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ORIGINAL ARTICLE

# Reduced dose of post-transplantation cyclophosphamide compared to ATG for graft-versus-host disease prophylaxis in recipients of mismatched unrelated donor hematopoietic cell transplantation: a single-center study

Yves Soltermann[1] · Dominik Heim[1] · Michael Medinger[2] · Helen Baldomero[1] · Jörg P. Halter[1] · Sabine Gerull[1] · Christian Arranto[1] · Jakob R. Passweg[1] · Martina Kleber[1,2]

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## Abstract

Post-transplantation cyclophosphamide (PTCy) demonstrated effectiveness to prevent GVHD after haploidentical hematopoietic cell transplantation (HCT). Reducing toxicities with a maximized efficacy is still challenging in HCT. In this retrospective study, we analyzed the safety and efficacy of transplantation from a 1-antigen HLA-mismatched unrelated donor (9/10 MMUD) in 80 patients with hematological disorders between 2010 and 2018; 22 patients received PTCy with a reduced dose of 40 mg/kg, cyclosporine A, and mycophenolate mofetil (MMF); 58 patients received anti-thymocyte globulin (ATG), cyclosporine A, and either methotrexate or MMF for GVHD prophylaxis. Cumulative incidence (CI) of acute GVHD grades II–IV in the PTCy group was significantly lower (15% vs. 50%, $p = 0.006$); however, CI of chronic GVHD was (not significantly) lower in the PTCy group (26% vs. 35%, $p = 0.137$). One-year OS was significantly longer ($p = 0.008$) in the PTCy group with a similar 1-year PFS ($p = 0.114$) in both groups. Rates of 1-year relapse and non-relapse mortality were similar. Median time to neutrophil engraftment was comparable in both GVHD prophylaxis groups (14 days vs. 16 days, respectively, $p = 0.107$). Our results show that a lower dose of PTCy-based prophylaxis is an effective and safe strategy to prevent acute GVHD in HCT with 9/10 MMUD compared to ATG.

**Keywords** Reduced post-transplantation cyclophosphamide · GVHD · Outcome

## Introduction

Allogeneic hematopoietic cell transplantation (HCT) is a curative option for many patients with hematologic disorders [1]. In patients with neither an HLA-identical sibling nor a matched unrelated donor, options include the use of haploidentical or mismatched unrelated (MMUD) donors [2]. HCT from mismatched donors are associated with increased graft-versus-host disease (GVHD) risks, increased non-relapse mortality (NRM), and shorter progression-free (PFS) and shorter overall survival (OS) [3, 4]. The standard GVHD prophylaxis regimen for MMUD HCT consists of a calcineurin inhibitor (cyclosporine A (CyA) or tacrolimus) with methotrexate or mycophenolate mofetil (MMF) often in combination with in vivo T cell depletion (TCD) such as anti-thymocyte globulin (ATG). GVHD prophylaxis with ATG produces superior results, lower incidence of acute and chronic GVHD, than GVHD prophylaxis regimens without ATG at the cost of greater risk of infections [5–7]. Post-transplantation high-dose cyclophosphamide (PTCy) has proven to be an effective strategy for GVHD prophylaxis in haploidentical donor transplants by eliminating early proliferating alloreactive T cells [8–14].

Based on the encouraging results with haploidentical transplants in overcoming negative effects of HLA disparity and given unsatisfactory outcome of patients transplanted with 9/10 MMUD, PTCy was also used in 9/10 MMUD HCT in

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✉ Martina Kleber
martina.kleber@usb.ch

[1] Division of Hematology, Department of Medicine, University Hospital Basel, Petersgraben 4, 4031 Basel, Switzerland

[2] Division of Internal Medicine, Department of Medicine, University Hospital Basel, Petersgraben 4, 4031 Basel, Switzerland

combination with CyA and methotrexate or MMF [5, 10, 15, 16]. Due to concerns of cyclophosphamide toxicity in this rather older population of 9/10 MMUD HCT patients, we evaluated a modified PTCy protocol using a reduced dose of 40 mg/kg cyclophosphamide on days + 3 and + 4 combined with other immunosuppressive drugs as GVHD prophylaxis in 9/10 MMUD HCT with either a myeloablative or a reduced-intensity conditioning regimen.

## Material and methods

### Patient population and study design

This study performed at the Division of Hematology of the University Hospital Basel analyzed data from 80 patients who received an allogeneic HCT from a 1-antigen HLA-mismatched unrelated donor (9/10 MMUD) for hematologic disorders. From 2016 to 2018, we consecutively analyzed 22 patients in a PTCy-based GVHD prophylaxis protocol and compared them with 58 consecutively treated patients with conventional GVHD prophylaxis with ATG (used as a control group) between 2010 and 2017.

