Comparison of mycophenolate mofetil and calcineurin inhibitor versus calcineurin inhibitor-based graft-versus-host-disease prophylaxis for matched unrelated donor transplant in acute myeloid leukemia. A study from the ALWP of the EBMT

Annalisa Paviglianiti, Myriam Labopin, Didier Blaise, Gerard Socie, Claude Eric Bulabois, Bruno Lioure, Patrice Ceballos, Igor Wolfgang Blau, Gaelle Guillerm, Johan Maertens, et al.

► To cite this version:

Annalisa Paviglianiti, Myriam Labopin, Didier Blaise, Gerard Socie, Claude Eric Bulabois, et al.. Comparison of mycophenolate mofetil and calcineurin inhibitor versus calcineurin inhibitor-based graft-versus-host-disease prophylaxis for matched unrelated donor transplant in acute myeloid leukemia. A study from the ALWP of the EBMT. Bone Marrow Transplantation, 2020, 10.1038/s41409-020-01155-z . hal-03111030

HAL Id: hal-03111030

<https://hal.sorbonne-universite.fr/hal-03111030>

Submitted on 15 Jan 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Comparison of mycophenolate mofetil and calcineurin inhibitor versus calcineurin inhibitor-based graft-versus-host-disease prophylaxis for matched unrelated donor transplant in acute myeloid leukemia. A study from the ALWP of the EBMT.

Annalisa Paviglianiti¹, Myriam Labopin¹, Didier Blaise², Gerard Socié³, Claude Eric Bulabois⁴, Bruno Lioure⁵, Patrice Ceballos⁶, Igor Wolfgang Blau⁷, Gaelle Guillermin⁸, Johan Maertens⁹, Patrice Chevallier¹⁰, Anne Huynh¹¹, Pascal Turlure¹², Eric Deconinck¹³, Edouard Forcade¹⁴, Arnon Nagler¹⁵, Mohamad Mohty¹

¹Sorbonne University, Service d'Hématologie Clinique et Thérapie cellulaire, Hôpital Saint-Antoine, INSERM UMRs 938, Paris, France

²Programme de Transplantation & Thérapie Cellulaire, Centre de Recherche en Cancérologie de Marseille, Institut Paoli Calmettes, Marseille, France

³Hopital St. Louis, Dept. of Hematology – BMT, Paris, France

⁴CHU Grenoble Alpes - Université Grenoble Alpes, Service d'Hématologie, Grenoble, France

⁵Techniciens d'Etude Clinique suivi de patients greffes, Nouvel Hopital Civil, Strasbourg, France

⁶CHU Lapeyronie, Département d'Hématologie Clinique, Montpellier, France

⁷Charité Universitätsmedizin Berlin, Campus Virchow Klinikum, Medizinische Klinik m. S. Hämatologie/Onkologie, Berlin, Germany

⁸C.H.R.U de Brest, Service Onco-Hématologie, Brest, France

⁹University Hospital Gasthuisberg, Dept. of Hematology, Leuven, Belgium

¹⁰CHU Nantes, Dept. D'Hématologie, Nantes, France

¹¹CHU - Institut Universitaire du Cancer Toulouse, Oncopole, I.U.C.T-O, Toulouse, France

¹²CHRU Limoges Service d'Hématologie Clinique, Limoges, France

¹³Hopital Jean Minjoz, Service d'Hématologie, Besançon, France

¹⁴CHU Bordeaux, Service d'hématologie et thérapie Cellulaire, F-33000 Bordeaux, France

¹⁵ Chaim Sheba Medical Center, Tel-Hashomer, Tel-Aviv University, Israel; Acute leukemia working party office, Paris

Corresponding author:

Annalisa Paviglianiti, MD
Hopital Saint Antoine
184, rue du Faubourg Saint Antoine,
75012, Paris, France
Tel : +33 1 49 28 26 24
Fax : +33 1 49 28 33 75
annalisa.paviglianiti@gmail.com

Abstract: 198 words

Main text: 2567 words

Number of tables: 2

Number of figures: 4

Supplemental table: 1

Number of references: 30

Keywords: matched unrelated donor, acute myeloid leukemia, Cyclosporine A, CsA, mycophenolate mofetil, MMF

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.

