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ABSTRACT

Post-transplant cyclophosphamide (PT-Cy) is effective for graft-versus-host disease (GVHD) prophylaxis, but it may cause dose-dependent toxicities, particularly in frail patients. Therefore, we compared the outcomes with a reduced PT-Cy total dose (70 mg/kg) to those with the standard PT-Cy dose (100 mg/kg) in haploidentical hematopoietic cell transplantation (HCT) patients aged ≥65 years and those with cardiac comorbidities. All consecutive patients with a hematological malignancy receiving peripheral blood stem cells (PBSCs) after a thiotepa-based conditioning with low-dose antithymocyte globulin were included. Thirty-three patients received PT-Cy at 70 mg/kg and 25 at 100 mg/kg. PT-Cy dose reduction did not increase the risk of GVHD and was associated with faster neutrophil and platelet recovery and lower cumulative incidences of bacteremia (38% versus 72%, p=0.004) and cardiac complications (12% versus 44%, p=0.028). At 2 years, GVHD-free, relapse-free survival (GRFS) was higher with the reduced dose compared to the standard dose (60% versus 33%, p=0.04). In conclusion, reducing PT-Cy total dose to 70 mg/kg is a safe and valid approach for elderly patients and those with cardiac comorbidities underdoing haploidentical HCT with PBSCs and low-dose antithymocyte globulin. The reduced PT-Cy dose was associated with improved hematological count recovery, lower incidence of toxicities, and higher GRFS.

INTRODUCTION

Since the first allogeneic hematopoietic cell transplantation (HCT) 65 years ago, remarkable progress has been made in reducing transplant-related morbidity and mortality, thus expanding the transplant option to older or more fragile patients (1). New sources of grafts and donors can now be used, such as umbilical cord blood and haploidentical cells, allowing a donor to be found for nearly all patients (2). The development of post-transplant cyclophosphamide (PT-Cy) has contributed considerably to the increase in the number of haploidentical HCT performed, particularly in Europe and the United States. PT-Cy has emerged as a powerful platform to overcome the human leucocyte antigen (HLA) barrier and reduce the incidences of acute and chronic graft-versus-host disease (GVHD) (3). PT-Cy is now a successful and widely used GVHD prophylaxis, considered the standard of care for haploidentical HCT, and is actively being investigated for HCT with other donor sources (3–13). However, in the context of haploidentical HCT, PT-Cy is associated with toxicities and organ damage, such as delayed engraftment and immune recovery (14,15), cardiac events (16), and hemorrhagic cystitis (HC) (4,17–19), especially in elderly patients. While toxicities can be correlated with the total cyclophosphamide dose (20), few studies have compared the safety and efficacy of different PT-Cy doses.

The tolerance induced by cyclophosphamide has been the subject of intensive research since the 1960s (21–24). Based on the findings of experimental studies, PT-Cy was evaluated in a phase I clinical trial in haploidentical HCT (25), and then in two phase II studies. One was conducted in Seattle, in 28 patients receiving a single PT-Cy dose of 50 mg/kg on day +3, and one in Baltimore, in 40 patients receiving two PT-Cy doses of 50 mg/kg on days +3 and +4 (3). PT-Cy dose was not associated with any significant difference in terms of overall survival (OS), relapse incidence or incidence of acute GVHD. Only the cumulative incidence of

extensive chronic GVHD tended to be lower with two doses of PT-Cy at 50 mg/kg compared to one dose (hazard ratio 0.21, 95% confidence interval (CI 95%) 0.04-1.01, p=0.05).

Published in 2008 by Luznik et al., these studies established PT-Cy as a safe and efficient platform for GVHD prophylaxis after haploidentical HCT (3). Most studies and clinical applications have since used two doses of PT-Cy at 50 mg/kg. While advances have been accomplished to further improve the results of haploidentical HCT, such as the development of other conditioning regimens and the addition of antithymocyte globulin (ATG) (8,26–30), the optimal dose of PT-Cy to reduce the incidence of GVHD while lessening the risk of toxicities has yet to be defined. Recent studies have shown that PT-Cy total dose can be safely reduced to 80 mg/kg (divided into two doses of 40 mg/kg) without increasing the incidence of GVHD in a haploidentical HCT setting with peripheral blood stem cells (PBSC) (31,32). Compared to a PT-Cy total dose of 100 mg/kg, platelet and neutrophil recovery were improved with 80 mg/kg. However, the dose reduction did not allow a significant reduction of non-hematologic toxic events and was not associated with improved survival. Based on these empirical evidences and laboratory findings (24), we hypothesized that a more substantial dose reduction to 70 mg/kg might be needed to achieve significant benefits.