The patient-, disease- and transplant characteristics, including the Pretransplant Assessment of Mortality (PAM) score, the European Group for Blood and Marrow Transplantation (EBMT) risk score, and the Hematopoietic Cell Transplantation Comorbidity Index (HCT-CI), are shown in Table 1. The Ethics Committee of Northwestern and Central Switzerland approved this study (EKNZ study number 2017-01019).

The primary study aims were to compare safety (time to engraftment), efficacy (cumulative incidence of acute GVHD (aGVHD) and chronic GVHD at 1 year (cGVHD)), and outcome after HCT (1-year non-relapse-related mortality (NRM), relapse rate, 1-year progression-free survival (PFS), and 1-year overall survival (OS)).

### Conditioning regimens and graft-versus-host disease prophylaxis

The myeloablative conditioning regimen (MAC, $n = 9$, 41%) in the PTCy group consisted of 5 mg/kg/day thiotepa (Thio) on days − 6 and − 5, and 0.8 mg/kg busulfan (Bu) and 50 mg/m$^2$/day fludarabine (Flu) on days − 4 through − 2. The reduced-intensity conditioning (RIC, $n = 13$, 59%) included 15 mg/kg/day cyclophosphamide (Cy) on days − 6 and − 5, 30 mg/m$^2$/day fludarabine daily on days − 6 through − 2, and total body irradiation (TBI) with 2 Gy on day − 1. GVHD prophylaxis in the PTCy group was cyclophosphamide (40 mg/kg, days + 3 and + 4 after HCT), cyclosporine A (CyA; 5 mg/kg/day, started on day 5), and MMF (15 mg/kg, Online Resource Table 1).

Patients in the control group received MAC regimens, including Cy/Bu ($n = 22$, 38%), Cy/TBI (> 8 Gy, $n = 6$, 10%), and other protocols ($n = 8$, 14%). RIC regimens consisted of Flu/Bu ($n = 12$, 21%), Cy/Flu/TBI 2 Gy ($n = 3$, 5%), and other protocols ($n = 7$, 12%). Reasons for using RIC were advanced age or comorbidities (Online Resource Table 1).

GVHD prophylaxis for patients in the control group consisted of anti-thymocyte globulin (Grafalon®), cyclosporine A or tacrolimus, and methotrexate (MTX) or MMF as published [17]. All patients received standard supportive care including antimicrobial prophylaxis as previously described [18, 19].

### Post-transplantation complications

Post-HCT complications were assessed, including acute and chronic GVHD, infections, and organ toxicities. Acute and chronic GVHD were diagnosed based on clinical symptoms and/or skin, oral mucosa, liver, or gut biopsies and were graded according to consensus criteria for acute and chronic GVHD [20–22].

Non-hematologic and non-infectious toxicity grades III–IV were analyzed according to the Common Terminology Criteria for Adverse Events (CTCAE, v4.0) of the US National Cancer Institute and National Institutes of Health from the day of HCT through day 360 after transplantation. Additionally, we evaluated acute bacterial, viral, and fungal infections that occurred within 100 days post-HCT.

### Engraftment and chimerism

Neutrophil engraftment was defined as the time interval from transplantation to the first of three consecutive days with an absolute neutrophil count (ANC) $\geq 0.5 \times 10^9$/L. Platelet engraftment was defined as the time interval between transplantation and the first of seven consecutive days with platelet count $\geq 20 \times 10^9$/L. Primary graft failure was defined as the failure to reach an ANC $> 0.5 \times 10^9$/L by day + 28 in three consecutive measurements. Chimerism analyses (PCR-based short tandem repeat analysis) were performed on peripheral blood and/or bone marrow samples at day 30 and day 100 after transplantation, and complete chimerism was defined as 100% donor DNA.

### Statistical analysis

Continuous variables were expressed as mean (± standard deviation (SD)) or median (interquartile range (IQR)), as appropriate; categorical variables were presented as rates/proportions. We assessed differences in demographic, clinical, and transplantation parameters using chi-squared or Fisher's exact test for categorical variables and Student's $t$ test or Mann-Whitney $U$ test for continuous variables, depending on data distributions. The cumulative incidences of acute and chronic GVHD, NRM, and relapse/

