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Abstract

The association of Cyclosporine A (CsA) and mycophenolate mofetil (MMF) has increased in the setting of reduced intensity conditioning (RIC). Nevertheless, the use of CsA or CsA+MMF has not been reported in a large and uniform cohort.

We analyzed 497 patients with acute myeloid leukemia in complete remission (CR) who underwent matched unrelated donor (MUD) hematopoietic stem cell transplantation (HSCT). All patients received a fludarabine busulfan RIC regimen and anti-thymocyte globulin (ATG) with either CsA alone or in combination with MMF.

The cumulative incidence (CI) of grade II-IV acute GvHD was 27% (95% CI 21-33%) for CsA and 33% (95% CI 27-38%) for CsA+MMF (p=0.25). The 2-year CI of chronic GvHD was 38% (95% CI 31-45%) and 33% (95% CI 28-39%) for the CsA and the CsA+MMF group, respectively (p=0.26).

On multivariate analysis, no statistically significant differences with respect to relapse incidence (RI), non-relapse mortality (NRM), leukemia free survival (LFS), overall survival (OS), acute and chronic GvHD were found between the two groups, also when conducting a subgroup analysis in peripheral blood stem cells (PBSC) recipients. Our results support the importance of randomized trial to identify patients who could benefit from the addition of MMF in MUD HSCT.

Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is an important and potentially curative treatment option for patients with acute myeloid leukemia (AML). However, long-term outcomes may be associated with an extent of non-relapse mortality (NRM). Acute graft-versus-host disease (aGvHD) is one of the most common causes of increased morbidity and mortality after transplant. In the setting of matched unrelated donor (MUD), GvHD prophylaxis strategies include the use of immunosuppressive drugs such as the calcineurin inhibitor cyclosporine A (CsA), but they are poorly standardized (1) (2).

CsA combined with another immunosuppressive agent i.e. methotrexate (MTX) or mycophenolate mofetil (MMF) has been shown to further decrease the incidence of severe aGVHD and to improve survival (3) (4).

MMF inhibits T cell activation and proliferation, and has been used in combination with CsA for GvHD prophylaxis, especially in the setting of reduced intensity regimens (5) (6) (7)**.** Compared to MTX, MMF is less toxic when used in combination with CsA (5) (8).

However, all of the published studies have included heterogeneous groups of patients who underwent HSCT for both lymphoid and myeloid malignancies and with diverse chemotherapy conditioning intensity regimes (9) (10) (11). None of the studies has compared MMF and CsA with CsA alone-based GvHD prophylaxis exclusively in a large cohort of patients with homogeneous disease.

In this study, we report the outcomes of two groups of patients who underwent MUD HSCT with fludarabine and busulfan for AML in complete remission (CR) and received CsA alone or CsA and MMF as GvHD prophylaxis.

Materials and Methods

This is a registry cohort study. We analyzed 497 adult patients, with a diagnosis of AML who underwent a 10/10 MUD HSCT and received CsA alone (n=183) or CsA+MMF (n=314) as GvHD prophylaxis. All patients underwent a reduced intensity conditioning (RIC) regimen with fludarabine 30mg/m2 from day -6 to day -2, busulfan 130mg/m2 from day -5 to day -4 and received anti-thymocyte globulin (ATG). Patient's human leucocyte antigen (HLA)-matched to the donor at the allele-level for HLA-A, HLA-B, HLA-C, HLA-DQB1 and HLA-DRB1 were included. Transplants were performed in European Society for Blood and Marrow Transplantation (EBMT) centers from 2007 to 2017. All patients provided written informed consent for the use of their data for clinical research, in accordance with the modified guidelines of the Declaration of Helsinki and the local ethics committee. The Review Board of the Acute Leukemia Working Party (ALWP) of the EBMT approved this study.

Patients with available cytogenetic data were classified according to the Medical Research Council (12).

The primary endpoint was the cumulative incidence (CI) of aGvHD and chronic GvHD (cGvHD), defined according to standard criteria(13) (14).

Secondary endpoints were neutrophil engraftment, non-relapse mortality (NRM), relapse incidence (RI), leukemia free survival (LFS), overall survival (OS) and graft-versus-host-disease free, relapse free survival (GRFS).

Neutrophil engraftment was defined as the first day of an absolute neutrophil count of 0.5×10^9/L lasting for 3 or more consecutive days. OS was defined as time from HSCT to death from any cause or last follow-up, whichever came first; patients alive at last follow

up were censored. LFS was defined as time from HSCT to relapse, death from any cause or last follow-up, whichever comes first; patients alive without disease at last follow-up were censored. NRM was defined as time to death from any cause not related to relapse and RI was defined as time from HSCT to relapse. GRFS was defined as the time from transplant to grade III-IV acute GVHD, extensive cGVHD, relapse, or death; whichever came first (15).