88

89 **Materials and Methods**

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

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

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

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

109 Neutrophil engraftment was defined as the first day of an absolute neutrophil count of
110 $0.5 \times 10^9/L$ lasting for 3 or more consecutive days. OS was defined as time from HSCT to
111 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 $\geq 90\%$.

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).

RI and NRM

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.

Acknowledgments

The authors thank all the EBMT transplant centers that have contributed data in this study.

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

References

1. Powles RL, Clink HM, Spence D, Morgenstern G, Watson JG, Selby PJ, et al. Cyclosporin A to prevent graft-versus-host disease in man after allogeneic bone-marrow transplantation. *Lancet Lond Engl*. 1980 Feb 16;1(8164):327–9.
2. Ruutu T, van Biezen A, Hertenstein B, Henseler A, Garderet L, Passweg J, et al. Prophylaxis and treatment of GVHD after allogeneic haematopoietic SCT: a survey of centre strategies by the European Group for Blood and Marrow Transplantation. *Bone Marrow Transplant*. 2012 Nov;47(11):1459–64.
3. Ram R, Herscovici C, Dahan D, Israeli M, Dreyer J, Peck A, et al. Tailoring the GVHD prophylaxis regimen according to transplantation associated toxicities-Substituting the 3rd dose of methotrexate to mycophenolate mofetil. *Leuk Res*. 2014 Aug;38(8):913–7.
4. Storb R, Deeg HJ, Whitehead J, Appelbaum F, Beatty P, Bensinger W, et al. Methotrexate and cyclosporine compared with cyclosporine alone for prophylaxis of acute graft versus host disease after marrow transplantation for leukemia. *N Engl J Med*. 1986 Mar 20;314(12):729–35.
5. Bolwell B, Sobecks R, Pohlman B, Andresen S, Rybicki L, Kuczkowski E, et al. A prospective randomized trial comparing cyclosporine and short course methotrexate with cyclosporine and mycophenolate mofetil for GVHD prophylaxis in myeloablative allogeneic bone marrow transplantation. *Bone Marrow Transplant*. 2004 Oct;34(7):621–5.
6. Neumann F, Graef T, Tapprich C, Vaupel M, Steidl U, Germing U, et al. Cyclosporine A and mycophenolate mofetil vs cyclosporine A and methotrexate for graft-versus-host disease prophylaxis after stem cell transplantation from HLA-identical siblings. *Bone Marrow Transplant*. 2005 Jun;35(11):1089–93.

