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Transplantation and Cellular Therapy

COMPARISON OF THREE STRATEGIES OF GVHD PROPHYLAXIS AFTER T-CELL REPLETE HAPLOIDENTICAL HEMATOPOIETIC TRANSPLANTATION: TACROLIMUS vs. CALCINEURIN INHIBITORS-MMF vs. SIROLIMUS-MMF

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Abstract:	Since the introduction of post-transplant cyclophosphamide (PTCy), haploidentical HSCT (HaploSCT) has become a real alternative for patients who lack other eligible donors. The standard GvHD prophylaxis after PTCy has been a calcineurin inhibitor plus MMF (up to day+35), but promising results with sirolimus (+/- MMF) and single-agent tacrolimus have been recently published. The current multicenter retrospective study compared the outcomes of 372 adult HaploSCT recipients who received TBF conditioning; PTCY and additional GVHD prophylaxis with one of three strategies, cohort-A: single-agent tacrolimus (N 222), cohort-B: CNI-MMF (N 49) and cohort-C: sirolimus-MMF (N 101). No differences in terms of grade II-IV (20%, 25%, and 30%) and III-IV (9%, 6%, and 15%) aGvHD at 100 days were found. However, cohort A had the lowest incidence of overall cGvHD [24%, 47%, and 52%, respectively (p 0.001)] and moderate-severe cGvHD [13%, 35%, and 33%, respectively (p 0.001)]. There were no differences in the 3-year overall survival, progression-free survival, NRM nor

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	relapse among the cohorts. In conclusion, our study suggests that single-agent tacrolimus, CNI+MMF and sirolimus+MMF GvHD prophylaxis lead to similar outcomes following HaploSCT with TBF and PTCy, with low incidence of grade III-IV aGvHD, albeit possible differences in cGVHD require further investigation.

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Monday, July 01 , 2024.

Transplantation and cellular therapy (TCT)

Dear Editor and reviewers,

First of all, I thank you on behalf of myself and co-authors for taking the time to read and propose areas of improvement for the first revised version of our manuscript.

We herein send the new version of our revised manuscript entitled “COMPARISON OF THREE STRATEGIES OF GVHD PROPHYLAXIS AFTER T-CELL REPLETE HAPLOIDENTICAL HEMATOPOIETIC TRANSPLANTATION: TACROLIMUS vs. CALCINEURIN INHIBITORS-MMF vs. SIROLIMUS-MMF” Ref.: Ms. No. TCT-D-24-00265. We have incorporated the suggestions made by the reviewers.

We look forward to hearing from you,

Yours trully,

Albert Esquirol Sanfeliu on behalf of all coauthors

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Answers to the reviewer 2:

- Patients & Methods. I would like to suggest to the Authors to better clarify the statement "as a general rule, the patients were excluded for general status (ECO >/=3), cardiac or lung dysfunction and hepatic or renal failure". It would be useful to clarify the presence of homogeneous selection criteria - which can thus prevent selection bias.

We included a new sentence to clarify the excluded criteria as the reviewer recommended and changed the original sentence "As a general rule, the patients were excluded for general status (ECOG>3), cardiac or lung dysfunction, and hepatic or renal failure" for the new sentence, "As a general rule patients were excluded due to poor general status (ECOG>3), cardiac dysfunction (LVEF < 40%), lung dysfunction (DLCO< 30%), hepatic failure (based on total bilirubin> x2 or AST/ALT > x5 the ULN) and renal dysfunction (creatinine > x1.5 the ULN). (pg. 4, line 110-113)

- Patients & Methods. The Authors declare "three different strategies of pharmacological GvHD prophylaxis started all at day +5, used according to the different center's rules". Please, could you specify if every single center (5 centers are listed) were using only one of the three regimen or whether each centre was free to choose one of the three prophylaxis options (if so, according to which criteria was each patient assigned to each cohort)?

In the first step, we designed a haploidentical clinical protocol based on TBF conditioning as per the Genova group published in 2013 (BBMT 19 (2013) 117-122). However, a few changes were included such as a peripheral blood source, preferably tacrolimus after PT-Cy as the only GvHD prophylaxis. As a consequence of the clinical protocol and non-clinical trial, all centers used TBF, but the GvHD prophylaxis was selected by each center. In the end, only two centers used tacrolimus as the only GvHD prophylaxis; one center started the haploidentical platform with tacrolimus alone but added MMF in several patients. The other centers used tacrolimus or tacrolimus-MMF in the first years but subsequently changed to sirolimus-MMF in all their patients.

- Statistic. Did you consider the "center effect"? Has the possibility of conducting a match-pair analysis between the three cohorts been considered?

This is an extremely good appreciation. However, because the type of GVHD prophylaxis was strongly linked to the variable center, we were unable to separate any potential "center effect" from the GVHD prophylaxis used.

- Discussion. It might be useful in the discussion to add a reference to the American experience recently presented by Elmariah and colleagues who consider - based on Lee Moffitt's phase II study - the retrospective comparison of Siroli and Tacro in PBSC / HAPLO / PTCy. I understand that the publication of this paper coincided with the closing of the work presented by Esquirol and colleagues.

Elmariah, H., Otoukesh, S., Kumar, A., Ali, H., Arslan, S., Shouse, G., Pourhassan, H., Nishihori, T., Faramand, R., Mishra, A., Khimani, F., Fernandez, H., Lazaryan, A., Nieder, M., Perez, L., Liu, H.,

Nakamura, R., Pidala, J., Marcucci, G., Forman, S. J., … Al Malki, M. M. (2024). Sirolimus Is an Acceptable Alternative to Tacrolimus for Graft-versus-Host Disease Prophylaxis after Haploidentical Peripheral Blood Stem Cell Transplantation with Post-Transplantation Cyclophosphamide. Transplantation and cellular therapy, 30(2), 229.e1-229.e11. https://urldefense.com/v3/__https://doi.org/10.1016/j.jtct.2023.11.010__;!!D9dNQwwGXtA!Xq68aPuwcMEWyiZiKpQ3k7eN0yYw750WfraG0tIlR2juGTHtm8H7yGnWTH2s1K_h2knGGrukbiig_MpxPN6D$

*This is a very interesting appreciation, and as the reviewer suggested we have included a new paragraph in the discussion: "A recent large retrospective study with 423 patients, including 84 (20%) patients with Siro-MMF and 339 (80%) patients with Tacro-MMF as GvHD prophylaxis after PTCy in the haploidentical setting has been published, and the results are similar to those of our study with respect to aGVHD and main transplantation outcomes. Thus, there were no differences in terms of grade II-IV aGvHD (45% vs. 47%), OS and DFS (63% and 64% and 47% vs 61%, respectively), NRM (27% vs 22%) and relapse (23% vs. 25%); however, unlike in our study, these authors found no differences in cGvHD at two years with Siro-MMF vs. Tacro-MMF (47% vs. 54%). As previously published, siro-MMF was associated with SOS, and Tacro-MMF had more incidence of thrombotic microangiopathy, nephrotoxicity, and neurotoxicity, which are well known toxicities which differ with sirolimus and tacromilus. [41] **(pg 12, line 381-391)***

Highlights (for review)
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COMPARISON OF THREE STRATEGIES OF GVHD PROPHYLAXIS AFTER T-CELL REPLETE HAPLOIDENTICAL HEMATOPOIETIC TRANSPLANTATION: TACROLIMUS vs. CALCINEURIN INHIBITORS-MMF vs. SIROLIMUS-MMF

Esquirol A[1], Pascual MJ[2], Montoro J[3], Piñana JL[4], Ferrà C[5], Herruzo B[2], Garcia-Cadenas I[1], Balaguer A[3], Perez A[4], M. Huguet[5], Redondo S[1], Villalba M[3], Hernandez-Boluda JC[4], Chorao P[3], Hernani R[4], Sanz J [3], Solano C[4], Sierra J[1], Martino R[1]

Running title: Different graft versus host disease prophylaxis cohorts with the same conditioning regimen in the haploidentical stem cell transplantation setting.

Key points: haploidentical stem cell transplantation, TBF conditioning, haploidentical GvHD prophylaxis, tacrolimus vs sirolimus.

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Word counts: Abstract: 198 **Text: 3423**

ABSTRACT

Since the introduction of post-transplant cyclophosphamide (PTCy), haploidentical HSCT (HaploSCT) has become a real alternative for patients who lack other eligible donors. The standard GvHD prophylaxis after PTCy has been a calcineurin inhibitor plus MMF (up to day+35), but promising results with sirolimus (+/- MMF) and single-agent tacrolimus have been recently published. The current multicenter retrospective study compared the outcomes of 372 adult HaploSCT recipients who received TBF conditioning; PTCY and additional GVHD prophylaxis with one of three strategies, cohort-A: single-agent tacrolimus (N 222), cohort-B: CNI-MMF (N 49) and cohort-C: sirolimus-MMF (N 101). No differences in terms of grade II-IV (20%, 25%, and 30%) and III-IV (9%, 6%, and 15%) aGvHD at 100 days were found. However, cohort A had the lowest incidence of overall cGvHD [24%, 47%, and 52%, respectively (p 0.001)] and moderate-severe cGvHD [13%, 35%, and 33%, respectively (p 0.001)]. There were no differences in the 3-year overall survival, progression-free survival, NRM nor relapse among the cohorts. In conclusion, our study suggests that single-agent tacrolimus, CNI+MMF and sirolimus+MMF GvHD prophylaxis lead to similar outcomes following HaploSCT with TBF and PTCy, with low incidence of grade III-IV aGvHD, albeit possible differences in cGVHD require further investigation.

INTRODUCTION

Haploidentical hematopoietic stem cell transplantation (haploSCT) with post-transplantation cyclophosphamide (PTCy) permitted crossing the HLA barrier [1,2] and became a real alternative for patients without HLA identical sibling or eligible unrelated donors [3-7]. Since the first experience of haploSCT based on T-cell repleted with PTCy [2], the number of haploSCT has progressively increased in the European registry [8].