CI curves were used in a competing risk setting to calculate probabilities of aGvHD and cGvHD, neutrophil recovery, NRM and RI. To study acute and chronic GVHD, we considered relapse and death to be competing events. Probabilities of OS, LFS and GRFS were calculated using the Kaplan-Meier method. Univariate analyses were done using the Gray's test for cumulative incidence functions and the log rank test for OS, GRFS, and LFS. A Cox proportional hazards model was used for multivariate regression. All variables differing significantly between the two groups or factors associated with one outcome in univariate analysis were included in the Cox model. In order to test for a centre effect, we introduced a random effect or frailty for each centre into the model(16) (17). All tests were 2-sided. The type I error rate was fixed at 0.05 for the determination of factors associated with time-to-event outcomes. A subgroup analysis planned from the beginning of the study was also performed on a homogeneous population of patients transplanted in CR1 using PBSC. Ninety-five percent confidence intervals (95% CI) were calculated. Analyses were performed with SPSS 24 (IBM SPSS Statistics for Windows, Version 19.0. IBM Corp, Armonk, NY) and R 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria) software packages.

Results

Patient, disease, and transplant characteristics according to GvHD prophylaxis are summarized in Table 1. A total of 497 patients with diagnosis of AML were included, of which 183 received CsA and 314 CsA+MMF. The median follow-up was 33 (inter quartile range [IQR] 18-60) months for the CsA group and 34 (IQR 18-54) months for the CsA+MMF group. There were no significant differences between the groups with respect to patient, disease and HSCT characteristics. The majority of patients had intermediate cytogenetic risk and a performance status ≥90%.

Engraftment

The CI of neutrophil engraftment at 60 days was 99.4% and 99.7% in the CsA and CsA+MMF group, respectively (p=0.14). The median time to achieve neutrophil engraftment was not different between the two groups: 18 (range: 2-45) days in the CsA and 19 (range: 7-37) days in the CsA+MMF cohort, p=0.092). Graft failure was observed in one patient in the CsA group and one patient in the CsA+MMF group experienced secondary graft rejection.

Acute and chronic GVHD

Overall, the 100-day CI of grade II-V aGvHD was 30% (95% CI 26-35%). The CI of grade II-IV aGvHD did not differ between the group of patients who received CsA alone and those who received CsA+MMF (27% versus 33%, p=0.25) (figure 1a).

The CI of grade III-IV aGvHD was 10% (95% CI 8-13%) at 100 day; it was 9% (95% CI 6-14%) for the CsA group and 11% (95% CI 8-14%) for the CsA+MMF group (p=0.60) (figure 1b).

The 2 year CI of cGvHD and extensive cGvHD were 35% (95% CI 31-40%) and 15% (95% CI 12-19%), respectively. According to GvHD prophylaxis, the CI of cGvHD was 38% (95% CI 31-45%) and 33% (95% CI 28-39%) in patients who received CsA alone and CsA+MMF, respectively (p=0.26) (figure 2a). The CI of extensive cGvHD was also comparable between the two groups, being 17% (95% CI 11-23) for patients who received CsA and 15% (95% CI 11-19) for those who had CsA+MMF (p=0.49) (figure 2b).

The results of the univariate analysis are reported in the Supplementary Table 1.

On multivariate analysis (table 2), there was no significant difference for risk of aGvHD between patients who had CsA alone versus CsA+MMF (Hazard ratio [HR] 1.18, 95% CI 0.79-1.78, p=0.41) nor for cGvHD (HR 0.79, 95% CI 0.53-1.17, p=0.25).

<u>RI and NRM</u>

The 2-year RI and NRM were 25% (95% CI 21-29%) and 19% (95% CI 16-23%), respectively. RI was not significantly different between recipient who had CsA alone (26% [95% CI 20-33%]) and CsA+MMF (24% [95% CI 19-29%]) (p=0.90) (figure 3b). No difference was also observed in NRM according to the GvHD prophylaxis group: 21% (95% CI 16-28%) for CsA alone and 18% (95% CI 14-22%) for CsA+MMF, p=0.52 (figure 3a).

On multivariate analysis (table 2), there was no difference between the two groups with respect to RI (HR 0.63, 95% CI 0.37-1.06, p=0.08) and NRM (HR 0.83, 95% CI 0.52-1.33, p=0.43). A positive donor CMV serology was associated with higher NRM (HR 2.03, 95% CI 1.31-3.14, p=0.001). Another factor associated with increased risk of NRM was older age (HR 1.39, 95% CI 1.05-1.85, p=0.02).

A total of 208 patients died (81 and 127 in the CsA and CsA+MMF groups, respectively). In the CsA group, 39% died of relapse, 25% of GvHD, 22% of infection, 15% for other causes. In the CsA+MMF group 43% of patients died of relapse, 23% of infection, 23% of GvHD, 11% for other causes.