We therefore aimed to compare the outcomes with a reduced PT-Cy total dose of 70 mg/kg to those with the standard PT-Cy total dose (100 mg/kg) in elderly patients and in patients with cardiac comorbidities undergoing haploidentical HCT.

METHODS

Patient selection

We report a retrospective cohort study conducted in our center (Sorbonne University, Saint Antoine Hospital, Paris). All consecutive patients undergoing HCT from January 2014 to February 2022 were included according to the following criteria: (1) age ≥ 65 years or patients of any age with a history of a cardiac event, (2) hematological malignancy, (3) haploidentical HCT, (4) PBSC, and (5) thiotepa-based conditioning regimen with ATG. Since 2014, our center policy for GVHD prophylaxis in haploidentical HCT has been PT-Cy at 100 mg/kg, divided into two doses, to all patients receiving PBSC. Since 2020, all patients who are aged ≥ 65 years or have a cardiovascular event history have received PT-Cy at 35 mg/kg/day on day +3 and day +4. All patients provided written informed consent for the use of their data for clinical research, in accordance with the modified Declaration of Helsinki and the local Ethical Committee guidelines.

Transplantation procedures

Conditioning regimens were thiotepa-based with a reduced intensity conditioning (RIC) regimen (thiotepa, busulfan, and fludarabine) in patients with controlled disease or myelofibrosis, and Flamsa-like (thiotepa – etoposide – cyclophosphamide, followed by fludarabine – busulfan) sequential conditioning in patients with progressive refractory disease, as previously published (8,26,33,34). GVHD prophylaxis consisted of pretransplant low-dose ATG (2.5 mg/kg in RIC and 5 mg/kg in sequential conditioning regimens), intravenous cyclosporine A, mycophenolate mofetil, and PT-Cy in all patients. Cyclosporine A was progressively tapered, starting from days +60 to +90 (depending on the disease risk and the occurrence of GVHD), and mycophenolate mofetil was stopped on day +35 after HCT.

Supportive care

All patients underwent systematic cardiac monitoring and received the same prophylaxis of cyclophosphamide-induced cystitis, as detailed in the *Supplementary Material*. Patients received recombinant human granulocyte colony-stimulating factor from the day after the second dose of PT-Cy to engraftment. Antimicrobial prophylaxis consisted of valacyclovir (or acyclovir) and fluconazole initiated at the start of the conditioning regimen, while amoxicillin and trimethoprim-sulfamethoxazole (or atovaquone) were started after engraftment. From March 2020, letermovir was given orally to cytomegalovirus (CMV)-seropositive patients starting on day +5 and continued until day +100 after HCT. Blood samples were monitored by quantitative real-time polymerase chain reaction (PCR) twice a week for CMV, and once a week for BK virus, Epstein-Barr virus (EBV), and *Toxoplasma gondii* until at least day +100 after HCT. Monitoring of BK virus in urine samples once a week by PCR, and invasive fungal infections twice a week by galactomannan antigen and (1,3)-ß-d-glucan tests, was also carried out during the same time period. PCR assays were repeated more frequently according to biological and clinical signs, as previously reported (18,19,35).

Definitions of endpoints

Neutrophil and platelet recovery were defined according to established guidelines (36). HCT-specific comorbidity index (HCT-CI) (37) and disease risk index (38) were assessed as previously published. Acute and chronic GVHD were diagnosed and graded according to standard criteria (39–41). OS was defined as the probability of survival (irrespective of the disease state), progression-free survival (PFS) as survival with no evidence of relapse or progression, GVHD-free, relapse-free survival (GRFS), as being alive with neither acute grade III-IV GVHD nor chronic GVHD requiring immunosuppressive systemic treatment, and without disease recurrence (42), and non-relapse mortality (NRM) as death without

evidence of relapse. The definitions of hematological count recovery, BK virus-associated HC, cardiac toxicity, and cytokine release syndrome (CRS) are detailed in the *Supplementary Material*.