PTCy-based graft versus host disease (GvHD) prophylaxis was introduced in haploSCT [2,9-14] and later also was used in related or unrelated donor HSCT [15-19], different timing of PTCy administration was investigated to improve the final outcomes [20,21]. The combination of a calcineurin inhibitors (CNI) plus MMF (up to day +35) was the initial standard pharmacological prophylaxis for GVHD after PTC in the bone marrow haploSCT setting. In an effort to reduce the side effects of CNI, the combination of sirolimus and MMF (siro-MMF) emerged as a CNI-free regimen in the haploSCT setting. Thus, a phase II study found a low incidence of grade II-IV acute GvHD (18.8%), moderate and severe chronic GvHD (18.8%) (cGvHD) and a high (72%) overall survival (OS) with this strategy [22]. Previously, several studies had reported the results using sirolimus as GvHD prophylaxis after SCT from other donors, usually in combination with tacrolimus and prior to the introduction of PTCy [23-29], and using it without tacrolimus (or another CNI) following the introduction of PTCy seemed a logical step in designing a CNI-free GVHD prophylaxis platform [30].

In the current study, we retrospectively analyzed the incidence of acute and chronic graft versus host disease (GvHD) and survival outcomes among three cohorts of GvHD prophylaxis (CNI alone, CNI-MMF, or sirolimus-MMF) started after PTCy in a large series of patients with the same conditioning regimen based on thiotepa busulphan and fludarabine (TBF).

PATIENTS AND METHODS

Patients

This is a retrospective study of 372 adult patients transplanted by haploidentical donor in the five Spanish university centers, between June 2013 and October 2022 using TBF conditioning. All patients gave written consent. As a general rule patients were excluded due to poor general status (ECOG>3), cardiac dysfunction (LVEF < 40%), lung dysfunction (DLCO< 30%), hepatic failure (based on total bilirubin> x2 or AST/ALT > x5 the ULN) and renal dysfunction (creatinine > x1.5 the ULN).

Conditioning regimen and Graft versus host disease prophylaxis

The conditioning regimen was homogeneous and included thiotepa (5 mg/kg IV) on days –7 and –6 (total dose 10 mg/kg), fludarabine (50 mg/m2 IV) on days –5, –4 and –3 (total dose 150 mg/m2) and busulfan (1 mg/kg/6 h on days –5, –4 and –3 or the equivalent IV dose given as a single administration of 3.2 mg/kg on the same days and immediately after fludarabine). In patients over than 55 years or with contraindications for a myeloablative regimen, the busulphan administration was reduced to two days and thiotepa dose was reduced from 10 to 5 mg/kg [14].

GvHD prophylaxis consists of PTCy on days +3 and +4 (50 mg/kg/day, total doses 100 mg/kg), and one of three different strategies of pharmacological GvHD prophylaxis started all at day +5, used according to the different centers' rules:

*Cohort A) single-agent tacrolimus (0.02 mg/kg as continuous iv) [14], adjusting the dose for target blood levels of 5-10 ug/L, and progressive discontinuation after 120 days until 180 days,

*Cohort B) calcineurin inhibitors (tacrolimus or cyclosporine A) with MMF at 1 g/8 h (orally or intravenously) until day+35. Forty-nine per cent of patients in this cohort received cyclosporine A with MMF (N 24),

*Cohort C) sirolimus 6 mg in the first day, and 2 mg daily onwards, with dose modification to achieve the targeted plasma levels between 8 and 16 ng/mL, progressive discontinuation after day +100 until +180, and MMF at 1 g/8 h until day +35 [30].

Supportive care and definitions

Details of the supportive care and definitions used in the study can be found in the supplemental materials (sup. mat.)

Statistical methods

SPSS statistics (IBM SPSS Statistics 21, New York, NY, USA) and R studio programs (R studio, Boston, MA, USA) were used for statistical analyses. OS was defined as the time from day 0 (stem cell infusion) to the date of death by any cause and PFS was the time from day 0 to disease progression or death. The Kaplan-Meier method was used to estimate the actuarial PFS and OS, and the log-rank test was used to study the univariate impact of any given variable on OS and PFS. The cumulative incidence (CumInc) estimate with competing risk(s) analysis was used to calculate the incidence of acute and chronic GvHD, NRM, relapse and engraftment. The competing risk for NRM was relapse, while for relapse it was NRM. Competing risks for acute and chronic GvHD were disease relapse and NRM up to 100 days after stem cell infusion for acute GvHD and until the last follow-up for chronic GvHD and competing risk for engraftment was dead without engraftment before 30 days or primary graft failure. Cox regression and Gray test were used for survival and cumulative incidence outcomes multivariate analysis; the variables introduced in these analyses were those with a P value $\leqslant 0.2$ in univariate analysis; independently of the p-value, the GvHD prophylaxis variable was included in all multivariate analysis. The variables tested in the univariate analyses were GvHD prophylaxis, two or three days of busulphan, previous alloSCT, lymphoid or myeloid underlying disease, HCT-CI-age [31], EBMT score [32], DRI [33], sex of the patient and donor, patient and donor CMV serology, donor age < 35 years. The patient's age and response at SCT were not included as independent variable since they are included in the HCT-CI-age, EBMT-score, and DRI. Chi-square test was used to compared categorical clinical variables differences between GvHD prophylaxis groups, while the ANOVA test was used to analyze differences in continuous variables. HCT-CI-age >3 scores, DRI (low-intermediate or high-very high), and EBMT-risk-score >4 were selected due to their strong impact on post-transplant outcomes, including the haploSCT setting. [34]

RESULTS

Patient and donors

Patient characteristics and differences between groups are shown in Table 1. The median time of follow-up in survivors is 34 months (range 1-123) for the whole cohort. Two hundred twenty-two patients (60%) received only tacrolimus (cohort A); calcineurin inhibitors and MMF (Tacro/Csa-MMF, cohort B) were used in 49 patients (13%) and 101 (27%) were treated with sirolimus-MMF (Siro-MMF, cohort C) in addition to PTCy.

In the overall series, one hundred fifty-five (42%) patients had acute myeloid leukemia, and 75 patients (20%) had myelodysplastic syndrome as the underlying disease. Peripheral blood as a source of stem cells was used in 69% (N 153) in group A, 2% (N 1) in group B, and 17% (N 17) in group C (p 0.001). Other noted differences between groups A and B were age over 55 years (51% vs 35%, p 0.06) and EBMT risk score > 4 (64% vs 47%, p 0.04). On the other hand, the major difference between groups A and C was having a HCT-CI-age > 3 (39% vs 69%, p 0.001) (Table 1).

Engraftment, toxicity and early mortality

Eleven patients (3%) presented primary graft failure (five patients in group A and 3 patients in each other group (p 0.3). The overall cumulative incidence (CumInc) of neutrophils recovery (>0.5x109/L) was 91% at 30 days without differences between the three groups (90% group A, 94% group B, and 92% group C, p 0.26). The median time of recovery of neutrophils was 20 days (range 11-48), 20 days (range 13-33), and 22 (range 13-41) for groups A, B, and C (p 0.13), respectively.

The overall CumInc of platelets count (>20x109/L) was 82% at 100 days, the median time to platelets recovery was 36 days (range 10-389) for group A, 37 days (range 13-173) for group B, and 38 days (range 12-263) for group C (p 0.6).

Seventeen patients died before 30 days of follow-up, the causes of early mortality were infections 8 patients, primary graft failure and acute refractory GvHD (one patient each), sinusoidal obstructive syndrome (two patients), refractory bleeding (two patients) and cardiac toxicity, sudden death, and multiorgan failure (one patient each).

For comparing the incidence of hemorrhagic cystitis (HC), thrombotic microangiopathy (TMA), sinusoidal obstructive syndrome (SOS), and CMV reactivation groups A and B were combined, the results are shown in Table 2. As shown, group C had a higher incidence of HC (p 0.001) and SOS (p 0.002).

Acute and chronic graft versus host disease

The overall CumInc of acute grade II-IV and III-IV GvHD were 23% and 10% at 100 days. No differences were found among groups in the incidence of grade II-IV acute GvHD, 20% (95% CI; 15% to 25%), 25% (95% CI; 12% to 37%), and 30% (95% CI; 21% to 39%) for group A, B and C, respectively (p 0.08), nor were their differences in grade III-IV acute GvHD; 9% (95% CI; 5% to 13%), 6% (95% CI; 0% to 13%) and 15% (95% CI; 8% to 22%) for group A, B and C, respectively (p 0.12). (Figure 1). The use of peripheral blood vs bone marrow increased the incidence of grade II-IV aGVHD in univariate analysis, with no other variable impacting this complication as shown in table 3.

The CumInc of overall and moderate-severe chronic GvHD were 33% and 20% at 34 months, respectively. The GvHD prophylaxis was the only variable with impact in the univariate analysis for both (Table 3). Group A: 24% (95% CI; 18% to 30%), B: 47% (95% CI; 29% to 64%) and C: 52% (95% CI; 41% to 62%) (p 0.001) for overall cGvHD and 13% (95% CI; 8% to 17%), 35% (95% CI; 17% to 53%) and 33% (95% CI; 22% to 44%) (p 0.001) for moderate and extensive cGvHD (Figure 2), respectively. Since no other variable showed impact by univariate analysis, a multivariate analysis of cGVHD could not be done, as shown in table 3.

To rule out any potential impact of bone marrow as stem cell source, a sub-analysis including only recipients of peripheral blood stem cell (PBSC) as stem cell source (N 285) was done (details in sup. mat. Table1). In brief, results are similar to the overall patient population. Thus, the overall incidence of grade II-IV and III-IV acute GVHD was 26% and 12%, respectively, and the incidence of overall and moderate-severe chronic GvHD was 34% and 22%. Results of the univariate and multivariate analyses of risk factors for cGVHD did not differ from those of the entire study population (sup. mat. Table 2 and 3).

Non-relapse mortality and relapse

The CumInc of non-relapse mortality at 34 months was 28%, without differences between three groups of GvHD prophylaxis, 30% (95% IC; 23-36) group A, 26% (95% IC; 13-39) group B and 26% (95% IC; 17-35) group C (p 0.68). The variables with an impact in the univariate analyses are shown in Table 4. In the multivariate analyses, HCT-CI-age < 2 and EBMT score < 3 had a positive statistical impact, with a trend for GvHD prophylaxis (group A, p 0.07) and CMV seropositive recipient (p 0.07).

The overall CumInc of relapse at 34 months was 20%, without differences between prophylaxis group, 20% (95% IC; 15-26) group A, 25% (95% IC; 11-39) group B and 16% (95% IC; 9-29) group C (p 0.55); The multivariate analyses showed DRI and prior alloHSCT as the only variables with an impact on relapse (Table 4).