OS, LFS and GRFS

The 2-year OS and LFS were 60% (95% CI 55-64%) and 56% (95% CI 52-61%), respectively. OS was similar between patients who had CsA alone (55% [95% CI 47-63%]) and CsA+MMF (62% [95% CI 57-68%]), p=0.67. No differences were detected for LFS (CsA group: 52% [95% CI 45-60%]; CsA+MMF: 59% [95% CI 53-64%], p=0.56) and GRFS (40% [95% CI 32-47%] for CsA alone and 48% [95% CI 42-54%] for CsA+MMF, p=0.37) (figure 3c, 3d and figure 4).

On multivariate analysis (table 2), there were no differences in survival rates according to GvHD prophylaxis. Patients with a donor positive CMV serology had a significantly lower OS (HR 1.66, 95% CI 1.22-2.26, p=0.001), LFS (HR 1.69, 95% CI 1.26-2.27, p<0.001) and GRFS (HR 1.75, 95% CI 1.34-2.28, p<0.001) compared with patients with a donor negative CMV serology.

Subgroup analysis for patients in CR1 who received PBSC

We observed no differences with respect to aGvHD, cGvHD, NRM, RI, OS, LFS and GRFS when analyzing patients in CR1 who received PBSC (CsA alone, n=138; CsA+MMF, n=257). In univariate analysis, patients who received CsA+MMF tended to have a lower cGvHD at 2 years (p=0.053), but this was not statistically significant in multivariate analysis (HR 0.67, 95% CI 0.42-1.05, p=0.08).

Discussion

We observed comparable outcomes in patients with AML undergoing a RIC ATG based 10/10 MUD HSCT with fludarabine and busulfan as the conditioning regimen when comparing two groups of GvHD prophylaxis (CsA alone versus CsA+MMF). The addition of MMF to CsA was not associated with a significant reduction of acute and chronic GvHD.

Since the introduction of the combination of MMF and CsA in the late 1990s, few studies have reported transplant outcomes of MUD HSCT, thus data are scarce. Moreover, published studies refer to a small number of patients.

In 2004, the outcomes of 34 patients who underwent RIC with a fludarabine and busulfan ATG-based preparative regimen and a HLA-identical sibling as graft source were compared for these two types of GvHD prophylaxis(18). Among them, 27 had hematological malignancies of which 16 received CsA alone and 11 received CsA and MMF. No differences in outcomes and specifically in aGvHD incidence were detected in this small group of patients.

Similarly, a brief report retrospectively described 35 patients with both myeloid and lymphoid malignancies who underwent a MUD HSCT with the same conditioning regimen (19). Of these, 31 were 10/10 matched with the recipient (CsA alone, n=16; CsA and MMF, n=15). Although a limited number of patients were described, the study reported a better OS and a lower CI of grade III-IV aGvHD in the CsA+MMF group. Another brief report showed the beneficial effect of ATG, CsA and MMF in the RIC setting for cGvHD on 20 patients, of whom 12 were 10/10 matched (20). A recent phase 3 trial investigating the addition of sirolimus to CsA+MMF for prophylaxis, reported higher CI

of grade II-IV aGvHD (up to 52%) in CsA+MMF arm and supported the benefit of adding silorimus in GvHD prophylaxis. However, in contrast with our study population, in this trial patients did not receive ATG (21).

In our study, the benefit of adding MMF regardless of the use of ATG could not be analyzed. Notably, all patients received ATG, a T-cell depleting agent. It might be possible that the association of ATG with the use of CsA reflects an adequate GvHD prophylaxis in our cohort and indirectly prevented us in estimating the supposed benefit of MMF addition, which selectively targets the activated lymphocytes(22). The protective effect of ATG in reducing cGVHD and improving GRFS has recently been demonstrated in a large phase clinical III trial(23), and ATG remains a recommended treatment for GvHD prevention in patients undergoing MUD or HLA-sibling HSCT.

Recently, the type of post-transplant immunosuppression was described in patients with AML in CR1 given PBSC from matched related donors with a RIC fludarabine based regimen. The CsA group (n=86) was compared to a group receiving CsA+MMF/MTX (n=66, CsA+MMF= 45) and to another group who did not receive ATG. The authors found that the addition of MMF or MTX to CsA did not reduce the risk of aGvHD, but significantly increased the risk of relapse, possibly due to the relatively reduced risk of cGvHD, leading to worse LFS an OS (24).

In our cohort, which is similar for disease type but in MUD setting, a subgroup analysis performed in patients in CR1 who received PBSC identified a tendency toward higher cGvHD at 2 years for patients who received CsA alone. However, this finding was not confirmed on multivariate analysis.

The intensity of GvHD prophylaxis did not modify the risk of relapse. We observed in both groups a satisfactory OS and GRFS, which reflects the quality of life without long-term complications related to the GvHD.

We observed a higher NRM in recipients for whom the donor had a positive CMV serology that resulted in a lower LFS, GRFS and OS. This finding is in line with previous reports described from the EBMT on outcomes in de novo acute leukemia (25) and from the Center for International Blood and Marrow Transplant Research (CIBMTR) who demonstrated that early CMV reactivation has a negative impact on transplant outcomes regardless of hematological disease type (26). Finally, in our cohort older age was associated with an increased risk of NRM (p=0.02) on multivariate analysis. This finding did not result in a lower OS, indicating that older age should not be considered a condition for withholding RIC HSCT in patients with AML in CR.

