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# Reduced Post-Transplant Cyclophosphamide Dose with Antithymocyte Globulin in Peripheral Blood Stem Cell Haploidentical Transplantation

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1 **ABSTRACT**

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

19 **INTRODUCTION**

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

36  
37 The tolerance induced by cyclophosphamide has been the subject of intensive research since  
38 the 1960s (21–24). Based on the findings of experimental studies, PT-Cy was evaluated in a  
39 phase I clinical trial in haploidentical HCT (25), and then in two phase II studies. One was  
40 conducted in Seattle, in 28 patients receiving a single PT-Cy dose of 50 mg/kg on day +3, and  
41 one in Baltimore, in 40 patients receiving two PT-Cy doses of 50 mg/kg on days +3 and +4  
42 (3). PT-Cy dose was not associated with any significant difference in terms of overall survival  
43 (OS), relapse incidence or incidence of acute GVHD. Only the cumulative incidence of

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

46  
47 Published in 2008 by Luznik et al., these studies established PT-Cy as a safe and efficient  
48 platform for GVHD prophylaxis after haploidentical HCT (3). Most studies and clinical  
49 applications have since used two doses of PT-Cy at 50 mg/kg. While advances have been  
50 accomplished to further improve the results of haploidentical HCT, such as the development  
51 of other conditioning regimens and the addition of antithymocyte globulin (ATG) (8,26–30),  
52 the optimal dose of PT-Cy to reduce the incidence of GVHD while lessening the risk of  
53 toxicities has yet to be defined. Recent studies have shown that PT-Cy total dose can be safely  
54 reduced to 80 mg/kg (divided into two doses of 40 mg/kg) without increasing the incidence of  
55 GVHD in a haploidentical HCT setting with peripheral blood stem cells (PBSC) (31,32).  
56 Compared to a PT-Cy total dose of 100 mg/kg, platelet and neutrophil recovery were  
57 improved with 80 mg/kg. However, the dose reduction did not allow a significant reduction of  
58 non-hematologic toxic events and was not associated with improved survival. Based on these  
59 empirical evidences and laboratory findings (24), we hypothesized that a more substantial  
60 dose reduction to 70 mg/kg might be needed to achieve significant benefits.

61  
62 We therefore aimed to compare the outcomes with a reduced PT-Cy total dose of 70 mg/kg to  
63 those with the standard PT-Cy total dose (100 mg/kg) in elderly patients and in patients with  
64 cardiac comorbidities undergoing haploidentical HCT.

65  
66  
67  
68

69 **METHODS**

70 **Patient selection**

71 We report a retrospective cohort study conducted in our center (Sorbonne University, Saint  
72 Antoine Hospital, Paris). All consecutive patients undergoing HCT from January 2014 to  
73 February 2022 were included according to the following criteria: (1) age  $\geq$  65 years or  
74 patients of any age with a history of a cardiac event, (2) hematological malignancy, (3)  
75 haploidentical HCT, (4) PBSC, and (5) thiotepa-based conditioning regimen with ATG. Since  
76 2014, our center policy for GVHD prophylaxis in haploidentical HCT has been PT-Cy at 100  
77 mg/kg, divided into two doses, to all patients receiving PBSC. Since 2020, all patients who  
78 are aged  $\geq$  65 years or have a cardiovascular event history have received PT-Cy at 35  
79 mg/kg/day on day +3 and day +4. All patients provided written informed consent for the use  
80 of their data for clinical research, in accordance with the modified Declaration of Helsinki and  
81 the local Ethical Committee guidelines.

82

83 **Transplantation procedures**

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

93

94 **Supportive care**

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

109

110 **Definitions of endpoints**

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

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

122

### 123 **Statistical analysis**

124 Patients' characteristics were compared using the Mann-Whitney test for continuous  
125 variables, and the chi-squared or Fisher's exact test for categorical variables. Cumulative  
126 incidence was used to estimate the endpoints of GVHD, relapse, NRM, platelet recovery, HC,  
127 CMV reactivation, EBV increased viral load > 5000 IU/mL, fungal infections, and cardiac  
128 events to accommodate competing risks. The competing events were relapse and death to  
129 study GVHD, relapse for NRM, and death for infectious or cardiac complications.