**Table 1** Patient- and transplant-related characteristics of all patients ($n = 80$), PTCy-based prophylaxis group and control group

| Variable | All patients ($n = 80$) $n$ (%) | PTCy group ($n = 22$) $n$ (%) | Control group ($n = 58$) $n$ (%) | $p$ values |
|---|---|---|---|---|
| Age (years, median (IQR)) | 52 (42–62) | 58 (44–67) | 50 (39–60) | 0.037 |
| Diagnosis | | | | 0.792 |
| Myeloid malignancy | 48 (60) | 12 (54) | 36 (62) | |
| Lymphoid malignancy | 28 (35) | 9 (41) | 19 (33) | |
| Bone marrow failure | 4 (5) | 1 (5) | 3 (5) | |
| Disease risk index | | | | 0.643 |
| Low | 8 (10) | 2 (9) | 6 (10) | |
| Intermediate | 48 (60) | 14 (64) | 34 (59) | |
| High | 20 (25) | 4 (18) | 16 (28) | |
| Very high | 4 (5) | 2 (9) | 2 (3) | |
| Conditioning regimen | | | | 0.088 |
| Myeloablative | 45 (56) | 9 (41) | 36 (62) | |
| Reduced intensity | 35 (44) | 13 (59) | 22 (38) | |
| HCT score pretransplant | | | | |
| KPS, median (IQR) | 90 (90–100) | 90 (80–90) | 90 (90–100) | 0.116 |
| HCT-CI, median (IQR) | 2 (0–3) | 2 (1–4) | 2 (0–3) | 0.171 |
| EBMT score, median (IQR) | 5 (3–6) | 5 (4–6) | 4 (3–5) | 0.092 |
| PAM score, median (IQR) | 21 (16–25) | 21 (19–25) | 21 (16–45) | 0.358 |
| Donor/recipient gender | | | | 0.008 |
| Female/female | 13 (16) | 2 (9) | 11 (19) | |
| Female/male | 15 (19) | 7 (32) | 8 (14) | |
| Male/female | 22 (28) | 1 (4) | 21 (36) | |
| Male/male | 30 (37) | 12 (55) | 18 (31) | |
| Donor/recipient CMV status | | | | 0.461 |
| Negative/negative | 26 (32) | 10 (46) | 16 (28) | |
| Negative/positive | 21 (26) | 4 (18) | 17 (29) | |
| Positive/negative | 7 (9) | 2 (9) | 5 (9) | |
| Positive/positive | 26 (33) | 6 (27) | 20 (34) | |
| HLA mismatch class | | | | 0.312 |
| Class I mismatch (HLA A, B, C) | 59 (74) | 18 (82) | 41 (71) | |
| Class II mismatch (HLA DRB1, DQB1) | 21 (26) | 4 (18) | 17 (29) | |
| HLA mismatch | | | | 0.397 |
| HLA A | 37 (46) | 12 (54) | 25 (43) | |
| HLA B | 12 (15) | 5 (23) | 7 (12) | |
| HLA C | 10 (13) | 1 (4) | 9 (16) | |
| HLA DR | 14 (17) | 3 (14) | 11 (19) | |
| HLA DQ | 7 (9) | 1 (5) | 6 (10) | |
| Graft source | | | | 0.947 |
| Bone marrow | 6 (8) | 1 (5) | 5 (9) | |
| Peripheral blood | 74 (92) | 21 (95) | 53 (91) | |
| CD34+ cell dose ($\times 10^6$/kg), median (IQR) | | | | |
| Bone marrow | 1.9 (1.2–2.6) | 2.6 (2.6–2.6) | 1.9 (0.9–2.6) | |
| Peripheral blood | 7.2 (6.0–9.4) | 7.0 (5.7–8.1) | 7.2 (5.6–9.4) | |
| Interval diagnosis to HCT (months, median, IQR) | 11 (6–38) | 13 (5–27) | 12 (6–41) | |
| Median follow-up in months, median (IQR) | 31 (22–59) | 24 (12–26) | 55 (37–64) | |

*CMV* cytomegalovirus, *EBMT* European Group for Blood and Marrow Transplantation, *HCT* hematopoietic stem cell transplantation, *HCT-CI* hematopoietic cell transplantation comorbidity index, *IQR* interquartile range, *KPS* Karnosfky performance status, *HLA* human leucocyte antigen, *PAM* pretransplant assessment of mortality, *PTCy* post-transplantation cyclophosphamide

progression were estimated accounting for competing risks [23]. Disease progression and death before GVHD were considered as competing risks in the estimation of GVHD incidence. Relapse was a competing risk in the estimation of the rate of NRM, and NRM was a competing risk in the estimation of relapse. Probabilities of PFS and OS were estimated using the Kaplan–Meier estimator. Log-rank test was used to compare the outcomes between the PTCy-based GVHD prophylaxis group and the control group. A Cox regression model was built including GVHD prophylaxis (ATG vs. PTCy) for all outcomes. Other variables considered were age, disease risk index, cytomegalovirus (CMV) serostatus, gender matching, and conditioning intensity. For each patient, follow-up was calculated from HCT until death from any cause or until last follow-up (censoring of survivors). These computations relied on standard software (SPSS Statistics v22, IBM, Chicago, IL, USA, and Stata SE v15, StataCorp LLC, College Station, TX, USA). All $p$ values are two-sided and the significance level was 0.05.