One of the limitations of our registry-based study is that some disease characteristics could be confounding factors. In order to overcome this limitation, we performed a subgroup analysis in a homogenous group of patients with AML in CR1 who had PBSC.

Although we acknowledge that due to the retrospective nature of the study recent specific molecular methods such as next generation sequencing or molecular status were not available, the results remained consistent with those of the entire study population.

Although measuring the active metabolite of MMF is not a standard practice, several pharmacokinetic studies on GvHD have demonstrated higher mycophenolic acid concentration in responders compared with non-responders(27). The optimal duration of MMF has not been established; however, some authors have reported lower incidences of GvHD after a prolonged treatment (28). Due to the retrospective nature

of the study, we are not able to report the dosing schedule and the duration of the two metabolites MMF and CsA.

A study conducted on 23 recipients undergoing MUD HSCT reported that interindividual variations in CsA levels after 2 hours intake were not associated with a higher risk of GvHD when cyclosporine trough (C0) guided the dosing (29). A more comprehensive study on 85 patients receiving a MUD HSCT with either reduced or myeloablative conditioning demonstrated that the lowest CsA concentration in the first and second weeks after HSCT was associated with a significantly higher risk of grade III-IV aGvHD (30). We were not able to further investigate these points in our study, but we did not observe significant differences in transplant outcomes between the two groups regarding aGvHD.

We acknowledge that the retrospective nature of our study could introduce another bias. Preference for a particular GvHD prophylaxis is largely based on uncontrolled, observational studies, and is often guided by physician or transplant center preference. Conflicting results regarding various clinical outcomes have been observed when comparing MMF against MTX regimens for aGvHD prophylaxis, and less is known regarding CsA compared with MMF+CsA. With an increasing number of RIC HSCTs being performed, it could be of importance to prospectively evaluate the comparative efficacy of the two prophylactic agents in the prevention of GvHD.

Randomized trials analyzing the use of MMF versus placebo in the setting of RIC ATG MUD HSCT should be considered to better assess which patients could eventually benefit from the addition of MMF for GvHD prophylaxis.

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Author contributions

AP and MM designed the study, AP, MM and AN wrote the manuscript, ML performed the statistical analysis, DB, GS, CEB, BL, PC, IWB, GG, JM, PC, AH, PT, ED, EF and AN provided cases for the study. All authors edited and approved the final version of the manuscript.

Conflict of interest

The authors have no conflict of interest to disclose

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Figure 1. Cumulative incidence of acute grade II-IV GvHD and grade III-IV GvHD according to CsA alone (black continuous line) or CsA+MMF GvHD (dashed line) prophylaxis.

Figure 2. Cumulative incidence of chronic GvHD and extensive chronic GvHD according to CsA alone (black continuous line) or CsA+MMF GvHD (dashed line) prophylaxis.

Figure 3. Cumulative incidence of non-relapse mortality, relapse incidence, leukemia free survival and overall survival according to CsA alone (black continuous line) or CsA+MMF GvHD (dashed line) prophylaxis.

Figure 4. Graft-versus-host-disease free, relapse free survival (GRFS) according to CsA alone (black continuous line) or CsA+MMF GvHD (dashed line) prophylaxis.

Table 1. Disease, patient and transplant characteristics.

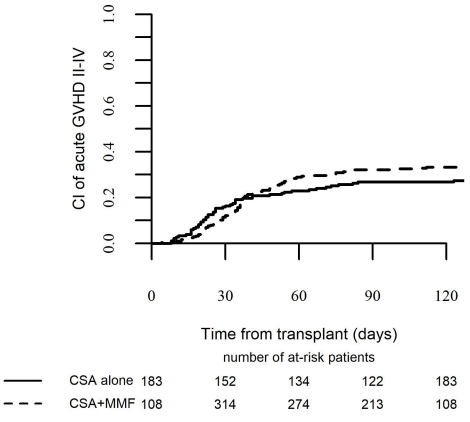
Table 2. Multivariate analysis.