One hundred sixty-one patients died at the end of follow-up (43%). Disease relapse was the cause of death in 34% of patients (N 55). The main causes of NRM were infections (N 54, 52%) and refractory acute GvHD (N 20, 19%). The causes of death and distribution are shown in Table 5. Infections were the most common causes of NRM in all prophylaxis groups: 58% vs 64% vs 31% for groups A, B, and C (p 0.04) respectively; while 42% of NRM in group C were assigned to acute GvHD, in contrast to 12% and 9% for group A and B (p 0.003), respectively.

Overall survival and progression-free survival

The overall and progression-free survival were 57% and 52% at 34 months, without differences between GvHD prophylaxis groups; A: 54% (95% IC; 51-57), B: 56% (95% IC; 48-64) and C: 63% (95% IC; 58-68) (p 0.4) for OS neither for PFS, group A: 51% (95% IC; 47-53), group B: 49% (95% IC; 41-57) and group C: 58% (95% IC; 53-63) (p 0.48).

In the univariate analysis, variables with positive impact on both OS and PFS were DRI (low-intermediate), low HCT-CI-age score (<2), low EBMT score (<3), and no prior alloSCT (Table 4). Multivariate analysis showed that all three pre-transplant scores (low and intermediate DRI, < 2 HCT-CI-age, and < 3 EBMT score) had a

statistically significant impact on OS and PFS, without differences between GvHD prophylaxis groups (Table 4).

Sub-analysis comparing PBSC recipients who received either single-agent tacrolimus or sirolimus-MMF as GvHD

For this sub-analysis, recipients of the two major GVHD prophylaxis strategies (tacrolimus alone versus sirolimus-MMF) who received PBSC as the stem cell source were selected and their outcomes compared. Two hundred thirty-seven patients were included in the sub-analysis, and their clinical differences are shown in the sup. mat. table 4.

The CumInc of acute grade II-IV and III-IV GvHD were 25% and 12% at 100 days, respectively. The only variable with impact in the multivariate analysis for acute grade II-IV GvHD was the GvHD prophylaxis (tacrolimus vs. sirolimus-MMF), as shown in table 6. The CumInc of overall chronic and moderate-extensive GvHD was 31% and 19% at 34 months, respectively. Likewise, only GvHD prophylaxis had a statistical impact in the multivariate analysis of moderate-extensive GvHD (sup. mat. table 5). Multivariate analyses for acute grade III-IV and overall chronic GvHD were not done since there were no variables with an impact in the univariate analyses at a $p<0.2$.

The CumInc of NRM and relapse were 29% and 20%, in line with the results in the overall study cohort. Again, variables with impact in the multivariate analysis for NRM were HCT-CI-age and EBMT score, while DRI and previous alloSCT had an independent impact on relapse. There was no impact on either outcome of GVHD prophylaxis used (tacrolimus alone vs siro-MMF), as shown in (sup. mat. table 5)

This variable also had no impact on OS and PFS, which were 54% and 50%, respectively, at 34 months for both GVHD strategies combined. Variables with an impact of OS and PFS in multivariate analyses were DRI, HCT-CI-age and EBMT score (sup. mat. table 5).

DISCUSSION

HaploSCT with PTCy has become a real alternative for patients who need an SCT and lack other eligible donors; even more, this transplant modality is considered as the first-choice at some institutions in patients highly likely to have a single-allele mismatched unrelated donors [35].

The conditioning regimen based on TBF in the haploSCT has been studied by different groups [14,36-38], and the outcomes in our cohorts appear in line with previous studies. The observed 61% OS (67% to 56%) and 53% PFS (58% to 51%); 21% NRM (28% to 18%) and 20% of relapse (22% to 16%) are within expected ranges, as is the CumInc of grade III-IV aGvHD [10% (14% to 4%)] and overall cGvHD [25% (29% to 22%)].

Calcineurin inhibitors (CNI) and MMF are considered as a standard of care in GvHD prophylaxis after PTCy haploSCT. A large heterogeneous registry study showed an OS of nearly 55% in the haploSCT with tacro-MMF after PTCy; 42% of CumInc of grade II-IV aGvHD at 6-months and 41% chronic GvHD at 2 years of follow-up [39].

There has been growing interest, however, in using CNI-free GVHD prophylactic regimens, especially since the introduction of PTCy. In a recent phase 2 trial, the results with sirolimus and MMF after PTCy as GvHD prophylaxis in haploidentical peripheral blood stem cell transplantation showed 20% and 9.4% CumInc of grade II-IV and III-IV aGvHD at 100 days, respectively and nearly 19% of moderate-severe cGvHD at one year. The survival outcomes (OS and PFS), NRM, and relapse were 71.7%, 59%, 18.8%, and 22% at one year of follow-up, respectively [22]. Previously, encouraging results using myeloablative conditioning followed by PT-Cy and sirolimus-MMF in haploSCT platform had been published, with low incidence of grade II-IV and III-IV aGvHD (15% and 7.5% respectively), and 20% of moderate-severe cGvHD, and the authors postulated a synergistic effect between PTCy and sirolimus on immune reconstitution [40].

The colleagues from Valencia had previously reported the results of sirolimus and MMF following PTCy as GvHD prophylaxis including transplants from HLA-identical siblings (N 64), unrelated (N 52), or haploidentical donors (N 42), with an overall low incidence of grade II-IV (27%) and III-IV (9%) aGvHD, and a 38%

incidence of moderate severe chronic GvHD. With this platform the 1-year incidence of relapse and NRM were low at 12% and 14%, respectively, and the OS and PFS were very promising (82% and 75%, respectively) [30]. Studying only recipients of HaploSCT, a similar rate of grade III-IV aGvHD was found when comparing GVHD prophylaxis with CsA-MMF and Siro-MMF (CsA-MMF: 12% vs siro-MMF: 12%, p 0.79) [41]. In addition, in this previous report the incidence of overall and moderate-severe cGvHD in CsA-MMF and Siro-MMF [41] recipients were similar to the current study results, since the CumInc of overall cGvHD was 47% and 52%, respectively, and the incidence of moderate-severe cGvHD was 35% and 33%, respectively.

In the current study, similar incidence of grade II-IV (20%, 25%, and 30%) and III-IV (9%, 6%, and 15%) acute GvHD at 100 days occurred in the three GvHD cohorts [A (tacrolimus), B (CNI-MMF) and C (sirolimus-MMF), respectively]. We found no differences in the incidence of acute and chronic GvHD by the stem cell source (bone marrow or PBSC), and the results of restricting the analysis to only those receiving PBSC were similar to the overall analyses including both stem cell sources. However, we acknowledge that bone marrow as stem cell source is a well-known protective variable for the development of GVHD, especially cGVHD, and thus our study results are applicable mainly to recipients of PBSC haploidentical transplantation.

A sub-analysis including only recipients of PBSC as stem cell source who received either single-agent tacrolimus or siro-MMF after PTCy was performed to exclude potential biases due to the heterogeneity of the overall study cohort and compare outcomes between the two main GVHD prophylaxis studied [17]. Results of this sub-analysis showed similar results as the overall study cohort, with no differences in survival, NRM and relapse between these two groups. Both acute and chronic GVHD, however, were lower in the tacrolimus compared to the siro-MMF group, as shown in Table 6.

Unlike the 13% of primary graft failure (PGF) reported in the first haploidentical approach [2], only three percent of both tacrolimus and sirolimus groups had a PGF, without differences in the incidence of engraftment. A high incidence of SOS was found in the siro-MMF group (13% vs. 4%), confirming prior findings [30]. On the other hand, the higher incidence of hemorrhagic cystitis in Siro-MMF recipients has

not been previously published. Due to the retrospective nature of the study, these differences may be partly due to center-specific diagnostics and data management differences and may not represent true differences between GVHD prophylactic strategies.

A recent large retrospective study with 423 patients, including 84 (20%) patients with Siro-MMF and 339 (80%) patients with Tacro-MMF as GvHD prophylaxis after PTCy in the haploidentical setting has been published, and the results are similar to those of our study with respect to aGVHD and main transplantation outcomes. Thus, there were no differences in terms of grade II-IV aGvHD (45% vs. 47%), OS and DFS (63% and 64% and 47% vs 61%, respectively), NRM (27% vs 22%) and relapse (23% vs. 25%); however, unlike in our study, these authors found no differences in cGvHD at two years with Siro-MMF vs. Tacro-MMF (47% vs. 54%). As previously published, siro-MMF was associated with SOS, and Tacro-MMF had more incidence of thrombotic microangiopathy, nephrotoxicity, and neurotoxicity, which are well known toxicities which differ with sirolimus and tacrolimus. [42]

In conclusion, our results show that the survival appears to be essentially the same when using any of the three studied GVHD prophylaxis regimens with TBF, PTCy and HaploSCT, thus supporting using sirolimus for GVHD prophylaxis in patients with a (relative) contraindication to receive a CNI. As expected, results are especially good in patients with early phase of their underlying disease and a limited number of comorbidities. Although the incidence of acute GVHD was similar between groups, the observed lower incidence of chronic GVHD in the single-agent tacrolimus cohort merits further investigation.

AUTHORS' CONTRIBUTIONS

A.E. designed the study, performed research, analyzed data, and wrote the manuscript; R.M. and J.S. designed the study and performed research; M.P, J.M., J.L.P., C.F., B.H., I.G.C., A.B., A.P., M.H., S.R., M.V., J.C.H.B, P.C., R.H., J.S., C.S. contributed essential data and revised the manuscript; and all authors approved the final version of

the manuscript. R.M. and J.S. contributed equally to this work and should be considered co-first authors.

COMPLIANCE WITH ETHICAL STANDARDS

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Table 1. Clinical differences according to different types of GvHD prophylaxis

Table 2. Engraftment, complications, and early deaths

Table 3: Grade II-IV, III-IV aGvHD and overall, moderate-severe cGvHD Univariate and multivariate analyses

Table 4: Survival outcomes, NRM, and relapse univariate and multivariate analysis

Table 5: Causes of non-relapse mortality

Table 6: Clinical differences according tacro vs sirolimus-MMF as GvHD prophylaxis

Figure 1: Acute GvHD (100 days)

Figure 2: Overall and Moderate-extensive Chronic GvHD (34 months)

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COMPARISON OF THREE STRATEGIES OF GVHD PROPHYLAXIS AFTER T-CELL REPLETE HAPLOIDENTICAL HEMATOPOIETIC TRANSPLANTATION: TACROLIMUS vs. CALCINEURIN INHIBITORS-MMF vs. SIROLIMUS-MMF

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Running title: Different graft versus host disease prophylaxis cohorts with the same conditioning regimen in the haploidentical stem cell transplantation setting.