Statistical analysis

Patients' characteristics were compared using the Mann-Whitney test for continuous variables, and the chi-squared or Fisher's exact test for categorical variables. Cumulative incidence was used to estimate the endpoints of GVHD, relapse, NRM, platelet recovery, HC, CMV reactivation, EBV increased viral load > 5000 IU/mL, fungal infections, and cardiac events to accommodate competing risks. The competing events were relapse and death to study GVHD, relapse for NRM, and death for infectious or cardiac complications. Probabilities of OS, PFS, and GRFS were calculated by the Kaplan–Meier method. Univariate comparisons were performed using log-rank and Gray's tests for cumulative incidence functions. Endpoints were censored at 2 years for all comparisons to take into account the difference in follow-up between the 2 groups. Results are expressed as the estimated percentage with a 95% CI. All tests were 2-sided. The type I error rate was fixed at 0.05 for the determination of factors associated with time-to-event outcomes. Statistical analyses were performed with R 4.0.2 (R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/).

RESULTS

Patient characteristics and transplantation modalities

Fifty-eight patients met the inclusion criteria: 33 received PT-Cy at 70 mg/kg and 25 at 100 mg/kg. Patient characteristics and transplantation modalities according to PT-Cy dose are outlined in Table 1. No significant difference was found between the two groups (PT-Cy at 70 mg/kg versus 100 mg/kg) in terms of age, sex, HCT-CI score, cardiovascular risk factors, type of hematological disease, disease risk index, disease status, type of conditioning regimen, CD34+ cell dose, and cyclosporine A treatment duration.

Hematological recovery

All patients achieved neutrophil engraftment, except one who died during the neutropenic phase, 12 days after HCT. Neutrophil and platelet recovery were significantly improved with PT-Cy at 70 mg/kg compared to 100 mg/kg. The median time from HCT to neutrophil recovery was 16 days (range, 13-19 days) with PT-Cy at 70 mg/kg, and 19 days (1-24 days) with 100 mg/kg (p=0.006). At days +30, +60, and +90 after HCT, the cumulative incidences of platelet recovery above 50 x 10^9/L were 75%, 88%, and 91% with 70 mg/kg of PT-Cy, respectively, compared to 36%, 64%, and 64% with 100 mg/kg of PT-Cy, respectively (p=0.011). The differences were not significant for platelet recovery above 20 x 10^9/L (p=0.07) (Table 2).

Transplantation-related cardiac and infectious complications

The main transplantation-related complications are summarized in Table 3. The 2-year cumulative incidence of cardiac events after HCT was significantly lower in patients who received 70 mg/kg of PT-Cy (12% versus 44%, p=0.028). Four cardiac events occurred between the graft infusion (day 0) and PT-Cy first administration (three in patients receiving

70 mg/kg of PT-Cy and one in patients receiving 100 mg/kg). Cardiac events occurred between PT-Cy first administration (day +3) and 2 years after HCT in one (3%) patient receiving PT-Cy at 70 mg/kg, and in 10 (40%) patients receiving PT-Cy at 100 mg/kg. The main complication observed was LVSD (2-year cumulative incidences: 9% versus 24% with 70 mg/kg and 100 mg/kg of PT-Cy, respectively, p=0.15). Among patients receiving PT-Cy at 70 mg/kg, only one (3%) patient developed LVSD after PT-Cy administration. This case occurred on day +6 after HCT and was associated with arrhythmia, although no clinical symptoms of heart failure were observed. In contrast, LSVD occurred in five (20%) patients following the administration of 100 mg/kg of PT-Cy, between days +6 and +38 after HCT, including two cases that were asymptomatic. The 2-year cumulative incidence of acute pulmonary edema was 3% with 70 mg/kg of PT-Cy and 20% with 100 mg/kg (p=0.041). Other cardiac events included arrhythmia (n=4, 7%), pericardial effusion (n=8, 14%) and acute coronary syndrome (n=2, 3%) with no significant difference with respect to the PT-Cy dose for these events. Cardiac events resolved in most cases (9 out of 11 with PT-Cy at 100 mg/kg and 4 out of 4 with 70 mg/kg) but was the direct cause of death of one patient who received 100 mg/kg of PT-Cy. The cumulative incidence of CRS was 48% in patients receiving 70 mg/kg of PT-Cy (grade 1: 13 patients, grade 2: 3 patients) and 40% in patients receiving 100 mg/kg (grade 1: 6 patients, grade 2: 3 patients) (p=0.5991). No case of grade 3-4 CRS were observed, and tocilizumab was not used.

