA+MTX (HR, 2.27; 95% CI, 1.51 to 3.40; $P < .001$) and Tac+MMF (HR, 1.83; 95% CI, 1.19 to 2.81; $P = .006$) and grade II-IV aGVHD (HR, 1.49; 95% CI, 1.07 to 2.08; $P = .019$) with CSA+MMF compared with CSA+MTX, but not reaching the predefined level of significance (Supplementary index).

Similar to the MRD cohort, MVA results for cGVHD were divided into those experiencing cGVHD at <4 months and ≥4 months post-transplantation given differential effects over time specific for this outcome. Relative to Tac+MTX, CSA+MTX was associated with higher rates of cGVHD at <4 months post-transplantation (HR, 1.62; $P < .001$). At ≥4 months after HCT, Tac+MMF was associated with higher cGVHD (HR, 1.47; $P < .001$) relative to Tac+MTX. Pairwise comparisons also demonstrated higher rates of cGVHD with Tac+MMF compared with CSA+MTX (HR, 1.73; 95% CI, 1.41 to 2.12; $P < .001$) at ≥4 months (Supplementary index).

TRM

The incidence of TRM at 1 year post-transplantation was highest for CSA+MMF (41%) compared with the other regimens (Tables 2 and 3). In MVA, CSA+MMF (HR, 3.09; $P < .001$) was significantly associated with higher TRM compared with Tac +MTX at <4 months post-transplantation. After 4 months, Tac +MMF (HR, 1.45; $P = .001$) was associated with increased TRM relative to Tac+MTX (Table 4, Figure 2B). Pairwise comparisons also demonstrate worse TRM with CSA+MMF relative to CSA +MTX (HR, 2.49; 95% CI, 1.58 to 3.91; $P < .001$) and Tac+MMF (HR, 3.03; 95% CI, 1.85 to 5.00; $P < .001$) at <4 months post-transplantation. After 4 months, Tac+MMF was also associated with worse TRM relative to CSA+MTX (HR, 1.45; 95% CI, 1.12 to 1.87; $P = .0053$) (Supplementary index).

Relapse

There were no significant associations between any of the GVHD prophylaxis regimens with respect to relapse relative to Tac+MTX or any other GVHD regimen in the URD cohort.