Key points: haploidentical stem cell transplantation, TBF conditioning, haploidentical GvHD prophylaxis, tacrolimus vs sirolimus.

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ABSTRACT

Since the introduction of post-transplant cyclophosphamide (PTCy), haploidentical HSCT (HaploSCT) has become a real alternative for patients who lack other eligible donors. The standard GvHD prophylaxis after PTCy has been a calcineurin inhibitor plus MMF (up to day+35), but promising results with sirolimus (+/- MMF) and single-agent tacrolimus have been recently published. The current multicenter retrospective study compared the outcomes of 372 adult HaploSCT recipients who received TBF conditioning; PTCY and additional GVHD prophylaxis with one of three strategies, cohort-A: single-agent tacrolimus (N 222), cohort-B: CNI-MMF (N 49) and cohort-C: sirolimus-MMF (N 101). No differences in terms of grade II-IV (20%, 25%, and 30%) and III-IV (9%, 6%, and 15%) aGvHD at 100 days were found. However, cohort A had the lowest incidence of overall cGvHD [24%, 47%, and 52%, respectively (p 0.001)] and moderate-severe cGvHD [13%, 35%, and 33%, respectively (p 0.001)]. There were no differences in the 3-year overall survival, progression-free survival, NRM nor relapse among the cohorts. In conclusion, our study suggests that single-agent tacrolimus, CNI+MMF and sirolimus+MMF GvHD prophylaxis lead to similar outcomes following HaploSCT with TBF and PTCy, with low incidence of grade III-IV aGvHD, albeit possible differences in cGVHD require further investigation.

INTRODUCTION

Haploidentical hematopoietic stem cell transplantation (haploSCT) with post-transplantation cyclophosphamide (PTCy) permitted crossing the HLA barrier [1,2] and became a real alternative for patients without HLA identical sibling or eligible unrelated donors [3-7]. Since the first experience of haploSCT based on T-cell repleted with PTCy [2], the number of haploSCT has progressively increased in the European registry [8].

PTCy-based graft versus host disease (GvHD) prophylaxis was introduced in haploSCT [2,9-14] and later also was used in related or unrelated donor HSCT [15-19], different timing of PTCy administration was investigated to improve the final outcomes [20,21]. The combination of a calcineurin inhibitors (CNI) plus MMF (up to day +35) was the initial standard pharmacological prophylaxis for GVHD after PTC in the bone marrow haploSCT setting. In an effort to reduce the side effects of CNI, the combination of sirolimus and MMF (siro-MMF) emerged as a CNI-free regimen in the haploSCT setting. Thus, a phase II study found a low incidence of grade II-IV acute GvHD (18.8%), moderate and severe chronic GvHD (18.8%) (cGvHD) and a high (72%) overall survival (OS) with this strategy [22]. Previously, several studies had reported the results using sirolimus as GvHD prophylaxis after SCT from other donors, usually in combination with tacrolimus and prior to the introduction of PTCy [23-29], and using it without tacrolimus (or another CNI) following the introduction of PTCy seemed a logical step in designing a CNI-free GVHD prophylaxis platform [30].

In the current study, we retrospectively analyzed the incidence of acute and chronic graft versus host disease (GvHD) and survival outcomes among three cohorts of GvHD prophylaxis (CNI alone, CNI-MMF, or sirolimus-MMF) started after PTCy in a large series of patients with the same conditioning regimen based on thiotepa busulphan and fludarabine (TBF).

PATIENTS AND METHODS

Patients

This is a retrospective study of 372 adult patients transplanted by haploidentical donor in the five Spanish university centers, between June 2013 and October 2022 using TBF conditioning. All patients gave written consent. As a general rule, the patients were excluded for general status (ECOG≥3), cardiac or lung dysfunction, and hepatic or renal failure.

Conditioning regimen and Graft versus host disease prophylaxis

The conditioning regimen was homogeneous and included thiotepa (5 mg/kg IV) on days –7 and –6 (total dose 10 mg/kg), fludarabine (50 mg/m2 IV) on days –5, –4 and –3 (total dose 150 mg/m2) and busulfan (1 mg/kg/6 h on days –5, –4 and –3 or the equivalent IV dose given as a single administration of 3.2 mg/kg on the same days and immediately after fludarabine). In patients over than 55 years or with contraindications for a myeloablative regimen, the busulphan administration was reduced to two days and thiotepa dose was reduced from 10 to 5 mg/kg [14].

GvHD prophylaxis consists of PTCy on days +3 and +4 (50 mg/kg/day, total doses 100 mg/kg), and one of three different strategies of pharmacological GvHD prophylaxis started all at day +5, used according to the different centers' rules:

*Cohort A) single-agent tacrolimus (0.02 mg/kg as continuous iv) [14], adjusting the dose for target blood levels of 5-10 ug/L, and progressive discontinuation after 120 days until 180 days,

*Cohort B) calcineurin inhibitors (tacrolimus or cyclosporine A) with MMF at 1 g/8 h (orally or intravenously) until day+35. Forty-nine per cent of patients in this cohort received cyclosporine A with MMF (N 24),

*Cohort C) sirolimus 6 mg in the first day, and 2 mg daily onwards, with dose modification to achieve the targeted plasma levels between 8 and 16 ng/mL, progressive discontinuation after day +100 until +180, and MMF at 1 g/8 h until day +35 [30].

Supportive care and definitions

Details of the supportive care and definitions used in the study can be found in the supplemental materials (sup. mat.)

Statistical methods

SPSS statistics (IBM SPSS Statistics 21, New York, NY, USA) and R studio programs (R studio, Boston, MA, USA) were used for statistical analyses. OS was defined as the time from day 0 (stem cell infusion) to the date of death by any cause and PFS was the time from day 0 to disease progression or death. The Kaplan-Meier method was used to estimate the actuarial PFS and OS, and the log-rank test was used to study the univariate impact of any given variable on OS and PFS. The cumulative incidence (CumInc) estimate with competing risk(s) analysis was used to calculate the incidence of acute and chronic GvHD, NRM, relapse and engraftment. The competing risk for NRM was relapse, while for relapse it was NRM. Competing risks for acute and chronic GvHD were disease relapse and NRM up to 100 days after stem cell infusion for acute GvHD and until the last follow-up for chronic GvHD and competing risk for engraftment was dead without engraftment before 30 days or primary graft failure. Cox regression and Gray test were used for survival and cumulative incidence outcomes multivariate analysis; the variables introduced in these analyses were those with a P value $\leqslant 0.2$ in univariate analysis; independently of the p-value, the GvHD prophylaxis variable was included in all multivariate analysis. The variables tested in the univariate analyses were GvHD prophylaxis, two or three days of busulphan, previous alloSCT, lymphoid or myeloid underlying disease, HCT-CI-age [31], EBMT score [32], DRI [33], sex of the patient and donor, patient and donor CMV serology, donor age < 35 years. The patient's age and response at SCT were not included as independent variable since they are included in the HCT-CI-age, EBMT-score, and DRI. Chi-square test was used to compared categorical clinical variables differences between GvHD prophylaxis groups, while the ANOVA test was used to analyze differences in continuous variables. HCT-CI-age >3 scores, DRI (low-intermediate or high-very high), and EBMT-risk-score >4 were selected due to their strong impact on post-transplant outcomes, including the haploSCT setting. [34]

RESULTS

Patient and donors

Patient characteristics and differences between groups are shown in Table 1. The median time of follow-up in survivors is 34 months (range 1-123) for the whole cohort. Two hundred twenty-two patients (60%) received only tacrolimus (cohort A); calcineurin inhibitors and MMF (Tacro/Csa-MMF, cohort B) were used in 49 patients (13%) and 101 (27%) were treated with sirolimus-MMF (Siro-MMF, cohort C) in addition to PTCy.

In the overall series, one hundred fifty-five (42%) patients had acute myeloid leukemia, and 75 patients (20%) had myelodysplastic syndrome as the underlying disease. Peripheral blood as a source of stem cells was used in 69% (N 153) in group A, 2% (N 1) in group B, and 17% (N 17) in group C (p 0.001). Other noted differences between groups A and B were age over 55 years (51% vs 35%, p 0.06) and EBMT risk score > 4 (64% vs 47%, p 0.04). On the other hand, the major difference between groups A and C was having a HCT-CI-age > 3 (39% vs 69%, p 0.001) (Table 1).

Engraftment, toxicity and early mortality

Eleven patients (3%) presented primary graft failure (five patients in group A and 3 patients in each other group (p 0.3). The overall cumulative incidence (CumInc) of neutrophils recovery (>0.5x109/L) was 91% at 30 days without differences between the three groups (90% group A, 94% group B, and 92% group C, p 0.26). The median time of recovery of neutrophils was 20 days (range 11-48), 20 days (range 13-33), and 22 (range 13-41) for groups A, B, and C (p 0.13), respectively.

The overall CumInc of platelets count (>20x109/L) was 82% at 100 days, the median time to platelets recovery was 36 days (range 10-389) for group A, 37 days (range 13-173) for group B, and 38 days (range 12-263) for group C (p 0.6).

Seventeen patients died before 30 days of follow-up, the causes of early mortality were infections 8 patients, primary graft failure and acute refractory GvHD (one patient each), sinusoidal obstructive syndrome (two patients), refractory bleeding (two patients) and cardiac toxicity, sudden death, and multiorgan failure (one patient each).

For comparing the incidence of hemorrhagic cystitis (HC), thrombotic microangiopathy (TMA), sinusoidal obstructive syndrome (SOS), and CMV reactivation groups A and B were combined, the results are shown in Table 2. As shown, group C had a higher incidence of HC (p 0.001) and SOS (p 0.002).

Acute and chronic graft versus host disease

The overall CumInc of acute grade II-IV and III-IV GvHD were 23% and 10% at 100 days. No differences were found among groups in the incidence of grade II-IV acute GvHD, 20% (95% CI; 15% to 25%), 25% (95% CI; 12% to 37%), and 30% (95% CI; 21% to 39%) for group A, B and C, respectively (p 0.08), nor were their differences in grade III-IV acute GvHD; 9% (95% CI; 5% to 13%), 6% (95% CI; 0% to 13%) and 15% (95% CI; 8% to 22%) for group A, B and C, respectively (p 0.12). (Figure 1). The use of peripheral blood vs bone marrow increased the incidence of grade II-IV aGVHD in univariate analysis, with no other variable impacting this complication as shown in table 3.

