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Clinical outcomes of three haploidentical transplantation protocols for hematologic malignancies based on data from the Chinese Bone Marrow Transplantation Registry Group

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Abstract

This study aimed to demonstrate the clinical outcomes of granulocyte colony-stimulating factor (G-CSF)/antithymocyte globulin (ATG), posttransplantation cyclophosphamide (PTCy) and PTCy combined with low-dose ATG (PTCy with ATG^{low})-based haploidentical transplantation protocols in patients with haematologic malignancies. The comparisons were conducted via propensity score matching (PSM) analysis to balance the basic characteristics among different groups and were based on the transplantation data reported to the Chinese Bone Marrow Transplantation Registry Group (CBMTRG) from January 2020 to December 2022. For each patient in the PTCy or PTCy with ATG^{low} group, patients (at a 1:2 ratio) from the G-CSF/ATG group were selected. In total, the PTCy group (n=122) was matched with G-CSF/ATG Group 1 (n=230), and the PTCy+ATG^{low} group (n=123) was matched with G-CSF/ATG Group 2 (n=226). Compared with those in the PTCy group, the incidences of 28-day neutrophil engraftment (P=0.005), 100-day platelet engraftment (P=0.002), median time to neutrophil engraftment (P<0.001) and platelet engraftment (P=0.011) were significantly greater in the G-CSF/ATG group. No significant differences were observed in acute graft-versus-host disease (aGvHD) incidence or relapse incidence. In addition, patients in the G-CSF/ATG group had lower nonrelapse mortality (NRM, P<0.001), 3-year overall survival (OS, P<0.001) and leukaemia-free survival

(LFS, P<0.001) rates than those in the PTCy group. Similarly, the G-CSF/ATG group achieved lower NRM (P<0.001) and better 3-year LFS (P=0.002) than the PT-Cy plus ATG^{low} group. In conclusion, G-CSF/ATG-based haplo-HSCT may be a preferential choice for the Chinese population with haematologic malignancies. In the future, a randomized controlled study is needed for further confirmation.

Keywords: haploidentical, G-CSF, ATG, PTCy

Introduction

Allogeneic haematopoietic stem cell transplantation (allo-HSCT) is an effective option for curing a variety of haematologic malignancies(1). In the last two decades, great advances have been made in haploidentical haematopoietic stem cell transplantation (haplo-HSCT), which has become the largest donor source of allo-HSCT in China(2). The mainstream T-cell-replete (TCR) haplo-HSCT models include the granulocyte colony-stimulating factor (G-CSF)/antithymocyte globulin (ATG)-based Beijing protocol and the posttransplantation cyclophosphamide (PTCy)-based Baltimore protocol(3). Recently, novel combination protocols, such as PTCy combined with low-dose ATG and G-CSF/ATG combined with low-dose PTCy, have been attempted(4-6). The rapid expansion of the abovementioned protocols has promoted the flourishing development of haplo-HSCT worldwide(7).

Several published studies have attempted to compare the clinical outcomes of different haploidentical protocols, but the conclusions have been inconsistent (8-10). Data from Taiwan have indicated that, compared with PTCy (n=26) or PTCy with low-dose ATG (n=42), the G-CSF/ATG protocol (n=110) had the most favourable neutrophil and platelet engraftment kinetics, the lowest nonrelapse mortality (NRM) and the highest overall survival (OS) rates(8). However, outcomes from the European Society for Blood and Marrow Transplantation (EBMT) contradict these findings. Among adults with acute myeloid leukaemia (AML), patients treated with PTCy (n=193) had

markedly lower NRM and better leukaemia-free survival (LFS) than those treated with G-CSF/ATG (n=115), but patients in the PTCy cohort were transplanted more recently and had relatively shorter follow-up periods of 18 months compared with 36 months in the G-CSF/ATG cohort(9). Subsequently, EBMT data focused on patients with acute lymphoblastic leukaemia (ALL) revealed the clinical prognostic advantage of the PTCy-based protocol (n=336), including a lower relapse rate and better LFS compared with the G-CSF/ATG cohort (n=98); however, the G-CSF/ATG cohort had a significantly greater proportion of refractory/relapsed disease at the time of transplantation (10). All of the above studies were limited by imbalanced characteristics before transplantation.