Table 1. Disease, patient and transplant characteristics

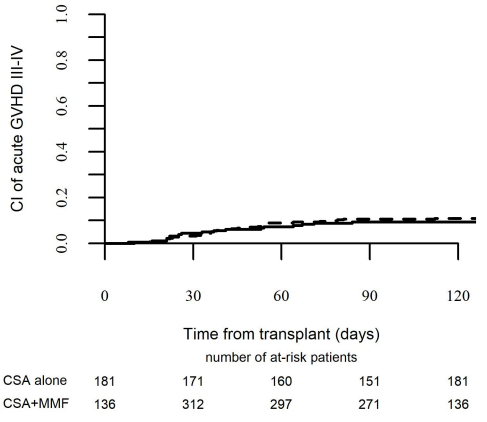
		CsA alone (n=183)	**CsA+MMF (n=314)**	***p-value***
age at HSCT, median (range)	years	59 (20-75)	60 (22-75)	0.84
year of HSCT, median (range)	years	2015 (2007-2017)	2014 (2007-2017)	0.08
disease state at HSCT	CR1 CR2	149 (81%) 34 (19%)	268 (85%) 46 (15%)	0.25
cytogenetics (MRC)	good intermediate poor missing	10 (6%) 121 (66%) 35 (19%) 17 (9%)	17 (5%) 209 (67%) 47 (15%) 41 (13%)	0.45
Karnofsky performance status	<90 >=90 missing	52 (32%) 113 (68%) 18	78 (27%) 212 (73%) 24	0.29
Graft source	BM PBSC	12 (7%) 171 (93%)	12 (4%) 302 (96%)	0.17
Gender	male female	108 (59%) 75 (41%)	160 (51%) 154 (49%)	0.08
Donor gender	male female missing	129 (70%) 54 (30%) 0	195 (63%) 113 (37%) 6	0.10
Gender matching	no female to male female to male missing	159 (87%) 24 (13%) 0	265 (85%) 46 (15%) 3	0.60
Patient CMV serology	negative positive missing	76 (42%) 107 (58%) 0	118 (38%) 192 (62%) 4	0.45
Donor CMV serology	negative positive missing	119 (65%) 63 (35%) 1	183 (60%) 124 (40%) 7	0.20

Abbreviations: HSCT, hematopoietic stem cell transplantation; CsA, cyclosporine A; MMF, mycophenolate mofetil; CR, complete remission; BM, bone marrow; PBSC, peripheral blood stem cells; CMV, cytomegalovirus; MRC, Medical Research Council

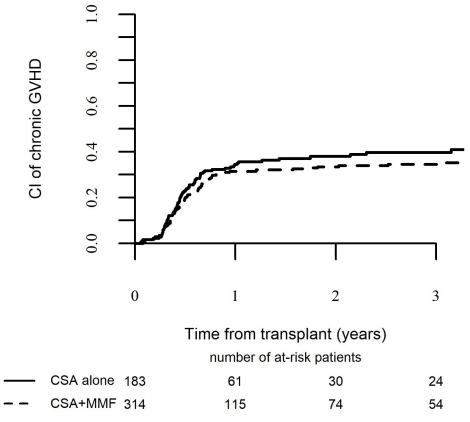
acute GVHD II-IV



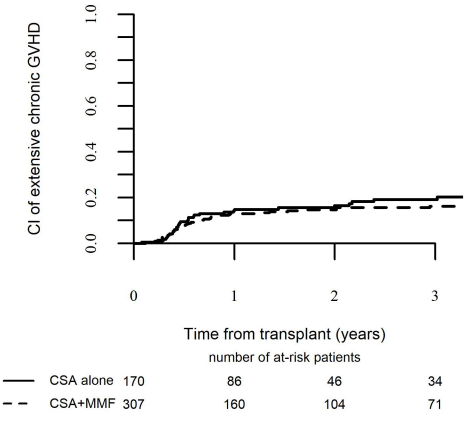
acute GVHD III-IV



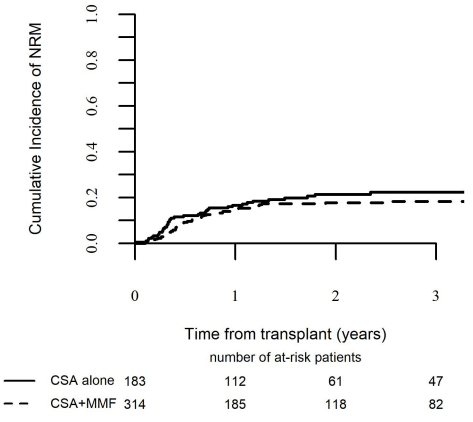
cGVHD



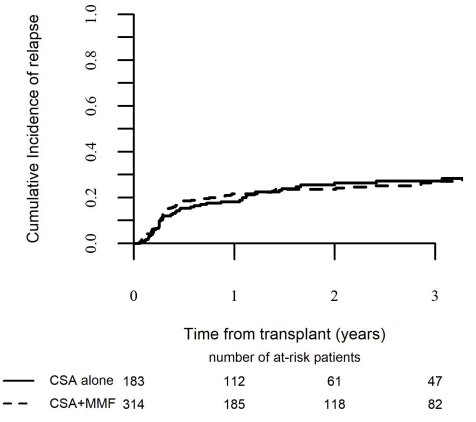
extensive cGVHD



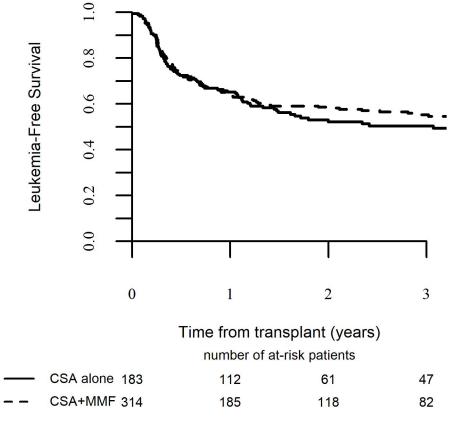
NRM


Cumulative Incidence of NRM
0.0
0.2
0.4
0.6
0.8
1.0
0
1
2
3
Time from transplant (years)
number of at-risk patients
CSA alone 183 112 61 47
CSA+MMF 314 185 118 82