130 Probabilities of OS, PFS, and GRFS were calculated by the Kaplan–Meier method. Univariate  
131 comparisons were performed using log-rank and Gray's tests for cumulative incidence  
132 functions. Endpoints were censored at 2 years for all comparisons to take into account the  
133 difference in follow-up between the 2 groups. Results are expressed as the estimated  
134 percentage with a 95% CI. All tests were 2-sided. The type I error rate was fixed at 0.05 for  
135 the determination of factors associated with time-to-event outcomes. Statistical analyses were  
136 performed with R 4.0.2 (R Core Team (2020). R: A language and environment for statistical  
137 computing. R Foundation for Statistical Computing, Vienna, Austria. URL [https://www.R-](https://www.R-project.org/)  
138 [project.org/](https://www.R-project.org/)).

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143



144 **RESULTS**

145 **Patient characteristics and transplantation modalities**

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

152

153 **Hematological recovery**

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

163

164 **Transplantation-related cardiac and infectious complications**

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

169 70 mg/kg of PT-Cy and one in patients receiving 100 mg/kg). Cardiac events occurred  
170 between PT-Cy first administration (day +3) and 2 years after HCT in one (3%) patient  
171 receiving PT-Cy at 70 mg/kg, and in 10 (40%) patients receiving PT-Cy at 100 mg/kg. The  
172 main complication observed was LVSD (2-year cumulative incidences: 9% versus 24% with  
173 70 mg/kg and 100 mg/kg of PT-Cy, respectively,  $p=0.15$ ). Among patients receiving PT-Cy at  
174 70 mg/kg, only one (3%) patient developed LVSD after PT-Cy administration. This case  
175 occurred on day +6 after HCT and was associated with arrhythmia, although no clinical  
176 symptoms of heart failure were observed. In contrast, LSVD occurred in five (20%) patients  
177 following the administration of 100 mg/kg of PT-Cy, between days +6 and +38 after HCT,  
178 including two cases that were asymptomatic. The 2-year cumulative incidence of acute  
179 pulmonary edema was 3% with 70 mg/kg of PT-Cy and 20% with 100 mg/kg ( $p=0.041$ ).  
180 Other cardiac events included arrhythmia ( $n=4$ , 7%), pericardial effusion ( $n=8$ , 14%) and  
181 acute coronary syndrome ( $n=2$ , 3%) with no significant difference with respect to the PT-Cy  
182 dose for these events. Cardiac events resolved in most cases (9 out of 11 with PT-Cy at 100  
183 mg/kg and 4 out of 4 with 70 mg/kg) but was the direct cause of death of one patient who  
184 received 100 mg/kg of PT-Cy. The cumulative incidence of CRS was 48% in patients  
185 receiving 70 mg/kg of PT-Cy (grade 1: 13 patients, grade 2: 3 patients) and 40% in patients  
186 receiving 100 mg/kg (grade 1: 6 patients, grade 2: 3 patients) ( $p=0.5991$ ). No case of grade 3-  
187 4 CRS were observed, and tocilizumab was not used.

188

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

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

202

### 203 **Graft-versus-host disease**

204 At day +180, the cumulative incidences of acute grade II-IV and grade III-IV GVHD were  
205 18% and 0% with PT-Cy at 70 mg/kg compared to 17% and 5% with 100 mg/kg, respectively  
206 (p=0.94 for grade II-IV and p=0.23 for grade III-IV acute GVHD). There was no difference in  
207 the cumulative incidence of chronic GVHD at 2 years with 27% and 29% for PT-Cy at 70  
208 mg/kg and 100 mg/kg, respectively (p=0.95) (Table 4). Moderate to severe chronic GVHD  
209 occurred in five (9%) patients and required immunosuppressive systemic therapy in six (10%)  
210 patients, with no significant difference with respect to PT-Cy dose.