Considering the above inconsistent results attributed to the mismatch of confounding factors, a previous study from Beijing applied the nested case□pair method to balance the basic characteristics between the G-CSF/ATG (n=176) and PTCy (n=44) groups. The outcomes from this study support the conclusions from Taiwan(11), indicating that the G-CSF/ATG group achieved better engraftment, LFS and OS as well as a lower incidence of NRM than the PTCy group. However, the study included a limited number of patients (44 patients in the PTCy group) and transplants at earlier years (between 2013 and 2018), and it included only the G-CSF/ATG and PTCy groups without the combination strategy(12).

Hence, we conducted the current study to compare the clinical outcomes

of G-CSF/ATG, PTCy and PTCy combined with low-dose ATG (PTCy+ATG^{low}) using a propensity score matching method to control for confounding bias based on data from the Chinese Bone Marrow Transplantation Registry Group (CBMTRG), aiming to maximize the balance of baseline data among the three groups.

Methods

Patient selection

The flowchart of patient selection is shown in Fig. 1. This work was a multicentre, retrospective trial based on the data in the CBMTRG. Informed consent was obtained from the patients or their families. The study was approved by the institutional review board of each centre.

To minimize selection bias and confounding bias, we employed propensity score matching (PSM) analysis for patient selection. Given the immaturity of multigroup PSM, we used PSM to compare the two groups. A ratio of 1:2 matching by PSM was calculated through logistic regression using the following variables: age at allo-HSCT, recipient sex, diagnosis, and haematopoietic cell transplantation-comorbidity index (HCT-CI)(13). The nearest-neighbour matching method using propensity scores was employed,

with a calliper of 0.20. The balance was verified by assessing standardized mean differences between these groups.

Transplantation procedure

G-CSF/ATG group: The modified busulfan-cyclophosphamide (Bu-Cy) plus ATG conditioning regimen included cytarabine 4 g/m^2/d i.v. on Days –10 to –9, Bu 3.2 mg/kg/d i.v. on Days –8 to –6, Cy 1.8 g/m^2/d i.v. on Days –5 to –4, Me-CCNU 250 mg/m^2/d orally on Day –3, and ATG 2.5 mg/kg/d i.v. on Days –5 to –2. The GvHD prophylaxis regimen consisted of cyclosporine A (CsA), mycophenolate mofetil (MMF), and short-term methotrexate (MTX) (14).

PTCy group: The dose of PTCy ranged from 30–50 mg/kg/day on Days +3 and +4. The conditioning regimens included Bu 3.2 mg/kg/day from Days -6 to -3, Flu 30 mg/m^2/day from Days -6 to -2, Ara-C 1 g/m^2/day from Days -6 to -2, or Bu 130 mg/m^2/day on Day -7, Flu 30 mg/m^2/day for 6 days and MEL 100 mg/m^2/day on Day -2. In addition to PTCy, the other GvHD prophylaxis regimens consisted of cyclosporine A, short-term MTX and MMF.

PTCy + low-dose ATG group: All patients in this group received a high dose of PTCy ranging from 30 to 50 mg/kg/day and an additional low dose of ATG. Rabbit ATG (thymoglobulin, Sanofi-Aventis) at 1 mg/kg/day on Day -2, 2 mg/kg/day on Day -1, or 1.5–2.5 mg/kg/day on Day +8 was administered. GvHD prophylaxis consisted of CsA and MMF in addition to PTCy and ATG. The conditioning regimen consisted of intravenous busulfan (Bu) 3.2 mg/kg/d on Days −6 to −3, fludarabine (Flu) 150 mg/m^2, and cytarabine (Ara-C) 1

g/m^2/d on Days −6 to −2 or Bu 130 mg/m^2/day on Day -7, Flu 180 mg/m^2, and MEL 100 mg/m^2/day on Day -2.