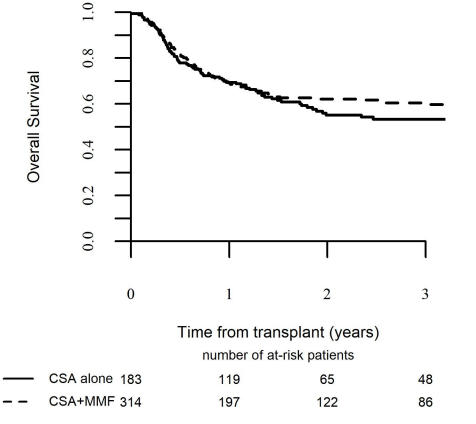
RI


Cumulative Incidence of relapse
0.0
0.2
0.4
0.6
0.8
1.0
0
1
2
3
Time from transplant (years)
number of at-risk patients
CSA alone 183 112 61 47
CSA+MMF 314 185 118 82

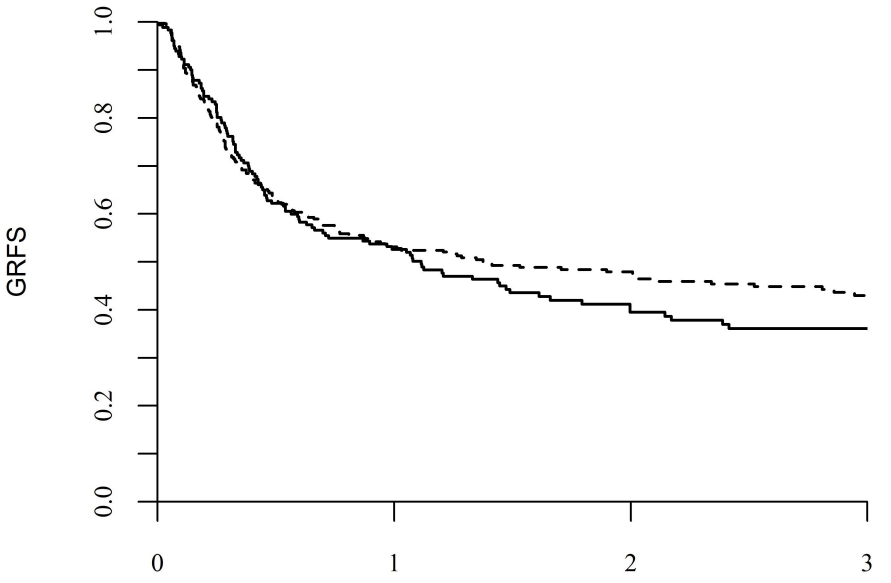
LFS


Leukemia-Free Survival
0.0
0.2
0.4
0.6
0.8
1.0
0
1
2
3
Time from transplant (years)
number of at-risk patients
CSA alone 183 112 61 47
CSA+MMF 314 185 118 82

OS


Overall Survival
0.0
0.2
0.4
0.6
0.8
1.0
0
1
2
3
Time from transplant (years)
number of at-risk patients
CSA alone 183 119 65 48
CSA+MMF 314 197 122 86