At two years, the cumulative incidence of post-HCT bacteremia was significantly lower with PT-Cy at 70 mg/kg than with 100 mg/kg (38% versus 72%, p=0.004). Bacteremia was caused by gram-negative bacteria in 4 (12%) patients receiving PT-Cy reduced dose and 10 (40%) patients receiving the standard dose (p=0.03). The cumulative incidence of BK virus-associated HC was 12% with 70 mg/kg of PT-Cy compared to 28% with 100 mg/kg of PT-Cy

(p=0.11). HC requiring bladder irrigation (grade ≥ 3) occurred in two (6%) and five (20%) patients treated with 70 mg/kg and 100 mg/kg of PT-Cy, respectively. The cumulative incidence of CMV reactivation was lower in patients who received 70 mg/kg of PT-Cy (19% versus 48%). However, letermovir prophylaxis for CMV was not given to patients who received 100 mg/kg of PT-Cy, while it was given to 28 (85%) patients receiving 70 mg/kg of PT-Cy. There was no significant difference in terms of EBV increase of viral load or fungal infections with respect to PT-Cy dose. Only one patient, who had received PT-Cy at 70 mg/kg, developed post-transplant lymphoproliferative disorder.

Graft-versus-host disease

At day +180, the cumulative incidences of acute grade II-IV and grade III-IV GVHD were 18% and 0% with PT-Cy at 70 mg/kg compared to 17% and 5% with 100 mg/kg, respectively (p=0.94 for grade II-IV and p=0.23 for grade III-IV acute GVHD). There was no difference in the cumulative incidence of chronic GVHD at 2 years with 27% and 29% for PT-Cy at 70 mg/kg and 100 mg/kg, respectively (p=0.95) (Table 4). Moderate to severe chronic GVHD occurred in five (9%) patients and required immunosuppressive systemic therapy in six (10%) patients, with no significant difference with respect to PT-Cy dose.

Relapse and survival outcomes

Disease progression or relapse occurred in 12 (21%) patients, within a median time of 8 months (range, 1-28). The 2-year relapse incidence was 30% with 70 mg/kg of PT-Cy compared to 21% with 100 mg/kg (p=0.89) (Table 4). At last-follow-up, 24 (41%) patients had died. The causes of deaths were attributed to hematological malignancy relapse (n=4, 12%), infection (n=5, 15%), and neurological complication (n=1, 3%) in patients receiving PT-Cy at 70 mg/kg, and hematological malignancy relapse (n=6, 24%), infection (n=4, 16%), GVHD (n=1, 4%), neurological complication (n=1, 3%), veno-occlusive disease (n=1, 3%),

and cardiac failure (n=1, 3%) in patients receiving PT-Cy at 100 mg/kg. The median follow-up among surviving patients was 17 months (interquartile range [IQR], 13-20) and 59 months (IQR, 51-66) in patients receiving PT-Cy at 70 mg/kg and 100 mg/kg, respectively. At 2 years, NRM (18% versus 33%, p=0.13), PFS (65% versus 46%, p=0.09), and OS (68% versus 52%, p=0.31) were similar with the reduced and the standard PT-Cy doses. However, GRFS was higher with 70 mg/kg of PT-Cy (60% versus 33%, p=0.04, Figure 1). Finally, the probability of survival at 2 years without severe GVHD, cardiac event, HC, or relapse, was improved with PT-Cy at 70 mg/kg (50% versus 25%, p=0.04).

DISCUSSION

The major findings of this study are that reducing the PT-Cy dose to 70 mg/kg is not only safe but also associated with higher GRFS in patients aged over 65 years or with cardiac comorbidities undergoing PBSC haploidentical transplantation compared to a standard PT-Cy total dose of 100 mg/kg. This represents a significant improvement for the patients, as GRFS is a composite endpoint that reflects health status and quality of life more precisely than OS or PFS. The reduction in PT-Cy resulted in remarkable decreases in several toxicities commonly induced or associated with high-dose cyclophosphamide, namely hematological count recovery, cardiotoxicity, BK virus-associated HC, and bacteremia.