Statistical analysis

Continuous variables were compared using the Wilcoxon rank-sum test or Mann□Whitney U test. Categorical variables were compared using the χ2 test or Fisher's exact test. Survival outcomes were described using the Kaplan□Meier method and compared using the log-rank test. The cumulative incidence (CIs) of engraftment, GvHD, relapse and NRM were estimated using competing risks to accommodate competing risks, and the Fine-Gray test was used to compare significant differences. Statistical analyses were primarily performed using the Statistical Package for SPSS software (Inc., USA) and the R software package (version 4.2.2; http://www.r-project.org).

Results

Patient characteristics

The study enrolled patients with haematologic malignancies who underwent their first haplo-HSCT between January 2020 and December 2022. In this study, all of the included patients were diagnosed with acute leukaemia or myelodysplastic syndrome (MDS). A total of 818 patients with complete medical records were enrolled for PSM. After PSM, G-CSF/ATG Group 1

(n=230) was matched with the PTCy group (n=122), whereas G-CSF/ATG Group 2 (n=226) was matched with the PTCy+ATG^{low} group (n=123).

The baseline patient characteristics after PSM are shown in Table 1 (G-CSF/ATG1 vs. PTCy) and Table 2 (G-CSF/ATG2 vs. PTCy+ATG^{low}). Except for number of HLA mismatches, MNCs, CD34+ cells or graft resources, the baseline characteristics did not significantly differ between the two groups. The median follow-up periods for survivors were 1057 days (range, 482–1574 days), 970 days (range, 515–1575 days), 1015 days (range, 482–1576 days), and 865 days (range, 497–1512 days) in G-CSF/ATG Group 1, the PTCy group, G-CSF/ATG Group 2, and the PTCy+ATG^{low} group, respectively.

Engraftment

G-CSF/ATG* vs*. PTCy

The cumulative incidence rates of neutrophil engraftment on Day 28 (98.3% [95% CI, 96.5–100.0%] vs. 97.5% [95% CI, 94.5–100%], P=0.005; Fig. 2A) and platelet engraftment on Day 100 (93.5% [95% CI, 90.2–96.7%] vs. 84.4% [95% CI, 77.9–90.9%]; P=0.002; Fig. 2B) in G-CSF/ATG Group 1 were significantly greater than those in the PTCy group. Moreover, the median times to neutrophil engraftment (12 days [range, 9–21 days] vs. 13 days [range, 9–22 days], P<0.001) and platelet engraftment (13 days [range, 7–73 days] vs. 14 days [range, 9–45 days], P=0.011) were shorter in the G-CSF/ATG group than in the PTCy group.

G-CSF/ATG* vs*. PTCy+ATG^{low}

The cumulative incidence of neutrophil engraftment on Day 28 (98.7% [95% CI, 97.1–100%] vs. 100%, P=0.784; Fig. 3A) or platelet engraftment on Day 100 (91.1% [95% CI, 87.4–94.9%] vs. 82.9% [95% CI, 76.2–89.7%]; P=0.337; Fig. 3B) did not significantly differ between G-CSF/ATG Group 2 and the PTCy+ATG^{low} group. No significant differences were observed in the median time to neutrophil engraftment (12 days vs. 13 days, P=0.247) or platelet engraftment (13 days vs. 13 days, P=0.330).

aGvHD

G-CSF/ATG* vs. *PTCy

No significant difference in aGvHD was observed among the groups. The 100-day cumulative incidences of Grade 2–4 GvHD were 28.6% (95% CI, 22.8–34.6%) and 28.7% (95% CI, 20.7–36.8%, P=0.972), and those of Grade 3–4 aGvHD were 10.8% (95% CI, 6.8–14.9%) and 13.1% (95% CI, 7.1–19.2%, P=0.494) in G-CSF/ATG Group 1 and the PTCy group, respectively.