GRFS



number of at-risk patients

——	CSA alone	181	90	47	35
- - - -	CSA+MMF	312	152	97	67

Supplemental table1. Univariate Analysis

	2 years					100 days		2 years	
	RI	**NRM**	**LFS**	**OS**	**GRFS**	**acute GvHD II-IV**	**acute GvHD III-IV**	**chronic GVHD**	**extensive chronic GvHD**
CSA alone	26% [20-33]	21% [16-28]	52% [45-60]	55% [47-63]	40% [32-47]	27% [21-33]	9% [6-14]	38% [31-45]	17% [11-23]
CSA+MMF	24% [19-29]	18% [14-22]	59% [53-64]	62% [56-68]	48% [42-54]	33% [27-38]	11% [8-14]	33% [28-39]	15% [11-19]
P value	0.89	0.52	0.55	0.67	0.37	0.25	0.60	0.26	0.49
age<59.6y	24% [19-29]	17% [13-22]	59% [52-65]	62% [55-68]	48% [41-54]	29% [23-35]	9% [6-13]	39% [32-45]	16% [12-21]
age>=59.6 y	25% [20-31]	21% [16-26]	54% [47-60]	58% [51-64]	42% [35-49]	32% [26-38]	11% [8-16]	32% [26-38]	14% [10-19]
P value	0.84	0.31	0.31	0.15	0.49	0.53	0.32	0.04	0.25
Year< 2014	20% [15-26]	22% [17-28]	58% [51-65]	61% [54-68]	47% [40-54]	29% [23-36]	9% [6-14]	37% [30-44]	16% [11-21]
Year>=2014	28% [22-33]	17% [13-22]	55% [49-62]	59% [53-65]	43% [37-49]	31% [26-37]	11% [8-15]	34% [28-39]	15% [11-20]
P value	0.10	0.15	0.65	0.91	0.25	0.72	0.71	0.35	0.96
CR1	23% [19-27]	19% [16-23]	57% [53-62]	61% [56-66]	45% [40-50]	32% [27-36]	11% [8-14]	36% [31-41]	15% [12-19]
CR2	31% [21-42]	19% [11-28]	50% [39-62]	53% [41-64]	43% [32-55]	24% [15-34]	8% [3-15]	32% [21-43]	15% [8-23]
P value	0.21	0.82	0.29	0.20	0.79	0.15	0.39	0.54	0.71
Cytogenetic good	27% [12-45]	4% [0-19]	68% [50-87]	67% [48-86]	55% [36-75]	22% [9-39]	4% [0-17]	39% [19-58]	13% [3-30]
intermediate	22% [17-27]	19% [15-24]	59% [53-64]	62% [57-68]	46% [40-51]	31% [26-36]	12% [8-15]	39% [34-45]	17% [13-22]
poor	37% [26-47]	15% [8-24]	48% [37-59]	50% [38-62]	39% [28-50]	33% [23-43]	9% [4-16]	24% [16-34]	12% [6-20]
P value	0.07	0.04	0.17	0.15	0.54	0.56	0.44	0.02	0.35
PS <90	23% [16-31]	17% [11-24]	60% [52-69]	62% [53-70]	47% [39-56]	32% [24-40]	12% [7-18]	37% [28-45]	16% [10-23]
PS>=90	25% [20-30]	21% [16-26]	54% [49-60]	59% [53-64]	45% [39-50]	30% [25-35]	10% [7-14]	35% [30-40]	14% [10-18]
P value	0.16	0.67	0.11	0.28	0.27	0.79	0.71	0.60	0.57
BM	25% [8-46]	18% [5-36]	57% [36-79]	57% [35-79]	46% [24-69]	17% [5-34]	4% [0-18]	31% [13-51]	6% [0-27]
PBSC	25% [21-29]	19%[16-23]	56% [52-61]	59% [55-64]	45% [40-49]	31% [27-35]	11% [8-13]	35% [31-40]	16% [13-19]
P value	0.89	0.79	0.68	0.72	0.39	0.25	0.72	0.59	0.09
Male	23% [18-28]	22% [17-28]	55% [49-61]	58% [51-64]	41% [34-47]	33% [27-38]	10% [7-14]	37% [31-43]	19% [14-24]
Female	27% [21-33]	15% [11-21]	58% [51-65]	62% [55-68]	50% [43-56]	28% [22-33]	11% [7-15]	32% [26-39]	11% [8-16]
P value	0.05	0.03	0.90	0.86	0.40	0.11	0.93	0.24	0.12
male donor	25% [21-30]	18% [14-22]	57% [51-63]	59% [53-65]	44% [39-49]	28% [24-33]	9% [6-13]	34% [29-40]	16% [12-21]
female donor	23% [17-30]	22% [16-29]	55% [47-62]	60% [53-68]	45% [38-53]	35% [28-42]	12% [7-17]	38% [30-45]	14% [9-21]
P value	0.63	0.14	0.44	0.59	0.88	0.06	0.31	0.61	0.75
no female to male MM	24% [20-29]	19% [15-23]	57% [52-62]	59% [55-65]	46% [41-51]	29% [25-34]	10% [7-13]	34% [30-39]	15% [12-19]
female to male MM	25% [16-36]	23% [14-34]	51% [39-63]	59% [48-71]	39% [27-50]	37% [26-48]	12% [6-21]	42% [30-54]	16% [8-27]

P value	0.69	0.14	0.41	0.65	0.38	0.07	0.36	0.21	0.83
Pat. CMV neg.	27% [21-34]	16% [11-21]	57% [50-64]	62% [55-69]	46% [39-53]	25% [19-32]	9% [6-14]	30% [24-37]	13% [9-19]
Pat. CMV pos	23% [18-28]	21% [16-26]	56% [50-62]	58% [52-64]	44% [38-50]	34% [28-39]	11% [7-14]	38% [33-44]	17% [13-22]
P value	0.50	0.19	0.75	0.26	0.37	0.08	0.83	0.07	0.15
Don. CMV neg.	22% [17-27]	14% [11-18]	64% [58-69]	67% [61-72]	53% [47-59]	28% [23-33]	8% [6-12]	34% [28-39]	13% [9-17]
Don. CMV pos	28% [21-35]	27% [21-34]	45% [37-53]	48% [41-56]	32% [25-39]	35% [28-42]	13% [9-18]	38% [31-45]	21% [15-27]
P value	0.25	0.0003	0.0001	<0.0001	<0.0001	0.07	0.12	0.25	0.02