### 211 **Relapse and survival outcomes**

212 Disease progression or relapse occurred in 12 (21%) patients, within a median time of 8  
213 months (range, 1-28). The 2-year relapse incidence was 30% with 70 mg/kg of PT-Cy  
214 compared to 21% with 100 mg/kg (p=0.89) (Table 4). At last-follow-up, 24 (41%) patients  
215 had died. The causes of deaths were attributed to hematological malignancy relapse (n=4,  
216 12%), infection (n=5, 15%), and neurological complication (n=1, 3%) in patients receiving  
217 PT-Cy at 70 mg/kg, and hematological malignancy relapse (n=6, 24%), infection (n=4, 16%),  
218 GVHD (n=1, 4%), neurological complication (n=1, 3%), veno-occlusive disease (n=1, 3%),

219 and cardiac failure (n=1, 3%) in patients receiving PT-Cy at 100 mg/kg. The median follow-  
220 up among surviving patients was 17 months (interquartile range [IQR], 13-20) and 59 months  
221 (IQR, 51-66) in patients receiving PT-Cy at 70 mg/kg and 100 mg/kg, respectively. At 2  
222 years, NRM (18% versus 33%, p=0.13), PFS (65% versus 46%, p=0.09), and OS (68% versus  
223 52%, p=0.31) were similar with the reduced and the standard PT-Cy doses. However, GRFS  
224 was higher with 70 mg/kg of PT-Cy (60% versus 33%, p=0.04, Figure 1). Finally, the  
225 probability of survival at 2 years without severe GVHD, cardiac event, HC, or relapse, was  
226 improved with PT-Cy at 70 mg/kg (50% versus 25%, p=0.04).

227

228

## 229 **DISCUSSION**

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

238

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

244 events, particularly LVSD, was high among patients receiving 100 mg/kg of PT-Cy, as  
245 expected in such a high-risk population. Notably, the observed incidence is in line with the  
246 findings of a large retrospective study involving 331 patients, which established PT-Cy and  
247 older age as major risk factors for early cardiac events (16), and another study reporting a  
248 43% incidence of congestive heart failure in high-risk patients (44). Besides the high-risk  
249 characteristics of the cohort, routine cardiac monitoring allowed for the detection of  
250 asymptomatic cardiac events, which likely contributed to increase to overall incidence of  
251 observed cardiac events. No difference in terms of cardiovascular risk factors, history of  
252 cardiac events, or anthracycline exposure between patients receiving PT-Cy at 70 mg/kg and  
253 100 mg/kg was found to contribute to the reduction of cardiac complications. Overall,  
254 reducing PT-Cy translated into significant improvement in terms of cardiac morbidity, and  
255 might also have favorably impacted survival since cardiac complications are associated,  
256 directly or not, with lower OS (16).

257

258 Allogeneic HCT outcomes are substantially related to the achievement of an acceptable  
259 hematological and immune restoration. Neutrophil engraftment constitutes an essential step in  
260 the early phase after HCT, as prolonged neutropenia is associated with extended in-hospital  
261 stay and increased incidences of severe infections and NRM (45). Although hematopoietic  
262 stem cells are relatively spared by cyclophosphamide, due to high levels of expression of  
263 aldehyde dehydrogenase (46), haploidentical HCT with PT-Cy is associated with a  
264 significantly longer time to neutrophil and platelet recovery (6). Reducing PT-Cy dose to 70  
265 mg/kg allowed faster hematological recovery for both neutrophils and platelets, compared to  
266 100 mg/kg of PT-Cy. The shorter neutropenic phase might also contribute to the lower  
267 incidence of bacteremia observed with PT-Cy dose reduction. In addition, delayed neutrophil

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

270

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

279

280 In a high-risk population with a median age of 69 years, HCT-CI  $\geq 2$  in 67% of patients,  
281 active disease in 70%, and high or very-high disease risk index in 64%, the 2-year incidence  
282 of NRM was relatively low at 18% with 70 mg/kg of PT-Cy. Interestingly, the 100-day NRM  
283 was nearly 3-fold lower in patients receiving 70 mg/kg PT-Cy compared to 100 mg/kg, and  
284 overall NRM continued to climb in the latter to 33% at 2 years. Our results in patients  
285 receiving 70 mg/kg of PT-Cy are in line with the NRM reported in studies using PT-Cy at 80  
286 mg/kg (NRM of 16% to 18%) (31,32), and compare favorably with most studies using 100  
287 mg/kg of PT-Cy in a haploidentical setting for elderly patients (NRM of 13% to 38%) (50–  
288 53).