Previous studies have reported that cyclophosphamide-induced cardiac events may be dose-dependent (43,20) and that PT-Cy may be associated with a higher incidence of such events in haploidentical HCT (16). In the present study, reducing the dose of PT-Cy to 70 mg/kg was associated with a significantly lower cumulative incidence of cardiac events, while no significant benefits had been reported with PT-Cy at 80 mg/kg (32). The incidence of cardiac

events, particularly LVSD, was high among patients receiving 100 mg/kg of PT-Cy, as expected in such a high-risk population. Notably, the observed incidence is in line with the findings of a large retrospective study involving 331 patients, which established PT-Cy and older age as major risk factors for early cardiac events (16), and another study reporting a 43% incidence of congestive heart failure in high-risk patients (44). Besides the high-risk characteristics of the cohort, routine cardiac monitoring allowed for the detection of asymptomatic cardiac events, which likely contributed to increase to overall incidence of observed cardiac events. No difference in terms of cardiovascular risk factors, history of cardiac events, or anthracycline exposure between patients receiving PT-Cy at 70 mg/kg and 100 mg/kg was found to contribute to the reduction of cardiac complications. Overall, reducing PT-Cy translated into significant improvement in terms of cardiac morbidity, and might also have favorably impacted survival since cardiac complications are associated, directly or not, with lower OS (16).

Allogeneic HCT outcomes are substantially related to the achievement of an acceptable hematological and immune restoration. Neutrophil engraftment constitutes an essential step in the early phase after HCT, as prolonged neutropenia is associated with extended in-hospital stay and increased incidences of severe infections and NRM (45). Although hematopoietic stem cells are relatively spared by cyclophosphamide, due to high levels of expression of aldehyde dehydrogenase (46), haploidentical HCT with PT-Cy is associated with a significantly longer time to neutrophil and platelet recovery (6). Reducing PT-Cy dose to 70 mg/kg allowed faster hematological recovery for both neutrophils and platelets, compared to 100 mg/kg of PT-Cy. The shorter neutropenic phase might also contribute to the lower incidence of bacteremia observed with PT-Cy dose reduction. In addition, delayed neutrophil

and platelet recovery can be risk factors, along with PT-Cy dose, GVHD, conditioning regimen intensity, for the development of more severe BK virus-associated HC (47,48).

The latter is one of the major causes of morbidity and prolonged in-hospital stay after HCT and is a possible cause of death. The reported incidence is 19 to 75% in haploidentical HCT with PT-Cy (17–19,49). Reducing PT-Cy dose to 70 mg/kg was associated with a lower cumulative incidence of BK virus-associated HC (12% versus 28% with 100 mg/kg of PT-Cy). Moreover, the incidence of HC requiring bladder irrigation was reduced by 3-fold. The limited number of events may explain why this considerable difference did not reach statistical significance. Although this result needs to be confirmed in larger cohort studies, it would allow a meaningful improvement in terms of quality of life for the patients.

In a high-risk population with a median age of 69 years, HCT-CI ≥ 2 in 67% of patients, active disease in 70%, and high or very-high disease risk index in 64%, the 2-year incidence of NRM was relatively low at 18% with 70 mg/kg of PT-Cy. Interestingly, the 100-day NRM was nearly 3-fold lower in patients receiving 70 mg/kg PT-Cy compared to 100 mg/kg, and overall NRM continued to climb in the latter to 33% at 2 years. Our results in patients receiving 70 mg/kg of PT-Cy are in line with the NRM reported in studies using PT-Cy at 80 mg/kg (NRM of 16% to 18%) (31,32), and compare favorably with most studies using 100 mg/kg of PT-Cy in a haploidentical setting for elderly patients (NRM of 13% to 38%) (50–53).

One of the main safety concerns when reducing PT-Cy dose is its impact on GVHD. In our study, that reduction was not associated with a higher incidence of acute or chronic GVHD of any grade. Patients receiving 70 mg/kg of PT-Cy had lower incidences of acute grade II-IV

GVHD and chronic GVHD than those receiving 80 mg/kg of PT-Cy in other studies (24-32% of acute grade II-IV GVHD and 28-41% of chronic GVHD) (31,32). Patients receiving PT-Cy at either 70 mg/kg or 100 mg/kg actually had lower incidences of GVHD compared to most studies using PBSC in a haploidentical setting with PT-Cy (36-44% of acute grade II-IV GVHD and 19-41% of chronic GVHD) (54–61). This result may be explained by the addition of pre-transplant low-dose ATG to PT-Cy for all patients, which can reduce the risk of GVHD, as shown in several studies (8,26,34,27). Regarding survival, reducing PT-Cy dose tended to result in higher PFS (p=0.09) and was significantly associated with higher GRFS. The 2-year OS (68%), PFS (65%), and GRFS (60%) in patients receiving 70 mg/kg of PT-Cy was higher than in studies with 100 mg/kg of PT-Cy in a haploidentical setting for elderly patients (OS of 39 to 55%, PFS of 35 to 58%, and PFS of 26 to 32%) (50–53). These findings confirm the safety and efficacy of reducing PT-Cy total dose to 70 mg/kg in this fragile population.