Abbreviations: CR, complete remission; CsA, cyclosporine A; MMF, mycophenolate mofetil; PS, performance status ; BM, bone marrow; PBSC, peripheral blood stem cell; CMV, cytomegalovirus; MM, mismatch; RI, relapse incidence ; NRM, non-relapse mortality; LFS, leukemia free survival, OS, overall survival; GvHD, graft-versus-host disease; GRFS, GvHD free ,relapse-free survival

Table 2. Multivariate analysis

	RI		NRM		LFS		OS		GRFS		Acute GvHD II-IV		chronic GvHD	
	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value
CSA+MMF versus CSA alone	0.63 (0.37-1.06)	0.08	0.83 (0.515-1.33)	0.43	0.74 (0.51-1.08)	0.12	0.84 (0.56-1.24)	0.37	0.80 (0.586-1.08)	0.14	1.18 (0.79-1.78)	0.41	0.79 (0.53-1.17)	0.25
Good Cytogenetics	1		1		1		1		1		1		1	
Intermediate	0.81 (0.35-1.89)	0.63	1.87 (0.43-8.09)	0.40	1.08 (0.52-2.22)	0.84	1.03 (0.49-2.13)	0.95	1.01 (0.54-1.89)	0.97	1.11 (0.43-2.85)	0.83	1.06 (0.49-2.27)	0.89
adverse	1.58 (0.62-3.98)	0.34	1.32 (0.27-6.44)	0.73	1.38 (0.63-3.03)	0.43	1.28 (0.57-2.86)	0.55	1.12 (0.56-2.24)	0.75	1.12 (0.40-3.12)	0.83	0.55 (0.23-1.36)	0.19
age (per 10y)	0.89 (0.73-1.08)	0.25	1.39 (1.05-1.85)	0.02	1.05 (0.89-1.24)	0.54	1.13 (0.96-1.35)	0.17	1.03 (0.89-1.19)	0.69	1.15 (0.94-1.4)	0.17	0.91 (0.77-1.07)	0.25
CR2 versus CR1	1.26 (0.76-2.1)	0.38	0.97 (0.53-1.81)	0.94	1.11 (0.75-1.65)	0.60	1.13 (0.75-1.72)	0.55	0.98 (0.68-1.4)	0.91	0.72 (0.42-1.24)	0.24	0.85 (0.54-1.34)	0.49
KPS>=90 versus KPS<90	1.2 (0.76-1.92)	0.44	1.2 (0.744-1.94)	0.45	1.23 (0.88-1.73)	0.23	1.19 (0.84-1.69)	0.34	1.15 (0.86-1.53)	0.35	0.93 (0.64-1.36)	0.72	0.98 (0.68-1.41)	0.90
PBSC vs BM	1.5 (0.53-4.28)	0.45	0.95 (0.34-2.66)	0.92	1.26 (0.60-2.65)	0.54	1.15 (0.52-2.53)	0.74	1.55 (0.78-3.09)	0.21	1.29 (0.52-3.23)	0.59	1.83 (0.78-4.28)	0.16
Female to male MM vs other	0.76 (0.42-1.36)	0.35	1.26 (0.73-2.16)	0.41	0.97 (0.65-1.45)	0.89	0.95 (0.62-1.44)	0.79	0.97 (0.68-1.4)	0.89	1.5 (0.95-2.36)	0.08	1.49 (0.96-2.31)	0.07
Patient CMV pos	0.67 (0.45-1.02)	0.06	1.06 (0.67-1.68)	0.79	0.83 (0.61-1.12)	0.22	0.95 (0.69-1.31)	0.75	0.90 (0.68-1.19)	0.46	1.22 (0.84-1.77)	0.29	1.28 (0.9-1.82)	0.17
Donor CMV pos	1.44 (0.97-2.15)	0.07	2.03 (1.31-3.14)	0.001	1.69 (1.26-2.27)	<0.001	1.66 (1.22-2.26)	0.001	1.75 (1.34-2.28)	<0.001	1.26 (0.88-1.8)	0.21	1.44 (1.02-2.04)	0.04
centre (frailty)		<0.001		0.24		0.006		0.006		0.13		0.21		0.06

Abbreviations: CR, complete remission; CsA, cyclosporine A; MMF, mycophenolate mofetil; KPS, Karnofsky performance status ; BM, bone marrow; PBSC, peripheral blood stem cells; CMV, cytomegalovirus; MM, mismatch; RI, relapse incidence ; NRM, non-relapse mortality; LFS, leukemia free survival, OS, overall survival; GvHD, graft-versus-host disease; GRFS, GvHD free, relapse-free survival.