The CumInc of overall and moderate-severe chronic GvHD were 33% and 20% at 34 months, respectively. The GvHD prophylaxis was the only variable with impact in the univariate analysis for both (Table 3). Group A: 24% (95% CI; 18% to 30%), B: 47% (95% CI; 29% to 64%) and C: 52% (95% CI; 41% to 62%) (p 0.001) for overall cGvHD and 13% (95% CI; 8% to 17%), 35% (95% CI; 17% to 53%) and 33% (95% CI; 22% to 44%) (p 0.001) for moderate and extensive cGvHD (Figure 2), respectively. Since no other variable showed impact by univariate analysis, a multivariate analysis of cGVHD could not be done, as shown in table 3.

To rule out any potential impact of bone marrow as stem cell source, a sub-analysis including only recipients of peripheral blood stem cell (PBSC) as stem cell source (N 285) was done (details in sup. mat. Table1). In brief, results are similar to the overall patient population. Thus, the overall incidence of grade II-IV and III-IV acute GVHD was 26% and 12%, respectively, and the incidence of overall and moderate-severe chronic GvHD was 34% and 22%. Results of the univariate and multivariate analyses of risk factors for cGVHD did not differ from those of the entire study population (sup. mat. Table 2 and 3).

Non-relapse mortality and relapse

The CumInc of non-relapse mortality at 34 months was 28%, without differences between three groups of GvHD prophylaxis, 30% (95% IC; 23-36) group A, 26% (95% IC; 13-39) group B and 26% (95% IC; 17-35) group C (p 0.68). The variables with an impact in the univariate analyses are shown in Table 4. In the multivariate analyses, HCT-CI-age < 2 and EBMT score < 3 had a positive statistical impact, with a trend for GvHD prophylaxis (group A, p 0.07) and CMV seropositive recipient (p 0.07).

The overall CumInc of relapse at 34 months was 20%, without differences between prophylaxis group, 20% (95% IC; 15-26) group A, 25% (95% IC; 11-39) group B and 16% (95% IC; 9-29) group C (p 0.55); The multivariate analyses showed DRI and prior alloHSCT as the only variables with an impact on relapse (Table 4).

One hundred sixty-one patients died at the end of follow-up (43%). Disease relapse was the cause of death in 34% of patients (N 55). The main causes of NRM were infections (N 54, 52%) and refractory acute GvHD (N 20, 19%). The causes of death and distribution are shown in Table 5. Infections were the most common causes of NRM in all prophylaxis groups: 58% vs 64% vs 31% for groups A, B, and C (p 0.04) respectively; while 42% of NRM in group C were assigned to acute GvHD, in contrast to 12% and 9% for group A and B (p 0.003), respectively.

Overall survival and progression-free survival

The overall and progression-free survival were 57% and 52% at 34 months, without differences between GvHD prophylaxis groups; A: 54% (95% IC; 51-57), B: 56% (95% IC; 48-64) and C: 63% (95% IC; 58-68) (p 0.4) for OS neither for PFS, group A: 51% (95% IC; 47-53), group B: 49% (95% IC; 41-57) and group C: 58% (95% IC; 53-63) (p 0.48).

In the univariate analysis, variables with positive impact on both OS and PFS were DRI (low-intermediate), low HCT-CI-age score (<2), low EBMT score (<3), and no prior alloSCT (Table 4). Multivariate analysis showed that all three pre-transplant scores (low and intermediate DRI, < 2 HCT-CI-age, and < 3 EBMT score) had a

statistically significant impact on OS and PFS, without differences between GvHD prophylaxis groups (Table 4).

Sub-analysis comparing PBSC recipients who received either single-agent tacrolimus or sirolimus-MMF as GvHD

For this sub-analysis, recipients of the two major GVHD prophylaxis strategies (tacrolimus alone versus sirolimus-MMF) who received PBSC as the stem cell source were selected and their outcomes compared. Two hundred thirty-seven patients were included in the sub-analysis, and their clinical differences are shown in the (sup. mat. table 4).

The CumInc of acute grade II-IV and III-IV GvHD were 25% and 12% at 100 days, respectively. The only variable with impact in the multivariate analysis for acute grade II-IV GvHD was the GvHD prophylaxis (tacrolimus vs. sirolimus-MMF), as shown in table 6. The CumInc of overall chronic and moderate-extensive GvHD was 31% and 19% at 34 months, respectively. Likewise, only GvHD prophylaxis had a statistical impact in the multivariate analysis of moderate-extensive GvHD (table 6). Multivariate analyses for acute grade III-IV and overall chronic GvHD were not done since there were no variables with an impact in the univariate analyses at a $p<0.2$.

The CumInc of NRM and relapse were 29% and 20%, in line with the results in the overall study cohort. Again, variables with impact in the multivariate analysis for NRM were HCT-CI-age and EBMT score, while DRI and previous alloSCT had an independent impact on relapse. There was no impact on either outcome of GVHD prophylaxis used (tacrolimus alone vs siro-MMF), as shown in (sup. mat. table 5)

This variable also had no impact on OS and PFS, which were 54% and 50%, respectively, at 34 months for both GVHD strategies combined. Variables with an impact of OS and PFS in multivariate analyses were DRI, HCT-CI-age and EBMT score (sup. mat. table 5).

DISCUSSION

HaploSCT with PTCy has become a real alternative for patients who need an SCT and lack other eligible donors; even more, this transplant modality is considered as the first-choice at some institutions in patients highly likely to have a single-allele mismatched unrelated donors [35].

The conditioning regimen based on TBF in the haploSCT has been studied by different groups [14,36-38], and the outcomes in our cohorts appear in line with previous studies. The observed 61% OS (67% to 56%) and 53% PFS (58% to 51%); 21% NRM (28% to 18%) and 20% of relapse (22% to 16%) are within expected ranges, as is the CumInc of grade III-IV aGvHD [10% (14% to 4%)] and overall cGvHD [25% (29% to 22%)].

Calcineurin inhibitors (CNI) and MMF are considered as a standard of care in GvHD prophylaxis after PTCy haploSCT. A large heterogeneous registry study showed an OS of nearly 55% in the haploSCT with tacro-MMF after PTCy; 42% of CumInc of grade II-IV aGvHD at 6-months and 41% chronic GvHD at 2 years of follow-up [39].

There has been growing interest, however, in using CNI-free GVHD prophylactic regimens, especially since the introduction of PTCy. In a recent phase 2 trial, the results with sirolimus and MMF after PTCy as GvHD prophylaxis in haploidentical peripheral blood stem cell transplantation showed 20% and 9.4% CumInc of grade II-IV and III-IV aGvHD at 100 days, respectively and nearly 19% of moderate-severe cGvHD at one year. The survival outcomes (OS and PFS), NRM, and relapse were 71.7%, 59%, 18.8%, and 22% at one year of follow-up, respectively [22]. Previously, encouraging results using myeloablative conditioning followed by PT-Cy and sirolimus-MMF in haploSCT platform had been published, with low incidence of grade II-IV and III-IV aGvHD (15% and 7.5% respectively), and 20% of moderate-severe cGvHD, and the authors postulated a synergistic effect between PTCy and sirolimus on immune reconstitution [40].

The colleagues from Valencia had previously reported the results of sirolimus and MMF following PTCy as GvHD prophylaxis including transplants from HLA-identical siblings (N 64), unrelated (N 52), or haploidentical donors (N 42), with an overall low incidence of grade II-IV (27%) and III-IV (9%) aGvHD, and a 38%

incidence of moderate severe chronic GvHD. With this platform the 1-year incidence of relapse and NRM were low at 12% and 14%, respectively, and the OS and PFS were very promising (82% and 75%, respectively) [30]. Studying only recipients of HaploSCT, a similar rate of grade III-IV aGvHD was found when comparing GVHD prophylaxis with CsA-MMF and Siro-MMF (CsA-MMF: 12% vs siro-MMF: 12%, p 0.79) [41]. In addition, in this previous report the incidence of overall and moderate-severe cGvHD in CsA-MMF and Siro-MMF [41] recipients were similar to the current study results, since the CumInc of overall cGvHD was 47% and 52%, respectively, and the incidence of moderate-severe cGvHD was 35% and 33%, respectively.

In the current study, similar incidence of grade II-IV (20%, 25%, and 30%) and III-IV (9%, 6%, and 15%) acute GvHD at 100 days occurred in the three GvHD cohorts [A (tacrolimus), B (CNI-MMF) and C (sirolimus-MMF), respectively]. We found no differences in the incidence of acute and chronic GvHD by the stem cell source (bone marrow or PBSC), and the results of restricting the analysis to only those receiving PBSC were similar to the overall analyses including both stem cell sources. However, we acknowledge that bone marrow as stem cell source is a well-known protective variable for the development of GVHD, especially cGVHD, and thus our study results are applicable mainly to recipients of PBSC haploidentical transplantation.

A sub-analysis including only recipients of PBSC as stem cell source who received either single-agent tacrolimus or siro-MMF after PTCy was performed to exclude potential biases due to the heterogeneity of the overall study cohort and compare outcomes between the two main GVHD prophylaxis studied [17]. Results of this sub-analysis showed similar results as the overall study cohort, with no differences in survival, NRM and relapse between these two groups. Both acute and chronic GVHD, however, were lower in the tacrolimus compared to the siro-MMF group, as shown in Table 6.

Unlike the 13% of primary graft failure (PGF) reported in the first haploidentical approach [2], only three percent of both tacrolimus and sirolimus groups had a PGF, without differences in the incidence of engraftment. A high incidence of SOS was found in the siro-MMF group (13% vs. 4%), confirming prior findings [30]. On the other hand, the higher incidence of hemorrhagic cystitis in Siro-MMF recipients has

not been previously published. Due to the retrospective nature of the study, these differences may be partly due to center-specific diagnostics and data management differences and may not represent true differences between GVHD prophylactic strategies.

In conclusion, our results show that the survival appears to be essentially the same when using any of the three studied GVHD prophylaxis regimens with TBF, PTCy and HaploSCT, thus supporting using sirolimus for GVHD prophylaxis in patients with a (relative) contraindication to receive a CNI. As expected, results are especially good in patients with early phase of their underlying disease and a limited number of comorbidities. Although the incidence of acute GVHD was similar between groups, the observed lower incidence of chronic GVHD in the single-agent tacrolimus cohort merits further investigation.