289

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

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

306  
307 Although our study is limited by the bias of its retrospective nature, the two comparative  
308 groups did not have significant differences in terms of age, sex, cardiovascular risk factors,  
309 HCT-CI score, type of hematological disease, disease risk index, type of conditioning  
310 regimen, CD34+ cell dose, and cyclosporine A treatment duration. Another limitation is the  
311 lack of temporal overlap between the two PT-Cy dose groups due to differences in the year of  
312 transplant. However, key transplant modalities such as conditioning regimens, GVHD  
313 prophylaxis associated with PT-Cy, supportive care procedures, and monitoring of infections  
314 and toxicities remained consistent throughout the study period. The only exception was the  
315 use of letermovir, which resulted in a lower incidence of CMV reactivation. The latter had no  
316 significant impact on platelet recovery, chronic GVHD, or GRFS but was associated with a  
317 higher risk of acute grade II-IV GVHD ( $p=0.05$ ) (Supplementary Table S1). The number of

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

321

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



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337

### 338 **Author contributions**

339 RD designed the study, recruited patients, collected, assembled, and analyzed data, performed  
340 the statistical analysis, and wrote the manuscript. MM designed the study, supervised  
341 research, analyzed data, and commented on the manuscript. SE, AC, and EC helped design  
342 the study, performed cardiac evaluations of the patients, analyzed data, and commented on the  
343 manuscript. ML analyzed data, performed the statistical analysis, and commented on the  
344 manuscript. AB and TL contributed to patient care and commented on the manuscript.  
345 FM, EB, AB, SS, RB, MC, ZVW, and OL recruited patients and commented on the  
346 manuscript. All authors reviewed the manuscript and approved its submission for publication  
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348

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353

### 354 **Availability of data and materials**

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

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598 **Figure Legends**

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

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629 **Table 1.** Patient characteristics and modalities of transplantation

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	<b>PT-Cy 70 mg/kg (n=33)</b>	<b>PT-Cy 100 mg/kg (n=25)</b>	<b>p-value</b>
<b>Recipient age (years), median (range)</b>	69 (21-74)	67 (15-76)	0.25
<b>Recipient gender (male/female), n (%)</b>	18 (55)/15 (45)	17 (68)/8 (32)	0.3
<b>Diagnosis, n (%)</b>			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)	
<b>Complete remission at transplant, n (%)</b>	10 (30)	8 (32)	0.89
<b>Disease risk index, n (%)</b>			0.68
Intermediate	12 (36)	10 (40)	
High	19 (58)	12 (48)	
Very-high	2 (6)	3 (12)	
<b>HCT-specific comorbidity index<sup>ψ</sup></b>			0.28
Median (range)	2 (0-5)	2 (0-3)	
Missing, n (%)	1 (3)	0 (0)	
<b>Cardiovascular risk factors, n (%)</b>			
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 <sup>‡</sup>	14 (42)	10 (40)	0.85
Diabetes	3 (9)	2 (8)	1
<b>Anthracycline exposure before HCT, n (%)</b>	21 (64)	20 (80)	0.18
<b>Cardiac event prior to HCT*, n (%)</b>	12 (36)	14 (56)	0.14
<b>LVSD at transplant, n (%)</b>	8 (24)	4 (16)	0.53
<b>Conditioning regimen, n (%)</b>			0.68
RIC: Thiotepa – busulfan – fludarabine	15 (45)	10 (40)	
Sequential thiotepa-based	18 (55)	15 (60)	
<b>CD34+ cell dose (10<sup>6</sup>/kg), median (range)</b>	6.97 (1.91-11.17)	7.38 (3.28-11.21)	0.8
<b>GVHD prophylaxis, n (%)</b>			
Cyclosporine A, MMF, and ATG	33 (100)	25 (100)	1
Cyclosporine A treatment duration, median (IQR)	141 (114-178)	135 (49-185)	0.38