## Results

### Patient characteristics

Patient and transplant characteristics of the PTCy group and the control group with ATG were compared and are summarized in Table 1. The median age of all patients was 52 years (IQR, 42–62 years). Patients in the PTCy group were older (median, 58 years; IQR, 44–67 years) than those in the control group (median, 50 years; IQR, 39–60 years; $p = 0.037$). The most common underlying hematological disease was myeloid neoplasm in the PTCy group and control group (54% vs. 62%, respectively), followed by lymphoid neoplasms (41% vs. 33%, respectively; Table 1).

Myeloablative conditioning was used in 41% of patients in the PTCy group and 62% in the control group ($p = 0.088$). Regarding donor/recipient gender, the PTCy group had more male/male (55% vs. 31%) and female/male (32% vs. 14%), and fewer female/female (9% vs. 19%) and male/female (4% vs. 36%) donor/recipient pairs than the control group ($p = 0.008$). Moreover, both groups were similar in the frequencies of donor/recipient CMV status, type of HLA mismatch, graft source, and the CD34+ cell dose.

Due to the study design, median follow-up was shorter for the PTCy group (24 months (IQR, 12–26 months)) than the control group (55 months (IQR, 37–64 months)), and was 31 months (IQR, 22–59 months) for all surviving patients.

### Acute and chronic GVHD

As depicted in Table 2, the CI of acute GVHD grades II–IV in the PTCy group (15%, 95% CI 4–33) was significantly lower than that of the control group (50%, 95% CI 37–62, $p = 0.006$) (Fig. 1a; Table 2). The overall 1-year CI of cGVHD was lower in the PTCy group (26%, 95% CI 9–46) than the control (35%, 95% CI 22–49, $p = 0.137$) (respectively, limited cGVDH 21% [95% CI 6–40] vs. 24% [95% CI 12–38], $p = 0.736$; extensive cGVHD 5% [95% CI 0.4–21] vs. 18% [95% CI 8–30], $p = 0.256$), but the differences did not reach statistical significance (Fig. 2b; Table 2). Multivariate analyses for acute and chronic GVHD are depicted in Table 3.

### Engraftment and chimerism

All patients engrafted. The time to neutrophil engraftment in PTCy vs. the control group was comparable (median 14 days [IQR 13–17 days] vs. 16 days [IQR 14–18 days], respectively; $p = 0.107$). Similarly, the time to platelet engraftment did not differ significantly between groups (median 15 days [IQR 12–20 days] vs. 13 days [IQR 11–15 days], respectively; $p = 0.165$). Chimerism analyses at day 30 and after 3 months are depicted in Table 2.

### Survival, NRM, and relapse rate

The 1-year OS was significantly higher in the PTCy group than the control group (91% [95% CI 68–98] vs. 64% [95% CI 50–75], $p = 0.008$, respectively). Moreover, in the PTCy group, the 1-year PFS was higher than the control group (77% [95% CI 53–90] vs. 57% [95% CI 43–68], respectively) but failed to reach statistical significance ($p = 0.114$) (Fig. 2a, Table 2). Multivariate analyses for overall survival, PFS, and NRM are depicted in Table 3.

In the PTCy group, the 1-year NRM was 5% (95% CI 3–19) vs. 28% (95% CI 17–39, $p = 0.075$) in the control group (Fig. 3b; Table 2). Additionally, the 1-year CI of relapse did not differ between the PTCy-based GVHD prophylaxis group (18%, 95% CI 5–37) and the control group (15%, 95% CI 8–26; $p = 0.740$) (Fig. 3a, Table 2).

### Post-transplantation complications

Grade III–IV toxicities occurring during the first 360 days after allogeneic HCT and acute infections and CMV reactivations occurring within 100 days after HCT are depicted in Table 4. The most common toxicities of all patients were mucositis (13%) and hemorrhage (12%), followed by pulmonary (12%) and cardiac (10%) toxicities. Of note, hemorrhages and hepatic complications were more pronounced in the PTCy group, whereas mucositis, pulmonary, and cardiac toxicities were more evident in the control group.