AUTHORS' CONTRIBUTIONS

A.E. designed the study, performed research, analyzed data, and wrote the manuscript; R.M. and J.S. designed the study and performed research; M.P, J.M., J.L.P., C.F., B.H., I.G.C., A.B., A.P., M.H., S.R., M.V., J.C.H.B, P.C., R.H., J.S., C.S. contributed essential data and revised the manuscript; and all authors approved the final version of the manuscript. R.M. and J.S. contributed equally to this work and should be considered co-first authors.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest: The authors declare no conflict of interest.

Grants: no

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575 **Table 1. Clinical differences according to different types of GvHD prophylaxis**
576 **Table 2. Engraftment, complications, and early deaths**
577 **Table 3: Grade II-IV, III-IV aGvHD and overall, moderate-severe cGvHD**
578 **Univariate and multivariate analyses**
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580 **analysis**
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582 **Table 6: Grade II-IV, III-IV aGvHD and overall, moderate-severe cGvHD**
583 **Univariate and multivariate analyses between tacro vs siro-MMF cohort**
584
585 **Figure 1: Acute GvHD (100 days)**
586 **Figure 2: Overall and Moderate-extensive Chronic GvHD (34 months)**
587

Click here to access/download;Table;Table 1 - Clinical characteristics.xls

Table 1. Clinical differences according to different types of GvHD prophylaxis

	group A (Ref) Tacrolimus		group B CNI-MMF		p value (A vs. B)	group C Sirolimus-MMF		p value (A vs. C)
	N (222)	%	N (49)	%		N (101)	%	
Pacient sex								
Male	123	55	32	65	0.26	62	61	0.33
Female	99	45	17	35		39	39	
Pacient age								
Median (range)	51	18-72	46	18-69	0.03	55	21-71	0.004
≤ 55 years	110	49	32	65	0.06	38	38	0.07
> 55 years	112	51	17	35		63	62	
Bu-conditioning								
Bu (x3 days)	81	36	26	53	0.02	27	27	0.1
Bu (x2 days)	141	64	23	47		74	73	
HCT-CI-age								
≤ 2	135	61	26	53	0.33	31	31	0.001
≥ 3	87	39	23	47		70	69	
EBMT score								
≤ 3	81	36	26	53	0.04	34	34	0.7
≥ 4	141	64	23	47		67	66	
DRI								
low-intermediate	128	58	30	61	0.74	65	64	0.27
high-very high	94	42	19	39		36	36	
Stem cell source (SC)								

Peripheral blood (PB) **Bone marrow (BM)**	153 69	69 31	48 1	98 2	0.001	84 17	83 17	0.001
Median PB-SC x10^6/kg (range)	5.2	2-10	6.2	2-13	0.001	7.4	3,7-16	0.001
Median BM-SC x10^6/kg (range)	2.4	1-8	2.5	-	-	3.9	2-10	0.001
Underlying disease **Myeloid** **Lymphoid**	 151 69	 69 31	 30 19	 61 39	 0.31	 69 32	 68 32	 0.9
LMA-MDS **other diseases**	140 82	63 37	28 21	57 43	0.51	64 37	63 37	1
Disease status at SCT **CR phase (1st and 2nd)** **Other phases**	 122 100	 55 45	 36 13	 73 27	 0.03	 65 36	 64 36	 0.14
Previous SCT **Non previous SCT** **alogeneic SCT**	 201 21	 91 9	 48 1	 98 2	 0.14	 96 5	 95 5	 0.2
Patient CMV seroloy **Negative** **Positive**	 46 176	 21 79	 5 44	 10 90	 0.11	 15 86	 15 85	 0.22
Donors' characteristics	**N**	**%**	**N**	**%**	**p value**	**N**	**%**	**p value**
Donor age **Median (range)**	 38	 14-74	 38	 19-69	 0.8	 38	 18-53	 1

Donor Sex								
male	136	61	32	65	0.63	65	64	0.62
female	86	39	17	35		36	36	
Donor direction								
other	174	78	38	78	0.85	82	81	0.65
female to male	48	22	11	22		19	19	
Donor CMV seroloy								
Negative	97	44	14	29	0.06	37	37	0.27
Positive	124	56	35	71		64	63	
CMV combination								
Re or Do IgG positive	124	56	35	71	0.06	90	89	0.71
IgG negative	97	44	14	29		11	11	

Grup A was used as a reference (Ref) to compare the clinical differences between group B and C. CNI calcineurin inhibitors. HCT-CI-age: hematopoietic stem cell transplantation comorbidity index. EBMT: European bone marrow transplantation score, DRI: disease risk index; SCT: stem cell transplantation; CR: complete remission; flu-bu: fludarabine-busulphan; flu-mel: fludarabine-melphalan GvHD: graft versus host disease; Tacro/CsA-MMF: tacrolimus or cyclosporine A and mycophenolate mofetil; Re: receptor; Do: donor; CMV: cytomegalovirus

Click here to access/download;Table;Table 2 - Engraftment and early complications.xls

Table 2. Engraftment, complications and early deaths

	group A+B CNI +/- MMF		group C Sirolimus-MMF		p value (A+B vs. C)
	N (271)	%	N (101)	%	
Engraftment					
Primary graft failure	8	3	3	3	0.7
Yes	253	93	96	95	
No	10	4	2	2	
Hemorragic cystitis					
No	206	76	56	55	0.001
Mild-moderate	46	17	31	31	
Severe	19	7	14	14	
Thrombotic microanghiopaty					
No	261	96	97	96	0.9
Yes	10	4	4	4	
Sinsusoidal obstructive sdr.					
No	260	96	88	87	0.004
Yes	11	4	13	13	
CMV reactivation					
No	108	40	35	35	0.6
Yes	159	59	65	64	
Disease	4	1	1	1	
Early death (<30 days)					
No	257	95	98	97	0.3
Yes	14	5	3	3	

Click here to access/download;Table;Table 3 - Acute and chronic GvDH.xlsx

Table 3: Grade II-IV, III-IV aGvHD and overall, moderate-severe cGvHD Univariate and multivariate analyses

Variables (N)	Grade II-IV acute GvHD				Grade III-IV acute GvHD			
	100 days				100 days			
	Univariate		Multivariate		Univariate		Multivariate	
	Probability % (95 % CI)	p value	HR (95% CI)	p value	Probability % (95 % CI)	p value	HR (95% CI)	p value
GvHD prophylaxis								
Tacrolimus (222)	20 (15-25)	0.08	NA	0.07	9 (5-13)	0.12	NA	0.2
Tacro/CsA - MMF (49)	25 (12-37)				6 (0-13)			
Sirolimus MMF (101)	30 (20-39)				15 (8-22)			
DRI								
Low-intermediate (223)	20 (15-26)	0.09	NA	0.09	8 (4-12)	0.09	NA	0.06
High-very high (149)	28 (20-35)				14 (8-19)			
HCT-CI-age								
≤ 2 (192)	21 (15-27)	0.2	NA	0.6	9 (5-13)	0.36	NA	NA
≥ 3 (180)	26 (19-32)				12 (7-16)			
EBMT-score								
≤ 3 (141)	24 (17-31)	0.6	NA	NA	10 (5-15)	0.7	NA	NA
≥ 4 (231)	23 (17-28)				10 (6-14)			
Stem cell source								
Peripheral Blood (285)	26 (21-31)	0.02	NA	0.09	12 (8-15)	0.1	NA	0.1
Bone Marrow (87)	15 (7-23)				6 (1-11)			

Busulphan								
x3 days (134)	23 (16-30)	0.7	NA	NA	9 (4 -14)	0.7	NA	NA
x2 days (238)	23 (18-29)				11 (7-15)			
Prior aloSCT								
No (345)	24 (19-28)	0.5	NA	NA	11 (7-14)	0.6	NA	NA
Yes (27)	18 (4-32)				7 (0-17)			
Donor Sex								
Male (233)	21 (16-26)	0.3	NA	NA	10 (6-14)	0.9	NA	NA
Female (139)	27 (19-34)				10 (5-15)			
CMV serology (patient)								
Negative (66)	24 (14-35)	0.9	NA	NA	12 (4-20)	0.6	NA	NA
Positives (306)	23 (18-28)				10 (7-13)			
CMV serology (donor)								
Negative (148)	21 (15-28)	0.4	NA	NA	12 (7-18)	0.08	NA	0.37
Positive (223)	24 (19-30)				9 (5-12)			

* The chronic GvHD (as overall as moderate-extensive) multivariate analysis was not done because any variable in the univariate analyses had p v

Overall chronic GvHD		Moderate-Severe chronic GvHD	
34 months		34 months	
Univariate		Univariate	
Probability % (95 % CI)	p value	Probability % (95 % CI)	p value
24 (18-30) 47 (29-64) 52 (41-62)	0.001	13 (8-17) 35 (17-53) 33 (22-44)	0.001
34 (28-41) 33 (25-41)	0.8	19 (14-25) 22 (15-30)	0.65
33 (26-40) 35 (28-43)	0.4	19 (13-25) 23 (15-30)	0.46
32 (24-41) 35 (28-41)	0.8	19 (12-26) 21 (16-27)	0.7
34 (28-40) 34 (24-45)	0.9	22 (16-27) 16 (8-25)	0.3

31 (23-40) 35 (29-42)	0.4	19 (12-26) 21 (16-27)	0.5
33 (28-39) 39 (21-58)	0.4	20 (15-24) 29 (11-47)	0.3
32 (26-38) 36 (28-44)	0.8	17 (12-23) 25 (17-33)	0.3
36 (24-48) 33 (28-39)	0.78	16 (6-25) 22 (16-27)	0.28
33 (25-41) 35 (28-41)	0.59	19 (12-26) 22 (16-28)	0.5

alor $\leq$ 2

Click here to access/download;Table;Table 4 - NRM, relapse and survival.xlsx

Table 4: Survival outcomes, NRM, and relapse univariate and multivariate analysis