Although our study is limited by the bias of its retrospective nature, the two comparative groups did not have significant differences in terms of age, sex, cardiovascular risk factors, HCT-CI score, type of hematological disease, disease risk index, type of conditioning regimen, CD34+ cell dose, and cyclosporine A treatment duration. Another limitation is the lack of temporal overlap between the two PT-Cy dose groups due to differences in the year of transplant. However, key transplant modalities such as conditioning regimens, GVHD prophylaxis associated with PT-Cy, supportive care procedures, and monitoring of infections and toxicities remained consistent throughout the study period. The only exception was the use of letermovir, which resulted in a lower incidence of CMV reactivation. The latter had no significant impact on platelet recovery, chronic GVHD, or GRFS but was associated with a higher risk of acute grade II-IV GVHD (p=0.05) (Supplementary Table S1). The number of

patients who received a reduced dose of PT-Cy is also limited, which might have precluded the observation of statistically significant improvements in terms of PT-Cy-associated toxicity, such as HC.

In conclusion, reducing PT-Cy dose to 70 mg/kg is a safe and valid approach in elderly patients and those with cardiac comorbidities underdoing haploidentical HCT with PBSC and low-dose ATG. Compared to PT-Cy at 100 mg/kg, neutrophil and platelet recoveries were improved, cumulative incidences of bacteremia, BK virus-associated HC, and cardiac complications were reduced, the risk of acute and chronic GVHD was not increased, and GRFS was higher with PT-Cy at 70 mg/kg. These encouraging findings provide compelling evidence to support the initiation of a prospective trial to evaluate the administration of PT-Cy at a total dose of 70 mg/kg in this specific population. Further studies should also investigate the potential benefits of reducing PT-Cy dose in patients aged younger than 60 years undergoing haploidentical or even HLA-matched donor transplantation. These studies may contribute to shed the light on crucial outcomes associated with the impact of PT-Cy dose reduction, such as immune reconstitution and the overall quality of life experienced by transplanted patients.

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Author contributions

RD designed the study, recruited patients, collected, assembled, and analyzed data, performed the statistical analysis, and wrote the manuscript. MM designed the study, supervised research, analyzed data, and commented on the manuscript. SE, AC, and EC helped design the study, performed cardiac evaluations of the patients, analyzed data, and commented on the manuscript. ML analyzed data, performed the statistical analysis, and commented on the manuscript. AB and TL contributed to patient care and commented on the manuscript. FM, EB, AB, SS, RB, MC, ZVW, and OL recruited patients and commented on the manuscript. All authors reviewed the manuscript and approved its submission for publication purposes.

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Availability of data and materials

The dataset supporting the conclusions of this article is available upon request to the corresponding author.

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Figure Legends

Figure 1. Kaplan-Meier estimates of graft-versus-host disease-free, relapse-free survival (GRFS) according to the total dose of post-transplant cyclophosphamide (PT-Cy).