Additionally, no differences were seen between the two GVHD prophylaxis groups concerning risk of acute bacterial, viral, and fungal infections; however, more fungal infections were seen in the control group within

**Table 2** Transplantation outcome of PTCy-based prophylaxis group and control group

| Variable | PTCy group ($n = 22$) | Control group ($n = 58$) | $p$ values |
|---|---|---|---|
| Acute GVHD cumulative incidence (95% CI), % | | | |
| Grades II–IV | 15 (4–33) | 50 (37–62) | 0.006 |
| Chronic GVHD 1-year cumulative incidence (95% CI), % | 26 (9–46) | 35 (22–49) | 0.137 |
| Limited chronic GVHD | 21 (6–40) | 24 (12–38) | 0.736 |
| Extensive chronic GVHD | 5 (0.4–21) | 18 (8–30) | 0.256 |
| 1-year OS rate (95% CI), % | 91 (68–98) | 64 (50–75) | 0.008 |
| 1-year PFS rate (95% CI), % | 77 (53–90) | 57 (43–68) | 0.114 |
| 1-year NRM cumulative incidence (95% CI), % | 5 (0.3–19) | 28 (17–39) | 0.075 |
| 1-year relapse cumulative incidence (95% CI), % | 18 (5–37) | 15 (8–26) | 0.740 |
| Time to neutrophil engraftment in days, median (IQR) | 14 (13–17) | 16 (14–18) | 0.107 |
| Time to platelet engraftment in days, median (IQR) | 15 (12–20) | 13 (11–15) | 0.165 |
| Chimerism at day 30, $n$ (%)[a] | | | |
| Mixed | 5/20 (25) | 4/51 (8) | |
| Full (100% donor DNA) | 15/20 (75) | 47/51 (92) | |
| Chimerism at day 100, $n$ (%)[b] | | | |
| Mixed | 3/18 (17) | 2/45 (4) | |
| Full (100% donor DNA) | 15/18 (83) | 43/45 (96) | |

*CI* confidence interval, *GVHD* graft-versus-host disease, *NRM* non-relapse-related mortality, *OS* overall survival, PFS progression-free survival, *PTCy* post-transplantation cyclophosphamide

[a] In the PTCy group, 1 patient died before chimerism assessment, and another patient had no chimerism analysis at day 30. Of the 20 remaining assessable patients, 5 patients had mixed chimerism at day 30. In the control group, 2 patients died before assessment and 5 patients had a missing chimerism analysis at day 30. Four of the remaining 51 assessable patients had mixed chimerism

[b] In the PTCy group, one patient had already died before day 30, and 3 patients had no chimerism analysis at day 100. Three of the remaining 18 patients in the PTCy group had mixed donor chimerism. In the control group, 8 patients died before chimerism assessment on day 100 and 5 patients did not have chimerism analysis performed between day 30 and 100. Only 2 of 45 assessable patients had a mixed donor chimerism

100 days post-transplant. The incidence of CMV reactivation was slightly higher in the control group, 40% compared to 32% in the PTCy group; however, these differences were not significant ($p = 0.518$).

## Immune reconstitution

Reconstitution of the immune system is depicted in Online Resource Table 2. Recovery of CD3+/CD4+ cells after HCT