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

634 <sup>ψ</sup>As previously published by Armand et al. (38)

635 <sup>‡</sup>Smoking was defined as ever-regular-, occasional- or ex-smoker, and compared to never-smoker

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



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

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	PT-Cy at 70 mg/kg	PT-Cy at 100 mg/kg	p-value
<b>Neutrophil engraftment, n (%)</b>	32 (97)	25 (100)	1
<b>Median time to ANC &gt; 0.5 x 10<sup>9</sup>/L, days (range)</b>	16 (13-19)	19 (1-24)	0.006
<b>Platelet count &gt; 20 x 10<sup>9</sup>/L, % [95% CI]</b>			
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
<b>Platelet count &gt; 50 x 10<sup>9</sup>/L, % [95% CI]</b>			
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

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

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

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

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

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

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672 \*Fungal infections included possible or probable invasive aspergillosis in 7 (12%) patients and toxoplasmosis in  
 673 2 (3%) patients (one patient had both invasive aspergillosis and toxoplasmosis).

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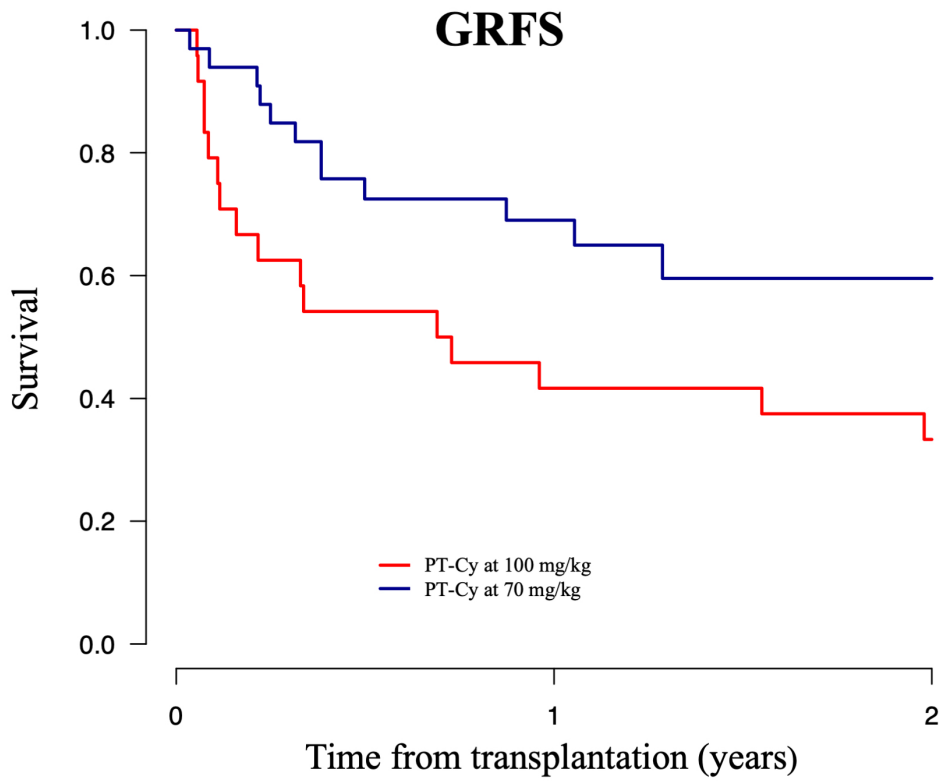
692 **Table 4.** Cumulative incidence of graft-versus-host disease and clinical outcomes according  
 693 to PT-Cy dose

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



PT-Cy at 100 mg/kg	25	10	8
PT-Cy at 70 mg/kg	33	19	5
		Number of patients at risk	

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