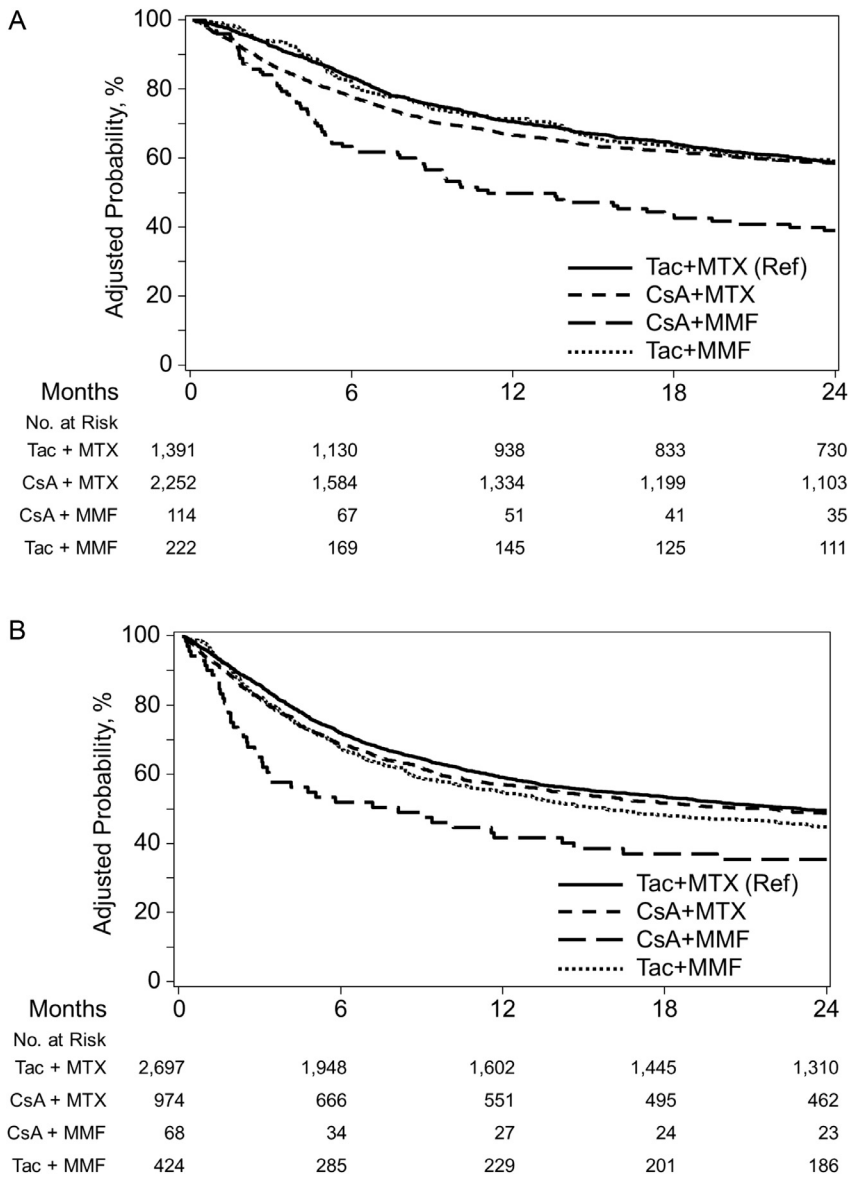

Figure 3. Adjusted OS by GVHD prophylaxis regimen in myeloablative MRD HCT (A) and myeloablative URD HCT (B).

DFS and OS

CSA+MMF recipients had poor 1-year DFS (36%) and OS (34%) compared with recipients of the other GVHD prophylaxis regimens (Tables 2 and 3). One-year GRFS was low at 10% for both CSA+MMF and Tac+MMF, compared with 16% for CSA +MTX and 17% for Tac+MTX (Tables 2 and 3).

In MVA, no prophylaxis regimen was associated with worse DFS relative to Tac+MTX. Older age, mismatched donor, donor age, poor performance status, disease, disease status, and conditioning regimen were associated with DFS (Supplementary index).

CSA+MMF (HR, 2.36; $P < .001$) and CSA+MTX (HR, 1.23; $P = .002$) were associated with worse OS compared with Tac +MTX at <4 months post-transplantation. After 4 months, Tac +MMF demonstrated worse OS (HR, 1.34; $P = .001$) compared with Tac+MTX (Figure 3B). Pairwise comparisons also demonstrated worse survival with CSA+MMF compared with CSA +MTX (HR, 1.92; 95% CI, 1.34 to 2.75; $P = .0004$) and Tac+MMF (HR, 1.96; 95% CI, 1.35 to 2.85; $P = .0005$). After 4 months, Tac +MMF was associated with worse survival compared with CSA +MTX (HR, 1.47; 95% CI, 1.18 to 1.81; $P = .0004$) (Supplementary index).

DISCUSSION

In this current large CIBMTR study, we found significantly worse aGVHD, TRM, GFRS, and OS with CSA+MMF compared with Tac+MTX in both MRD and URD myeloablative allogeneic HCT settings. Patients receiving CSA+MMF also had inferior TRM and survival outcomes compared with those receiving CSA+MTX or Tac+MMF. Overall, these findings indicate significant inferiority of CSA+MMF compared with the other combinations of Tac or CSA and MMF or MTX in both MRD and URD myeloablative transplantation.

The combination of MMF and Tac or CSA has previously been shown in retrospective and small prospective studies to be associated with significantly faster engraftment, shorter hospital stay, and less mucositis compared with MTX [8,11,23]. Our present analysis confirms generally faster engraftment rates with MMF compared with MTX, and also indicates superior engraftment with Tac compared with CSA. Previous

studies, including 3 large prospective randomized trials, have also demonstrated a significantly lower incidence of aGVHD for patients receiving Tac compared with those receiving CSA, although these findings did not translate into a survival benefit [24-26]. Similar to those studies, we evaluated MRD and MUD cohorts separately, given the known differences in GVHD outcomes and differences in patterns of use of GVHD prophylaxis. Our findings also suggest a more potent immunosuppressant effect of Tac, as demonstrated by significantly worse grade II-IV aGVHD, early (<4 months) cGVHD, and TRM with CSA+MTX compared with Tac+MTX in the MRD cohort and worse early cGVHD and OS in the URD setting. Likewise, CSA+MMF was also associated with worse TRM, DFS, and OS compared with Tac+MMF, with a trend toward worse GVHD in MRD recipients, whereas in the URD setting, CSA+MMF was associated with worse grade III-IV aGVHD, TRM, and OS compared with Tac +MMF. In fact, the immunosuppressive effect of Tac may be powerful enough to compensate for the inferiority of MMF, given the lack of statistically significant differences between Tac+MMF and Tac+MTX in the MRD setting for any GVHD and survival outcomes. Thus, Tac+MMF may be a reasonable substitute in recipients of MRD HCT when there is a concern for engraftment or severe mucosal toxicity.

In both the MRD and URD cohorts, CSA+MMF was associated with worse TRM and OS compared with CSA+MTX, secondary to significantly worse severe aGVHD, further supporting the superiority of MTX over MMF. We did not detect any differences in cGVHD in the CSA+MMF group, possibly due to the relatively higher proportion of patients receiving bone marrow grafts. Although GVHD and survival were similar with Tac+MMF and Tac+MTX in the MRD setting, Tac+MMF was associated with higher cGVHD, higher TRM, and worse survival at ≥4 months post-transplantation compared with Tac+MTX in the URD group, with a trend toward worse aGVHD outcomes. The composite endpoint of GRFS also showed significantly worse outcomes with CSA+MMF compared with the other regimens in the MRD setting, and both CSA+MMF and Tac+MMF had poorer GRFS compared with MTX-containing regimens in URD HCT, again indicating an advantage of MTX over MMF for GVHD prophylaxis.