- 335 7. Piñana JL, Valcárcel D, Fernández-Avilés F, Martino R, Rovira M, Barba P, et al. MTX or
336 mycophenolate mofetil with CsA as GVHD prophylaxis after reduced-intensity
337 conditioning PBSCT from HLA-identical siblings. *Bone Marrow Transplant*. 2010
338 Sep;45(9):1449–56.
- 339 8. Kiehl MG, Schäfer-Eckart K, Kröger M, Bornhäuser M, Basara N, Blau IW, et al.
340 Mycophenolate mofetil for the prophylaxis of acute graft-versus-host disease in stem
341 cell transplant recipients. *Transplant Proc*. 2002 Nov;34(7):2922–4.
- 342 9. Yerushalmi R, Shem-Tov N, Danylesko I, Shouval R, Nagler A, Shimoni A. The
343 combination of cyclosporine and mycophenolate mofetil is less effective than
344 cyclosporine and methotrexate in the prevention of acute graft-versus host disease
345 after stem-cell transplantation from unrelated donors. *Am J Hematol*. 2017
346 Mar;92(3):259–68.
- 347 10. Chhabra S, Liu Y, Hemmer MT, Costa L, Pidala JA, Couriel DR, et al. Comparative
348 Analysis of Calcineurin Inhibitor-Based Methotrexate and Mycophenolate Mofetil-
349 Containing Regimens for Prevention of Graft-versus-Host Disease after Reduced-
350 Intensity Conditioning Allogeneic Transplantation. *Biol Blood Marrow Transplant J Am*
351 *Soc Blood Marrow Transplant*. 2019 Jan;25(1):73–85.
- 352 11. Kharfan-Dabaja MA, Hamadani M, Sibai H, Savani BN. Managing Hodgkin lymphoma
353 relapsing after autologous hematopoietic cell transplantation: a not-so-good cancer
354 after all! *Bone Marrow Transplant*. 2014 May;49(5):599–606.
- 355 12. Grimwade D, Hills RK, Moorman AV, Walker H, Chatters S, Goldstone AH, et al.
356 Refinement of cytogenetic classification in acute myeloid leukemia: determination of
357 prognostic significance of rare recurring chromosomal abnormalities among 5876
358 younger adult patients treated in the United Kingdom Medical Research Council trials.
359 *Blood*. 2010 Jul 22;116(3):354–65.
- 360 13. Filipovich AH, Weisdorf D, Pavletic S, Socie G, Wingard JR, Lee SJ, et al. National
361 Institutes of Health consensus development project on criteria for clinical trials in
362 chronic graft-versus-host disease: I. Diagnosis and staging working group report. *Biol*
363 *Blood Marrow Transplant J Am Soc Blood Marrow Transplant*. 2005 Dec;11(12):945–
364 56.
- 365 14. Glucksberg H, Storb R, Fefer A, Buckner CD, Neiman PE, Clift RA, et al. Clinical
366 manifestations of graft-versus-host disease in human recipients of marrow from HL-A-
367 matched sibling donors. *Transplantation*. 1974 Oct;18(4):295–304.
- 368 15. Ruggeri A, Labopin M, Ciceri F, Mohty M, Nagler A. Definition of GvHD-free, relapse-
369 free survival for registry-based studies: an ALWP-EBMT analysis on patients with AML
370 in remission. *Bone Marrow Transplant*. 2016 Apr;51(4):610–1.
- 371 16. Andersen PK, Klein JP, Zhang MJ. Testing for centre effects in multi-centre survival
372 studies: a Monte Carlo comparison of fixed and random effects tests. *Stat Med*. 1999
373 Jun 30;18(12):1489–500.