Table 1. Patient characteristics and modalities of transplantation

	PT-Cy 70 mg/kg (n=33)	**PT-Cy 100 mg/kg** (n=25)	**p-value**
Recipient age (years), median (range)	69 (21-74)	67 (15-76)	0.25
Recipient gender (male/female), n (%)	18 (55)/15 (45)	17 (68)/8 (32)	0.3
Diagnosis, n (%)			0.3
Acute myeloid leukemia	20 (61)	17 (68)	
Myelodysplastic syndrome	8 (24)	3 (12)	
Myeloproliferative neoplasm	2 (6)	0 (0)	
Lymphoma	3 (9)	5 (20)	
Complete remission at transplant, n (%)	10 (30)	8 (32)	0.89
Disease risk index, n (%)			0.68
Intermediate	12 (36)	10 (40)	
High	19 (58)	12 (48)	
Very-high	2 (6)	3 (12)	
HCT-specific comorbidity index[ψ]			0.28
Median (range)	2 (0-5)	2 (0-3)	
Missing, n (%)	1 (3)	0 (0)	
Cardiovascular risk factors, n (%)			
None	2 (6)	1 (4)	1
Male > 50 years or female > 60 years old	27 (82)	21 (84)	0.83
Obesity	6 (18)	4 (16)	1
Hypertension	8 (24)	9 (36)	0.33
Dyslipidemia	2 (6)	3 (12)	0.42
Smoking[‡]	14 (42)	10 (40)	0.85
Diabetes	3 (9)	2 (8)	1
Anthracycline exposure before HCT, n (%)	21 (64)	20 (80)	0.18
Cardiac event prior to HCT*, n (%)	12 (36)	14 (56)	0.14
LVSD at transplant, n (%)	8 (24)	4 (16)	0.53
Conditioning regimen, n (%)			0.68
RIC: Thiotepa – busulfan – fludarabine	15 (45)	10 (40)	
Sequential thiotepa-based	18 (55)	15 (60)	
CD34+ cell dose (10^6/kg), median (range)	6.97 (1.91-11.17)	7.38 (3.28-11.21)	0.8
GVHD prophylaxis, n (%)			
Cyclosporine A, MMF, and ATG	33 (100)	25 (100)	1
Cyclosporine A treatment duration, median (IQR)	141 (114-178)	135 (49-185)	0.38

Abbreviations: PT-Cy: post-transplant cyclophosphamide, n: number of patients, HCT: hematopoietic cell transplant, LVSD: left ventricular systolic dysfunction, RIC: reduced intensity conditioning, GVHD: graft-versus-host disease, MMF: mycophenolate mofetil, ATG: antithymocyte globulin, IQR: interval quartile range.

[ψ]As previously published by Armand et al. (38)

[‡]Smoking was defined as ever-regular-, occasional- or ex-smoker, and compared to never-smoker

*Cardiac events included LVSD (n=12), coronary syndromes (n=7), arrythmia (n=6), or pericardial effusion (n=1).

Table 2. Neutrophil and platelet engraftment according to PT-Cy dose

	PT-Cy at 70 mg/kg	PT-Cy at 100 mg/kg	p-value
Neutrophil engraftment, n (%)	32 (97)	25 (100)	1
Median time to ANC > 0.5 x 10^9/L, days (range)	16 (13-19)	19 (1-24)	0.006
Platelet count > 20 x 10^9/L, % [95% CI]			
At day + 30	94% [74-99]	77% [52-90]	
At day + 60	94% [74-99]	86% [60-96]	
At day + 90	97% [58-100]	86% [60-96]	0.07
Platelet count > 50 x 10^9/L, % [95% CI]			
At day + 30	75% [55-87]	36% [18-55]	
At day + 60	88% [68-96]	64% [41-80]	
At day + 90	91% [71-97]	64% [41-80]	0.011

Abbreviations: PT-Cy: post-transplant cyclophosphamide, ANC: absolute neutrophil count, 95% CI: 95% confidence interval.

Table 3. Cumulative incidence of cardiac and infectious complications at two years according to PT-Cy dose

	PT-Cy at 70 mg/kg		PT-Cy at 100 mg/kg		p-value
	n	% [95% CI]	n	% [95% CI]	
Cardiac event after HCT	4	12% [4-26]	11	44% [24-63]	0.028
Bacteremia	13	38% [21-54]	18	72% [49-86]	0.004
BK virus hemorrhagic cystitis	4	12% [4-26]	7	28% [12-46]	0.11
CMV reactivation‡	6	19% [8-34]	12	48% [27-66]	0.014
EBV increased viral load	14	43% [25-59]	14	56% [34-73]	0.25
Fungal infection*	5	16% [6-30]	3	12% [3-28]	0.67

Abbreviations: PT-Cy: post-transplant cyclophosphamide, n: number of patients, 95% CI: 95% confidence interval, HCT: hematopoietic cell transplantation, CMV: cytomegalovirus, EBV: Epstein-Barr virus.

‡CMV prophylaxis with letermovir was administrated to 28 (85%) patients who received PT-Cy at 70 mg/kg and to none of those who received PT-Cy at 100 mg/kg ($p < 0.001$).

*Fungal infections included possible or probable invasive aspergillosis in 7 (12%) patients and toxoplasmosis in 2 (3%) patients (one patient had both invasive aspergillosis and toxoplasmosis).