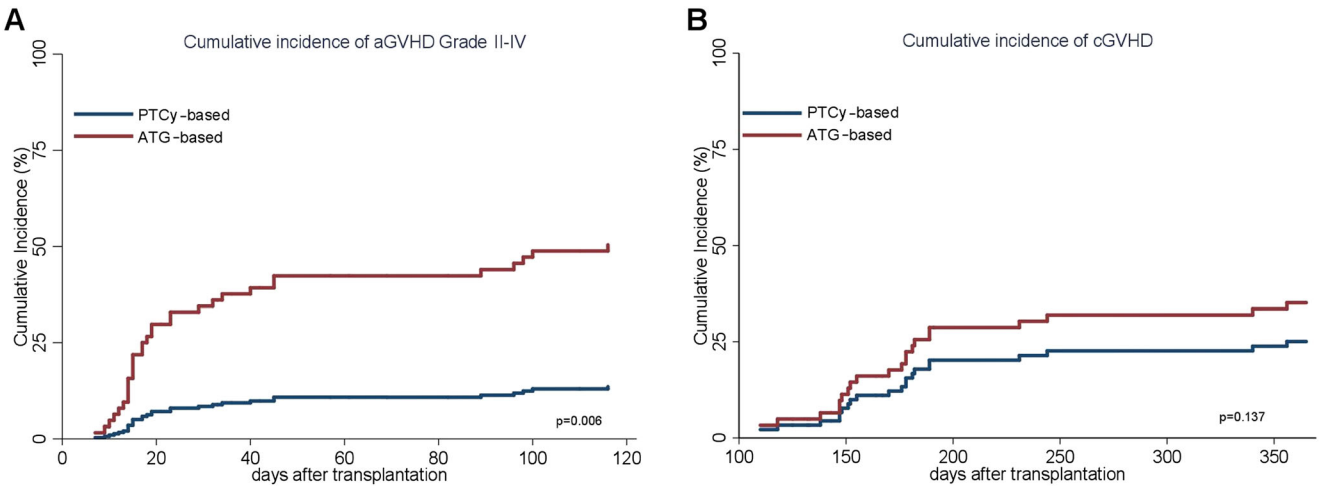


**Fig. 1** Cumulative incidence of graft-versus-host disease (GVHD). **a** Cumulative incidence of acute GVHD grades II–IV in the PTCy group (blue line) compared to the ATG group (red line). **b** Cumulative incidence of chronic GVHD at 1 year in the PTCy group (blue line) compared to the ATG group (red line)

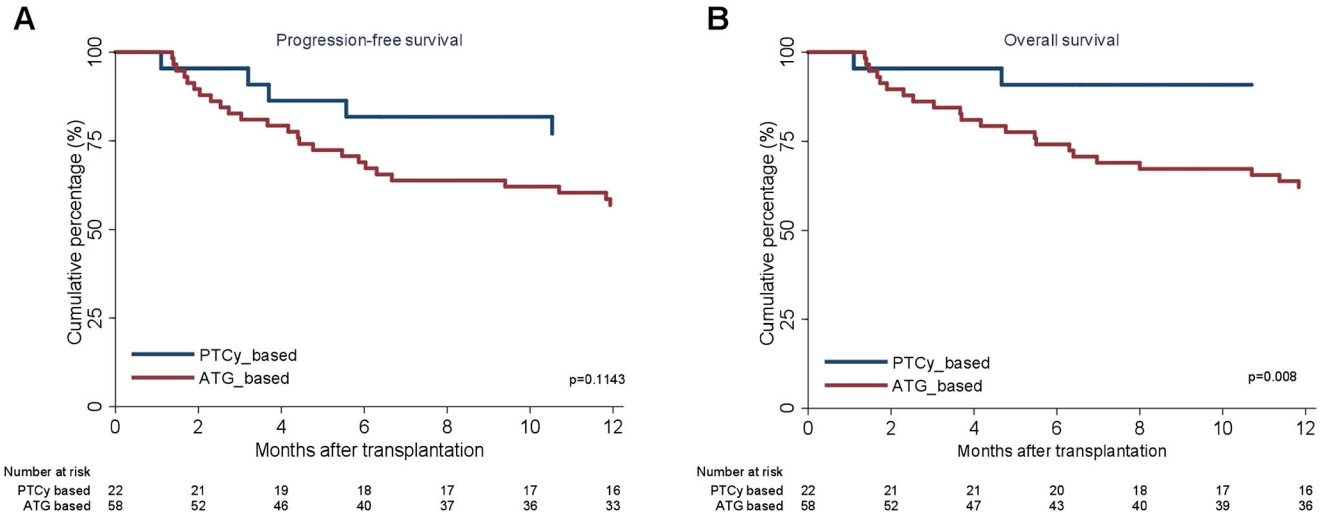


**Fig. 2** Kaplan–Meier curves for progression-free survival (PFS) and overall survival (OS). **a** Cumulative percentage of 1-year PFS in the PTCy group (blue line) compared to the ATG group (red line). **b** Cumulative percentage of 1-year OS in the PTCy group (blue line) compared to the ATG group (red line)

was significantly faster in the PTCy group than the control group: on day 100, 169 cells/μL (IQR 142–289) vs. 58 cells/μL (IQR 26–110), respectively ($p < 0.001$), and on day 365, 368 cells/μL (IQR 299–545) vs. 239 cells/μL (IQR 123–340), respectively ($p = 0.011$). In contrast, we observed no significant difference in the recovery of CD3+/CD8+ cell counts on day 100 between the two GVHD prophylaxis groups.

## Discussion

Identifying an HLA-matched donor remains key for patients with an indication for HCT [24]. Without a matched donor, options include haploidentical or mismatched unrelated donors; both are associated with increased GVHD risk and impaired overall outcome [3, 4]. The main unresolved challenges of HCT are the risk of GVHD and relapse after allogeneic HCT, which justifies focusing on the optimization and management of post-HCT complications, including the reduction of treatment-related toxicities and improvement of post-transplant strategies to address these important concerns. After encouraging results using PTCy for GVHD prophylaxis in the haploidentical HCT setting, several studies investigated the efficacy of PTCy at a dose of 50 mg/kg in patients undergoing MMUD HCT [5, 8–10, 15, 16, 25, 26]. Evaluation of lower PTCy doses, to limit toxicities, after allo-HCT is still lacking [27].