Variables (N)	Overall survival				Progression free survival			
	Univariate		Multivariate		Univariate		Multivariate	
	Probability % (95 % CI)	p value	HR (95% CI)	p value	Probability % (95 % CI)	p value	HR (95% CI)	p value
GvHD prophylaxis								
Tacrolimus (222) *	54 (51-57)	0.4	NA	0.5	51 (47-53)	0.5	NA	0.6
Tacro/CsA - MMF (49)	56 (48-64)				49 (41-57)			
Sirolimus MMF (101)	63 (58-68)				58 (53-63)			
DRI								
Low-intermediate (223)*	65 (62-68)	0.001	0,6 (0,4-0,8)	0.001	61 (57-65)	0.001	0,6 (0,44-0,8)	0.001
High-very high (149)	44 (40-48)				39 (35-43)			
HCT-CI-age								
≤ 2 (192)*	64 (60-68)	0.001	0,65 (0,47-0,88)	0.006	59 (55-63)	0.004	0,7 (0,5-0,9)	0.02
≥ 3 (180)	50 (46-54)				46 (42-50)			
EBMT-score								
≤ 3 (144)*	72 (64-76)	0.001	0,5 (0,35-0,7)	0.001	66 (62-70)	0.001	0,5 (0,4-0,7)	0.001
≥ 4 (231)	48 (44-52)				44 (40-48)			
Busulphan								
x3 days (134)*	67 (63-71)	0.003	NA	0.14	61 (57-65)	0.006	NA	0.16
x2 days (238)	50 (46-54)				47 (43-51)			
Prior aloSCT								
No (345)*	59 (56-62)	0.02	NA	0.7	54 (51-57)	0.013	NA	0.5

Yes (27)	36 (27-45)				33 (24-42)			
Stem cell source								
Peripheral blood (285)*	54 (51-57)	0.05	NA	0.3	50 (47-53)	0.08	NA	0.4
Bone marrow (87)	64 (58-70)				60 (54-66)			
Donor Sex								
Male (233)*	60 (56-64)	0.08	NA	0.45	54 (50-58)	0.15	NA	0.6
Female (139)	52 (48-56)				49 (45-53)			
CMV serology (patient)								
Negative (66)*	61 (55-67)	0.13	NA	0.1	57 (51-63)	0.16	NA	0.13
Positives (306)	56 (53-59)				51 (48-54)			

* Reference. NA: not applicable. Other variables tested in the univariate analysis: patients sex; lymphoid vs myeloid underlying disease; donor female; CI

Non relapse mortality				Relapse			
Univariate		Multivariate		Univariate		Multivariate	
Probability % (95 % CI)	**p value**	**HR (95% CI)**	**p value**	**Probability % (95 % CI)**	**p value**	**HR (95% CI)**	**p value**
30 (23-36) 26 (13-39) 26 (17-35)	0.68	NA	0.07	20 (15-26) 25 (11-39) 16 (8-24)	0.55	NA	0.6
25 (20-31) 33 (25-40)	0.06	NA	0.19	14 (9-19) 28 (21-36)	0.003	0,6 (0,35-0,9)	0.03
20 (14-26) 37 (30-44)	0.001	0,5 (0,3-0,7)	0.002	21 (15-28) 18 (12-24)	0.3	NA	NA
17 (10-23) 35 (29-42)	0.001	0,4 (0,26-0,66)	0.004	17 (10-24) 21 (15-27)	0.5	NA	NA
19 (12-26) 34 (27-40)	0.003	NA	0.11	20 (13-27) 20 (14-25)	0.89	NA	NA
29 (24-34)	0.4	NA	NA	18 (13-22)	0.001	0,4 (0,2-0,7)	0.004

22 (7-38)				45 (26-64)			
29 (24-34) 26 (16-35)	0.31	NA	NA	21 (16-27) 14 (6-22)	0.2	NA	0.6
26 (20-32) 32 (24-40)	0.09	NA	0.4	20 (15-26) 18 (12-25)	0.58	NA	NA
20 (10-30) 30 (25-35)	0.07	NA	0.07	23 (12-34) 19 (14-24)	0.67	NA	NA

MV donor serology

Click here to access/download;Table;Table 5 - Causes of NRM.xlsx

Table 5: Causes of non relapse mortality

	group A Tacrolimus		group B Tacro/Csa-MMF		group C Sirolimus-MMF	
Causes of NRM	**N (67)**	**%**	**N (11)**	**%**	**N (26)**	**%**
Infections (54)	39	*58*	7	*64*	8	*31*
Refractory acute GvHD (20)	8	*12*	1	*9*	11	*42*
Chronic GvHD (4)	4	*6*	0	-	0	-
Primary graft failure (6)	3	*5*	1	*9*	2	*8*
Severe idiopathic encephalopathy (5)	5	*7*	0	-	0	-
Sudden death (3)	3	*5*	0	-	0	
Refractory bleeding (3)	2	*3*	0	-	1	*4*
Sinusoidal obstructive syndrom (3)	2	*3*	1	*9*	0	-
Thrombotic microangiopathy (1)	1	*1*	0	-	0	-
Cardiac complication (1)	0	-	0	-	1	*4*
Unknown (4)	0	-	1	*9*	3	*11*

Table 6 GvHD between tacro vs siro-MMF cohort

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Table 6 (sub analysis): Grade II-IV, III-IV aGvHD and overall, moderate-severe cGvHD Univariate and multivariate analyses between tacro v

Variables (N 237)	Grade II-IV acute GvHD				Grade III-IV acute GvHD		Overall chror
	100 days				100 days		34 mon
	Univariate		Multivariate		Univariate		Univari
	Probability % (95 % CI)	p value	HR (95% CI)	p value	Probability % (95 % CI)	p value	Probability % (95 % CI)
GvHD prophylaxis							
Tacrolimus (153) *	22 (15-28)	0.03	0,6 (0,3-0,9)	0.001	10 (5-15)	0.05	22 (15-28)
Sirolimus MMF (84)	33 (23-43)				18 (10-26)		51 (39-63)
DRI							
Low-intermediate (130)*	24 (17-31)	0.49	NA	NA	11 (5-16)	0.34	32 (24-40)
High-very high (107)	28 (20-37)				15 (8-22)		30 (21-39)
HCT-CI-age							
≤ 2 (114)*	24 (16-31)	0.56	NA	NA	11 (5-16)	0.36	30 (22-39)
≥ 3 (123)	28 (20-36)				15 (8-21)		33 (24-41)
EBMT-score							
≤ 3 (84)*	27 (18-37)	0.54	NA	NA	14 (7-22)	0.74	32 (22-43)
≥ 4 (153)	25 (18-32)				12 (7-17)		31 (23-38)
Busulphan							
x3 days (83)*	23 (14-32)	0.71	NA	NA	11 (4-18)	0.74	28 (18-38)
x2 days (154)	27 (20-34)				14 (8-19)		34 (26-42)

Prior aloSCT							
No (211)*	27 (21-33)	0.16	NA	0.18	13 (9-18)	0.39	30 (24-37)
Yes (26)	15 (2-29)				8 (0-18)		38 (19-58)
Donor Sex							
Male (147)*	25 (18-32)	0.76	NA	NA	13 (8-18)	0.88	30 (22-38)
Female (90)	28 (19-37)				12 (5-19)		33 (23-43)
CMV serology (patient)							
Negative (47)*	18 (15-40)	0.97	NA	NA	15 (5-25)	0.62	35 (21-50)
Positives (190)	25 (19-32)				12 (7-17)		30 (23-37)

* The grade III-IV acute and overall chronic GvHD (as overall as moderate-extensive) multivariate analysis was not done because any variable variables tested in the univariate analysis: patients sex; lymphoid vs myeloid underlying disease; donor female; CMV donor serology

vs siro-MMF cohort

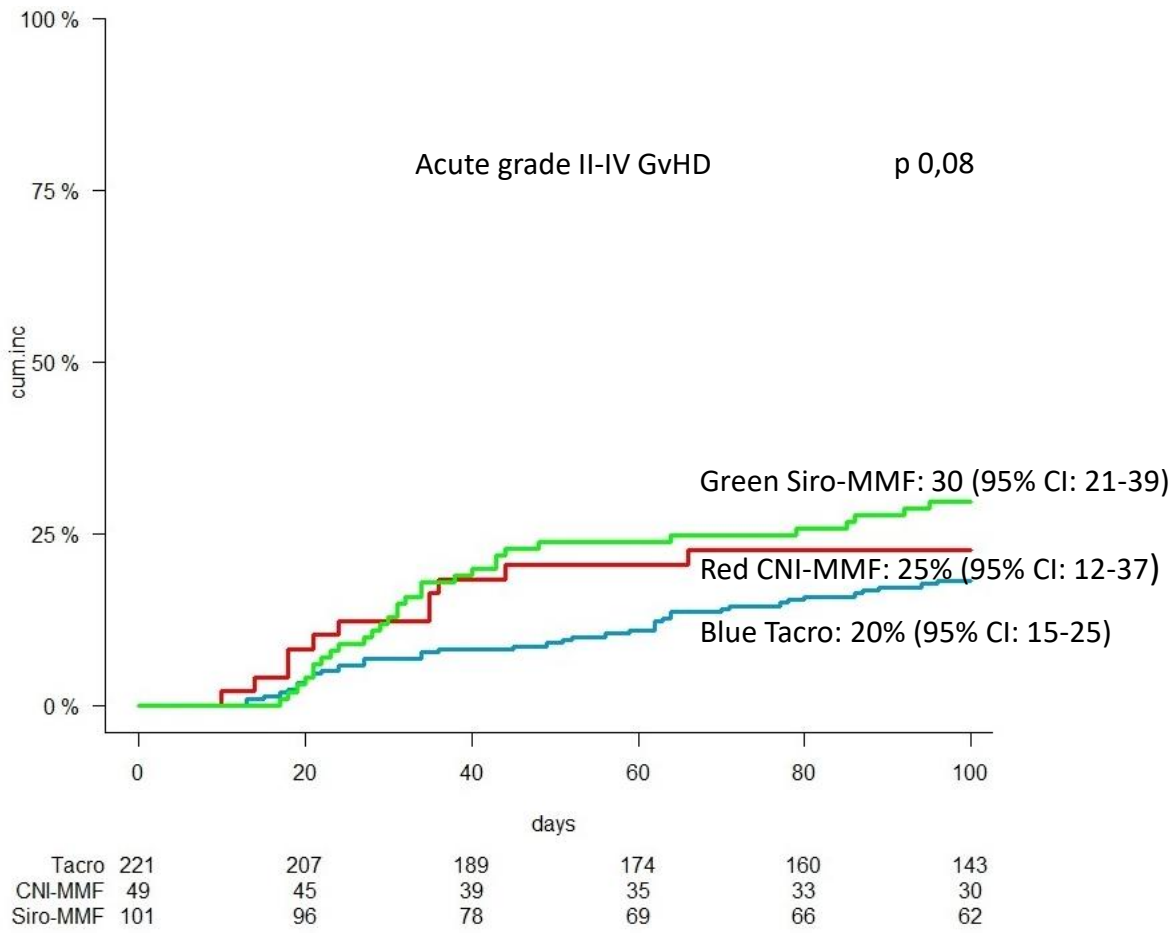
nic GvHD	Moderate-Severe chronic GvHD			
ıths	34 months			
ate	Univariate		Multivariate	
p value	Probability % (95 % CI)	p value	HR (95% CI)	p value
0.001	12 (6-17) 35 (22-47)	0.001	0,3 (0,2-0,6)	0.001
0.82	21 (13-28) 18 (10-26)	0.5	NA	NA
0.6	18 (11-26) 21 (13-29)	0.7	NA	NA
0.41	19 (10-28) 20 (13-26)	0.9	NA	NA
0.47	12 (5-19) 23 (16-31)	0.08	NA	0.36