692 **Table 4.** Cumulative incidence of graft-versus-host disease and clinical outcomes according
693 to PT-Cy dose

	PT-Cy at 70 mg/kg % [95% CI]	**PT-Cy at 100 mg/kg** % [95% CI]	**p-value**
Acute GVHD at day +180			
Grade II-IV	18% [7-33]	17% [5-34]	0.94
Grade III-IV	0%	5% [0-19]	0.23
Chronic GVHD at 2 years			
All grades	27% [12-43]	29% [12-49]	0.95
Requiring systemic treatment	6% [1-19]	17% [5-35]	0.36
Non-relapse mortality			
At day + 100	9% [2-22]	25% [10-44]	
At 2 years	18% [6-36]	33% [16-52]	0.13
Relapse incidence at 2 years	30% [7-58]	21% [7-39]	0.89
Progression-free survival at 2 years	65% [43-80]	46% [26-64]	0.09
Overall survival at 2 years	68% [30-78]	52% [31-59]	0.31
GRFS at 2 years	60% [39-75]	33% [16-52]	0.04

694 *Abbreviations*: PT-Cy: post-transplant cyclophosphamide, 95% CI: 95% confidence interval, GVHD: graft-
695 versus-host disease, GRFS: GVHD-free, relapse-free survival.
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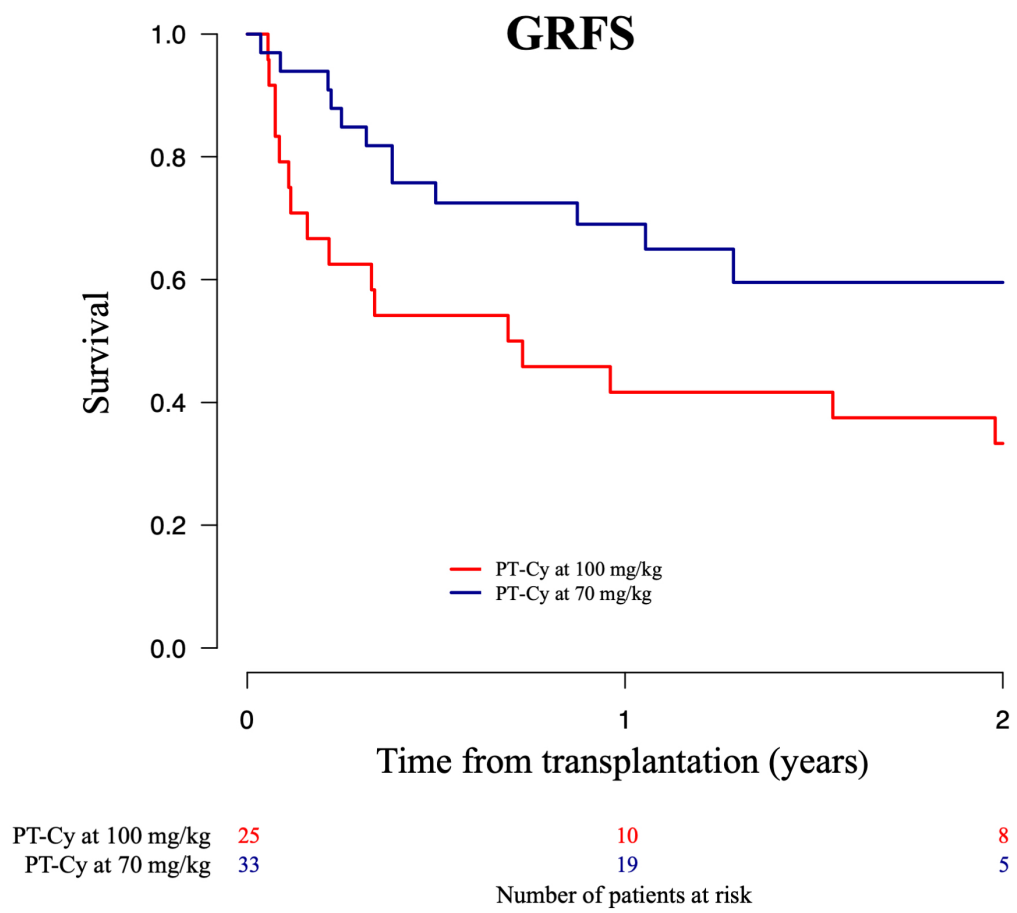
718 **Figure 1.**



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