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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 ≥ 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.

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66
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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)- β -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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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 ≥ 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 ≥ 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
347 purposes.

348

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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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	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: Thiotepe – busulfan – fludarabine	15 (45)	10 (40)	
Sequential thiotepe-based	18 (55)	15 (60)	
CD34+ cell dose (10⁶/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

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 ^ψAs previously published by Armand et al. (38)

635 [‡]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

639

	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 10⁹/L, days (range)	16 (13-19)	19 (1-24)	0.006
Platelet count > 20 x 10⁹/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 10⁹/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

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]	
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

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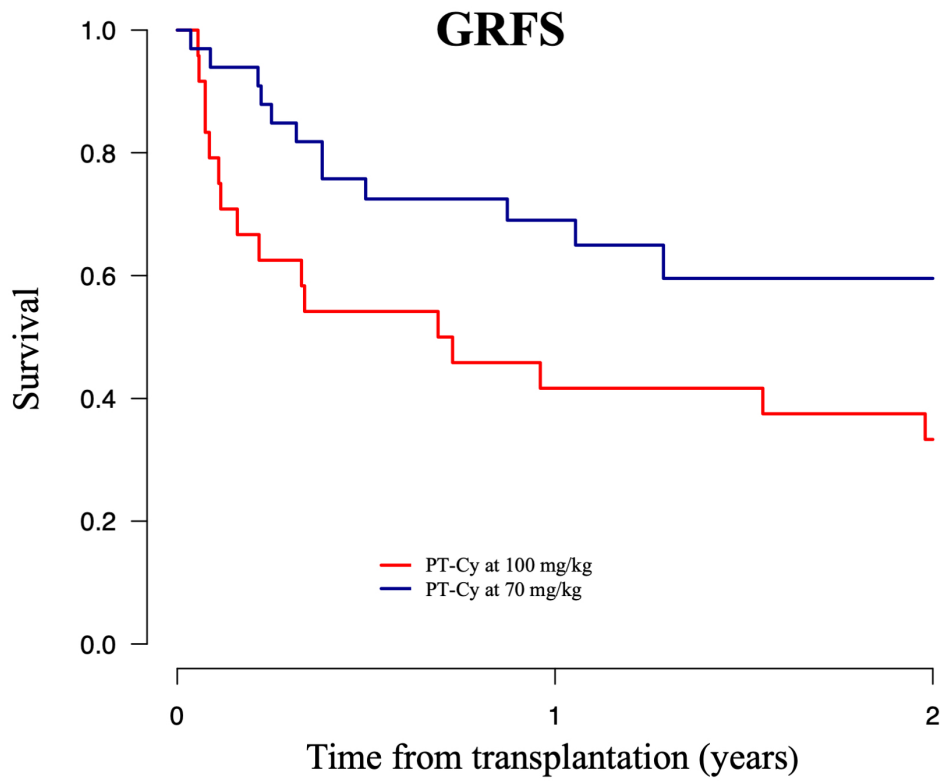
692 **Table 4.** Cumulative incidence of graft-versus-host disease and clinical outcomes according
 693 to PT-Cy dose

	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.**



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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