- 374 17. Hougaard P. Frailty models for survival data. *Lifetime Data Anal.* 1995;1(3):255–73.
- 375 18. Mohty M, de Lavallade H, Faucher C, Bilger K, Vey N, Stoppa A-M, et al.
376 Mycophenolate mofetil and cyclosporine for graft-versus-host disease prophylaxis
377 following reduced intensity conditioning allogeneic stem cell transplantation. *Bone*
378 *Marrow Transplant.* 2004 Sep;34(6):527–30.
- 379 19. Brissot E, Chevallier P, Guillaume T, Delaunay J, Ayari S, Dubruille V, et al. Prophylaxis
380 with mycophenolate mofetil and CsA can decrease the incidence of severe acute GVHD
381 after antithymocyte globulin-based reduced-intensity preparative regimen and allo-
382 SCT from HLA-matched unrelated donors. *Bone Marrow Transplant.* 2010
383 Apr;45(4):786–8.
- 384 20. Rodriguez R, Nademanee A, Palmer JM, Parker P, Nakamura R, Snyder D, et al.
385 Thymoglobulin, CYA and mycophenolate mofetil as GVHD prophylaxis for reduced-
386 intensity unrelated donor hematopoietic cell transplantation: beneficial effect seen on
387 chronic GVHD. *Bone Marrow Transplant.* 2010 Jan;45(1):205–7.
- 388 21. Sandmaier BM, Kornblit B, Storer BE, Olesen G, Maris MB, Langston AA, et al. Addition
389 of sirolimus to standard cyclosporine plus mycophenolate mofetil-based graft-versus-
390 host disease prophylaxis for patients after unrelated non-myeloablative haemopoietic
391 stem cell transplantation: a multicentre, randomised, phase 3 trial. *Lancet Haematol.*
392 2019 Aug;6(8):e409–18.
- 393 22. Vogelsang GB, Arai S. Mycophenolate mofetil for the prevention and treatment of
394 graft-versus-host disease following stem cell transplantation: preliminary findings.
395 *Bone Marrow Transplant.* 2001 Jun;27(12):1255–62.
- 396 23. Kröger N, Solano C, Wolschke C, Bandini G, Patriarca F, Pini M, et al. Antilymphocyte
397 Globulin for Prevention of Chronic Graft-versus-Host Disease. *N Engl J Med.* 2016 Jan
398 7;374(1):43–53.
- 399 24. Rubio MT, Labopin M, Blaise D, Socié G, Contreras RR, Chevallier P, et al. The impact of
400 graft-versus-host disease prophylaxis in reduced-intensity conditioning allogeneic stem
401 cell transplant in acute myeloid leukemia: a study from the Acute Leukemia Working
402 Party of the European Group for Blood and Marrow Transplantation. *Haematologica.*
403 2015 May;100(5):683–9.
- 404 25. Schmidt-Hieber M, Labopin M, Beelen D, Volin L, Ehninger G, Finke J, et al. CMV
405 serostatus still has an important prognostic impact in de novo acute leukemia patients
406 after allogeneic stem cell transplantation: a report from the Acute Leukemia Working
407 Party of EBMT. *Blood.* 2013 Nov 7;122(19):3359–64.
- 408 26. Teira P, Battiwalla M, Ramanathan M, Barrett AJ, Ahn KW, Chen M, et al. Early
409 cytomegalovirus reactivation remains associated with increased transplant-related
410 mortality in the current era: a CIBMTR analysis. *Blood.* 2016 19;127(20):2427–38.
- 411 27. Jacobson PA, Huang J, Wu J, Kim M, Logan B, Alousi A, et al. Mycophenolate
412 pharmacokinetics and association with response to acute graft-versus-host disease

- 413 treatment from the Blood and Marrow Transplant Clinical Trials Network. Biol Blood
414 Marrow Transplant J Am Soc Blood Marrow Transplant. 2010 Mar;16(3):421–9.
- 415 28. Nishikawa S, Okamura A, Yamamori M, Minagawa K, Kawamori Y, Kawano Y, et al.
416 Extended mycophenolate mofetil administration beyond day 30 in allogeneic
417 hematopoietic stem cell transplantation as preemptive therapy for severe graft-versus-
418 host disease. Transplant Proc. 2009 Nov;41(9):3873–6.
- 419 29. Barkholt L, Remberger M, Bodegård H, Ringdén O, Böttiger Y. Cyclosporine A (CsA) 2-h
420 concentrations vary between patients without correlation to graft-versus-host disease
421 after allogeneic haematopoietic stem cell transplantation. Bone Marrow Transplant.
422 2007 Oct;40(7):683–9.
- 423 30. Malard F, Szydlo RM, Brissot E, Chevallier P, Guillaume T, Delaunay J, et al. Impact of
424 cyclosporine-A concentration on the incidence of severe acute graft-versus-host
425 disease after allogeneic stem cell transplantation. Biol Blood Marrow Transplant J Am
426 Soc Blood Marrow Transplant. 2010 Jan;16(1):28–34.

427

428

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

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

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

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

438 **Table 1.** Disease, patient and transplant characteristics.

439 **Table 2.** Multivariate analysis.

Table 1. Disease, patient and transplant characteristics

		CsA alone (n=183)	CsA+MMF (n=314)	<i>p-value</i>
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

		Time from transplant (days)				
		number of at-risk patients				
—	CSA alone	183	152	134	122	183
- - -	CSA+MMF	108	314	274	213	108

acute GVHD III-IV

		Time from transplant (days)				
		number of at-risk patients				
—	CSA alone	181	171	160	151	181
- - -	CSA+MMF	136	312	297	271	136

cGVHD

extensive cGVHD

NRM

RI

LFS

OS

GRFS

		Time from transplant (years)			
		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 Intermediate adverse	1		1		1		1		1		1		1	
	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
	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.