There are several important considerations to take into account in this analysis, and given the retrospective nature of this evaluation, we also acknowledge several limitations. The reason for choosing MMF over MTX (or Tac over CSA) as GVHD prophylaxis is unknown in this study. We recognize this may reflect biases of specific institutional protocols or a preference for a GVHD regimen with less mucositis or faster engraftment due to patient comorbidities. This potential preferential bias may be reflected by the larger number of patients in the MTX cohorts, particularly recipients of URD HCT, compared with MMF-based regimens. These preferential differences are also highlighted by imbalances in other baseline characteristics (e.g., year of transplantation, use of ATG, graft source) between patient groups, which could potentially impact outcomes. Although all these factors were evaluated in MVA, it may be difficult to fully account for all the differences, and this must be considered when interpreting our results. The dosing and duration of MMF and MTX used were also unknown in this dataset, representing a major limitation of this study. Although measuring levels of the active metabolite of MMF, mycophenolic acid (MPA), is not standard practice, pharmacokinetic studies in GVHD treatment have shown significantly higher MPA concentrations in responders compared with nonresponders [27,28]. Previous studies have also demonstrated increased rates of severe GVHD and NRM in patients with lower MPA steady-state concentrations compared with those with higher levels, especially in recipients of URD HCT [29,30]. Although 45 mg/kg/day has been suggested as the optimal dose for the prevention of acute GVHD [10], other studies have demonstrated superiority of higher MMF doses administered 3 times daily (3 g) rather than the more common twice daily (2 g) [31,32]. Interestingly, pharmacokinetic analyses have also demonstrated differences in MPA clearance between CSA and Tac, with CSA increasing clearance of MPA by 33.8% compared with Tac, necessitating higher doses of MMF when used in combination with CSA [33]. This is a potentially important consideration when interpreting the differences in outcomes between CSA/MMF and Tac/MMF. Unfortunately specific dosing of MMF and levels of MPA in this study are unknown, and thus the potential effects of increased dosing, interaction with CNIs, and concentrations of MMF on GVHD outcomes limit our ability to draw conclusions. In addition, the duration of MMF therapy also likely plays an important role, in that there may have been differences in practice in this study. The optimal duration of MMF therapy after HCT has not been established; however, a prolonged course of MMF has been associated with low reported rates of GVHD [7,34]. Although longer duration of MMF therapy potentially may account for the generally lower incidence of cGVHD within the first few months after HCT, there likely was significant variation in dosing and duration of MMF that we were unable to take into account in this analysis.

Standard dosing of MTX is 15 mg/m^{2}on day 1, followed by 10 mg/m^{2}on days 3, 6, and 11, but there likely were at least some modifications to this dosing schedule, which would have been included in our analysis. Standard doses of MTX are often held or reduced due to severe mucositis or other toxicities, and the subsequent effect of this on GVHD outcomes is unclear [35]. In addition, a reduced dosage scheme of 5 mg/m^{2}on days 1, 3, 6, and 11 is frequently used with the aim of decreasing the risk of mucosal and hepatic complications. This regimen has proven effective in combination with CSA or Tac as GVHD prophylaxis, with historically similar outcomes as seen with standard dosing [36-39]. However, these reduced doses of MTX have never been directly compared with standard dosing of MTX, and thus never been proven to be equivalent, but would have been included in this analysis. Finally, we do not have any data on the dosing or duration of Tac or CSA. There was likely variation in practice regarding goal trough levels and duration of prophylactic therapy, which may have affected the incidence of GVHD.

We also recognize that there are several important secondary outcomes when evaluating MMF versus MTX that we were not able to analyze in this retrospective study. MTX is part of a regimen known to be associated with significant morbidities, including severe mucositis, prolonged hospitalization, use of total parenteral nutrition, delayed hematopoietic recovery, and liver and renal toxicities. Although the data captured in this analysis are insufficient to adequately address these questions, despite many attempts to substitute MTX in GVHD prophylaxis, the superiority of this regimen has not yet been demonstrated [8,10,40,41].

In conclusion, despite the limitations of a retrospective analysis, this study, the largest study reported to date evaluating MMF-based versus MTX-based GVHD prophylaxis, demonstrates significantly inferior outcomes with CSA+MMF compared with all the other GVHD regimens in both the related donor and URD settings. Although no combination of CNI and MMF or MTX was found to be superior to Tac+MTX, our findings also confirm the potent immunosuppressive effect

of Tac and suggest that Tac+MMF may be a reasonable substitute in the related donor setting.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.bbmt.2019.05.019.

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