We used PTCy at a reduced dose of 40 mg/kg along with CyA and MMF for GVHD prophylaxis in 9/10 HLA-matched donor HCT and compared the outcomes to those of a control cohort of patients who received transplants from 9/10 MMUD with ATG-based GVHD prophylaxis. We selected this lower dose of cyclophosphamide to reduce its toxicity. In previously published studies, the CI rates of aGVHD grades II–IV with PTCy-based GVHD prophylaxis range from 19 to 37% in patients transplanted from 9/10 MMUD [5, 15, 16, 25, 26]. In our PTCy cohort, the CI of aGVHD grades II–IV was low (15%), translating to longer OS, lower incidence of chronic GVHD, and lower NRM. However, differences in the latter two outcomes were not significant and may be due to the limited size of the PTCy group.

Additionally, the superior 1-year OS of 91% was remarkably higher than published reports of 60 to 75% [15, 25]. The 1-year PFS was lower in the PTCy group but not significantly different from controls. These results indicate at least equal effectiveness of the PTCy-based regimen compared with the regimen of the control group and demonstrate 1-year PFS

**Table 3** Multivariable Cox proportional hazards model for different outcomes in patients treated with ATG (control group) vs. PTCy GVHD prophylaxis

| Outcome | Variables | HR (95% CI) | *p* value |
|---|---|---|---|
| Overall survival | PTCy vs. ATG* | 0.236 (0.069–0.812) | 0.022 |
| Acute GVHD | PTCy vs. ATG** | 0.230 (0.069–0.763) | 0.016 |
| Chronic GVHD | PTCy vs. ATG*** | 0.426 (0.132–1.372) | 0.153 |
| NRM | PTCy vs. ATG**** | 0.129 (0.017–0.996) | 0.050 |
| PFS | PTCy vs. ATG***** | 0.534 (0.288–1.253) | 0.150 |

All models with treatment groups included plus the following additional variables significantly or with borderline significance associated with outcome: *Overall survival adjusted for DRI (p = 0.105); **aGVHD adjusted for donor/recipient CMV serostatus (p = 0.097); ***cGVHD adjusted for donor/recipient gender (p = 0.041), CMV status (p = 0.010), age at HCT (p = 0.041); ****Non-relapse mortality (NRM) no additional significant variables, *****progression-free survival (PFS) adjusted for DRI (p = 0.101)

**Table 4** Transplant-related toxicities and infections

| Variable | All patients ($n = 80$) $n$ (%) | PTCy group ($n = 22$) $n$ (%) | Control group ($n = 58$) $n$ (%) | $p$ values |
|---|---|---|---|---|
| Toxicity grades III–IV until day 360 after HCT | | | | |
| Hemorrhage | 10 (12) | 3 (14) | 7 (12) | 0.850 |
| Mucositis | 10 (13) | 2 (9) | 8 (14) | 0.570 |
| Pulmonary toxicity | 10 (12) | 2 (9) | 8 (14) | 0.570 |
| Cardiac toxicity | 8 (10) | 1 (5) | 7 (13) | 0.317 |
| Hepatic toxicity | 4 (5) | 2 (9) | 2 (3) | 0.301 |
| Dermatologic toxicity | 3 (4) | 1 (5) | 2 (3) | 0.818 |
| Renal toxicity | 7 (9) | 0 (0) | 7 (12) | 0.088 |
| Acute infections until day 100 after HCT | | | | |
| Acute bacterial infection | 27 (34) | 8 (36) | 19 (33) | 0.761 |
| Acute viral infection | 28 (35) | 8 (36) | 20 (35) | 0.875 |
| Acute fungal infection | 4 (5) | 0 (0) | 4 (7) | 0.206 |
| CMV reactivation | 30 (38) | 7 (32) | 23 (40) | 0.518 |

*CMV* cytomegalovirus, *HCT* hematopoietic cell transplantation, *PTCy* post-transplantation cyclophosphamide

rates (77%) superior to the prior reports (PFS using PTCy regimens in transplant recipients of 9/10 MMUD ranged from 47 to 65%) [15, 25].

In contrast to prior reports of prolonged neutrophil engraftment after PTCy prophylaxis, with a reduced dose of 40 mg/kg PTCy, we observed a relatively brief median time to neutrophil engraftment of 14 days, shorter than the median of 16 days in the control group [5, 17, 27, 28]. It was noteworthy, in our study, that the graft source was primarily PBSC; however, in prior reports, the primary graft source was bone marrow of 9/10 MMUD [5, 15, 25]. Moreover, post-transplantation toxicity and infection rates in the PTCy group were lower than the control group, especially concerning organ damage, including pulmonary and cardiac toxicities, with no fungal infections and similar incidence of bacterial and viral infections in both GVHD prophylaxis groups.