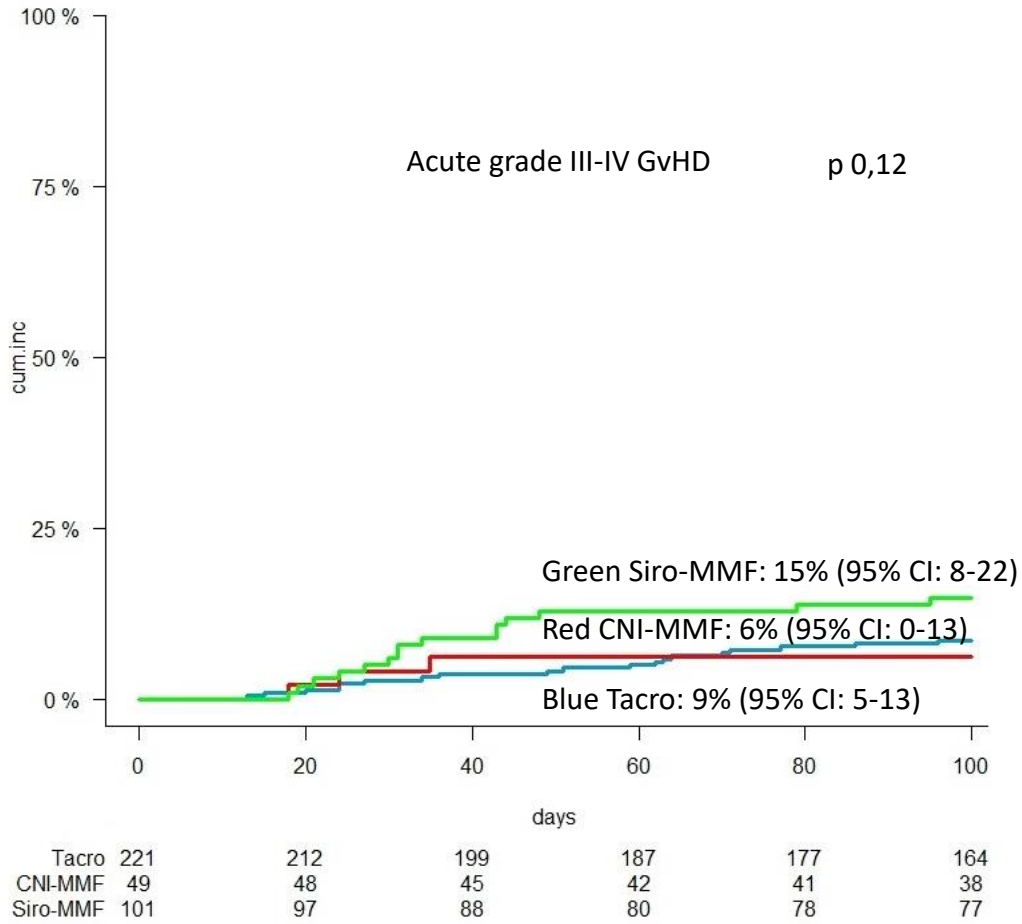
0.31	18 (13-24) 27 (9-45)	0.34	NA	NA
0.76	19 (12-25) 21 (12-30)	0.78	NA	NA
0.7	13 (2-23) 21 (15-27)	0.21	NA	0.39

: in the univariate analyses had p valor $\leq$ 2. * NA: not applicable Other

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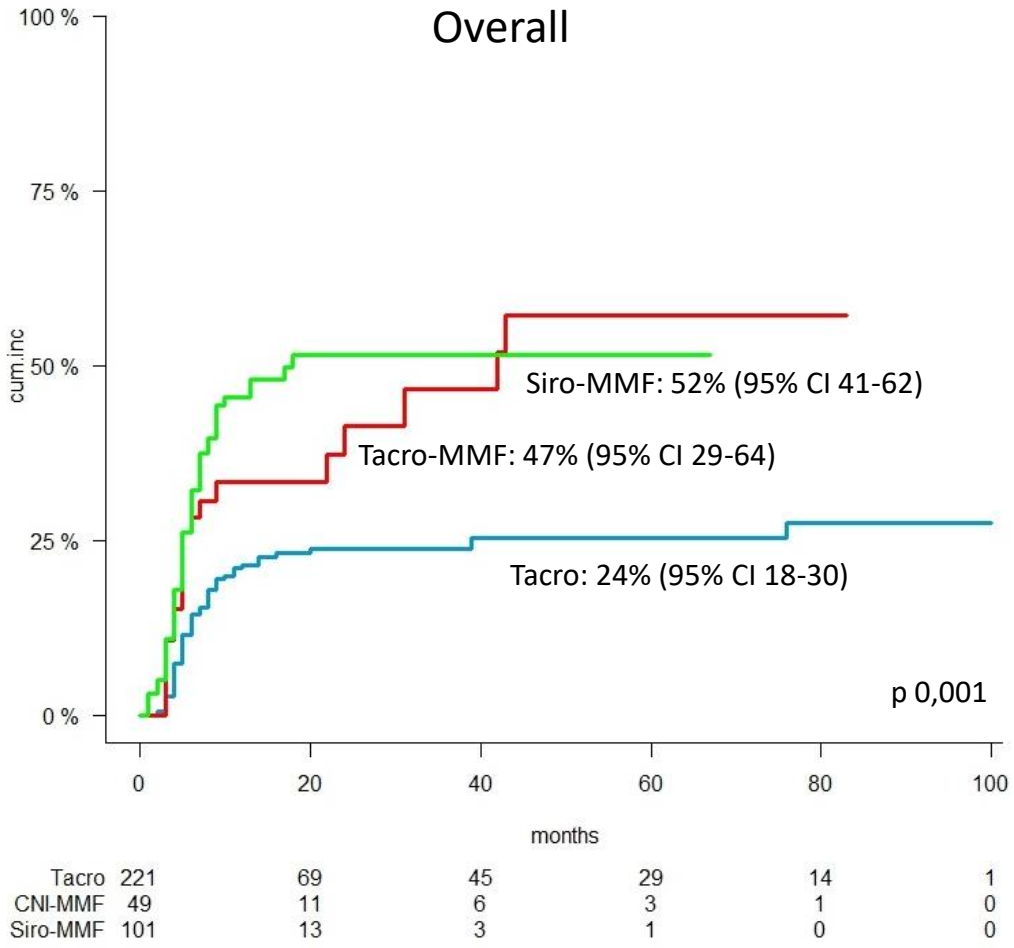
Figure 1: Acute GvHD (100 days)

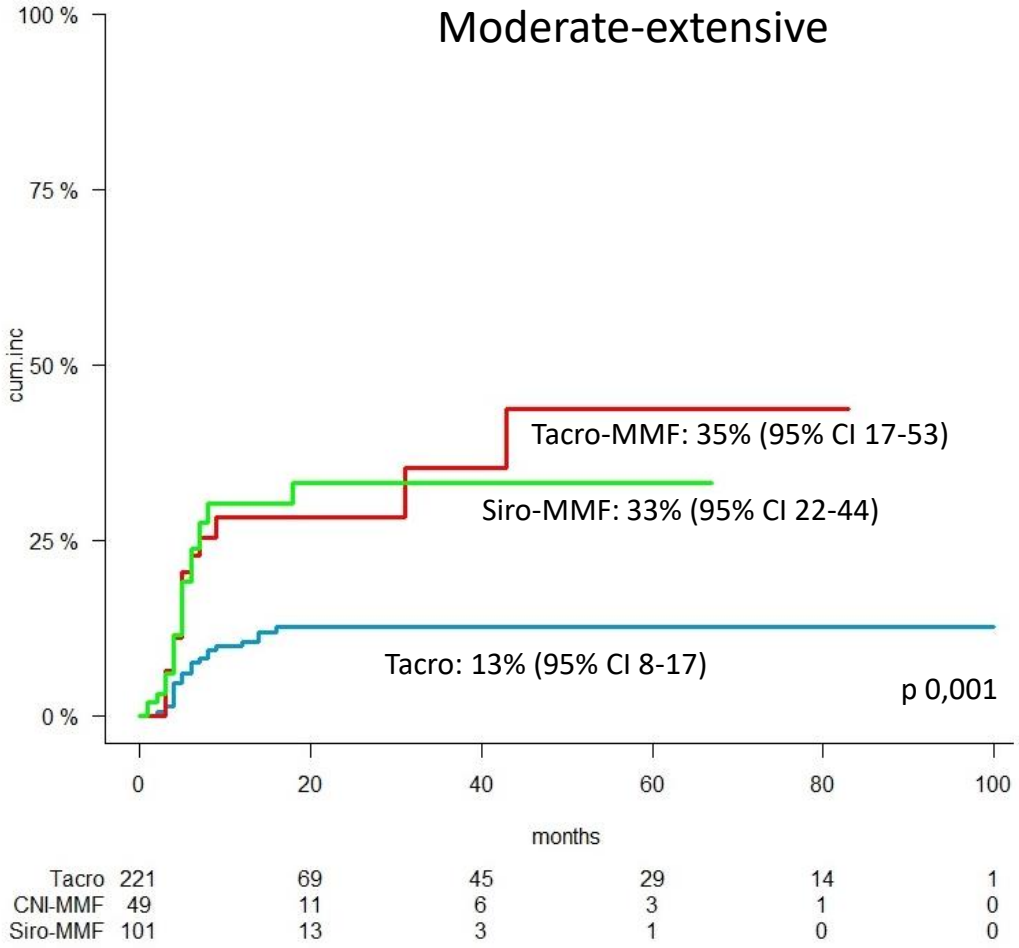




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Figure 2: Overall and Moderate-extensive Chronic GvHD (34 months)





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