Because post-HCT infectious complications are primarily responsible for greater NRM, rapid immune reconstitution is crucial to lower NRM. Mehta et al. described intensively the importance and the pathophysiology of the two phases of T cell reconstitution after allogeneic HCT [29]. We observed significantly higher CD4+ cell counts on day 100 and day 365 post-transplant in the PTCy group than the control group, which may be explained by the mechanism of action of PTCy, which selectively destroys early proliferating alloreactive donor and recipient T cells, particularly CD8+ T cells, and not CD4+ T cells [30]. In contrast, previous studies showed that ATG decreases CD4+ and CD8+ T cell counts [31]. Moreover, aGVHD impairs the thymus-dependent phase of

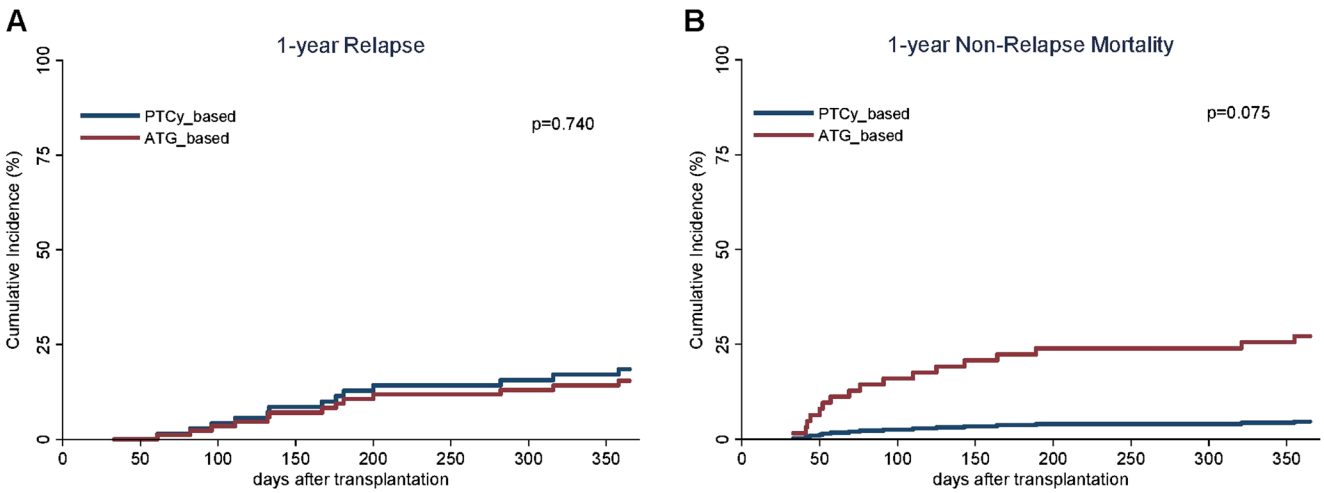


**Fig. 3** **a** 1-year cumulative incidence of relapse in the PTCy group (blue line) compared to the ATG group (red line). **b** 1-year cumulative incidence of non-relapse-related mortality in the PTCy group (blue line) compared to the ATG group (red line)

the T cell reconstitution, in which CD4+ T cells proliferate, by damaging thymic epithelial cells [29, 32, 33]. Consequently, a high CI of aGVHD impairs CD4+ T cell reconstitution. These mechanisms support our findings of a higher CD4+ T cell count in the PTCy group, as well as the significantly reduced CI of aGVHD in the PTCy group.

Our study has several limitations. In addition to the inherent flaws of a retrospective study, including a heterogeneous patient cohort and differences between both GVHD prophylaxis groups, the patient number in the PTCy group was limited. We cannot exclude selection bias, but all patients were treated consecutively. Due to limited patient numbers and imbalances between GVHD prophylaxis groups, results should be used for hypothesis generation and need to be evaluated within an enlarged prospective study.

In conclusion, our results are the first to prove the capability of PTCy using 2 days of a reduced dose of 40 mg/kg in 9/10 MMUD HCT. The results demonstrate PTCy as an effective method for reducing the risk of acute GVHD with favorable toxicities and significantly improved overall survival rates compared to ATG-based GVHD prophylaxis.

## Compliance with ethical standards

The Ethics Committee of Northwestern and Central Switzerland approved this study (EKNZ study number 2017-01019).

**Conflict of interest** The authors declare that they have no conflict of interest.

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