G-CSF/ATG* vs. *PTCy+ATG^{low}

The 100-day cumulative incidences of Grade 2–4 aGvHD were 25.2% (95% CI, 19.5–30.9%) and 27.9% (95% CI, 19.9–35.9%, P=0.548), and those of Grade 3–4 aGvHD were 10.2% (95% CI, 6.2–14.1%) and 14.8% (95% CI, 8.4–21.1%, P=0.173) in G-CSF/ATG Group 2 and the PTCy+ATG^{low} group, respectively.

cGvHD

G-CSF/ATG* vs. *PTCy

No significant difference in cGvHD was observed among the groups. The 3-year cumulative incidences of chronic GvHD were 33.4% (95% CI, 26.9–39.9%) and 28.3% (95% CI, 18.9–37.6%, *P*=0.322), and those of moderate and severe cGvHD were 14.6% (95% CI, 9.7–19.5%) and 10.6% (95% CI, 3.7–17.5%, *P*=0.252) in G-CSF/ATG Group 1 and the PTCy group, respectively.

G-CSF/ATG* vs. *PTCy+ATG^{low}

The 100-day cumulative incidences of cGvHD were 35.1% (95% CI, 28.3–41.9%) and 24.5% (95% CI, 15.2–33.8%, P=0.091), and those of moderate and severe cGvHD were 17.4% (95% CI, 11.9–22.8%) and 8.1% (95% CI, 2.7–13.4%, P=0.061) in G-CSF/ATG Group 2 and the PTCy+ATG^{low} group, respectively.

Viraemia

CMV viraemia (59.1% vs. 53.3%, P=0.291; 56.6% vs. 57.7%, P=0.845) or EBV viraemia (13.5% vs. 13.9%, P=0.906; 15.9% vs. 19.5%, P=0.397) did not significantly differ between the G-CSF/ATG1 and PTCy groups or between the G-CSF/ATG2 and PTCy+ATG^{low} groups within 100 days.

Relapse and NRM

G-CSF/ATG* vs. *PTCy

At the time of the last follow-up, 31 patients (13.5%) in G-CSF/ATG Group 1 and 13 patients (10.7%) in the PTCy group had relapsed. The 3-year cumulative incidences of relapse (CIRs) for patients in G-CSF/ATG Group 1

and the PTCy group were 14.3% (95% CI, 9.5–19.1%) and 10.9% (95% CI, 5.3–16.6%, P=0.501; Fig. 2E), respectively. The 3-year NRM significantly differed between the two groups (7.8% [95% CI, 4.3–11.3%] in the G-CSF/ATG cohort vs. 26.2% [95% CI, 18.1–34.3%] in the PTCy cohort, P<0.001; Fig. 2F).

G-CSF/ATG* vs. *PTCy+ATG^{low}

Thirty patients (13.3%) and 11 patients (9.0%) in G-CSF/ATG Group 2 and the PTCy+ATG^{low} group, respectively, relapsed. The 3-year CIR was comparable between the two groups (14.8% [95% CI, 9.7–19.9%] vs. 9.0% [95% CI, 3.9–14.0%], P=0.271; Fig. 3E). Compared with that in the PTCy+ATG group, the 3-year NRM in G-CSF/ATG Group 2 was lower (10.2% [95% CI, 6.2–14.1%] vs. 27.6% [95% CI, 19.7–35.6%], P<0.001; Fig. 3F).

OS and LFS

G-CSF/ATG* vs. *PTCy

During the follow-up period, 37 (16.1%), 41 (33.6%), 41 (18.1%) and 43 (35.0%) patients died in G-CSF/ATG Group 1, the PTCy group, G-CSF/ATG Group 2 and the PTCy+ATG^{low} group, respectively. The causes of death are summarized in Table 3, and relapse was the leading cause of death in the G-CSF/ATG group, whereas infection was the leading cause in the PTCy group and the PTCY+ATG group. The 3-year OS rates were 84.1% (95% CI, 79.4–89.0%) in G-CSF/ATG Group 1 and 65.2% (95% CI, 57.0–74.7%) in the PTCy group (P<0.001, Fig. 2G). The 3-year LFS rates were 77.8% (95% CI, 72.5–

83.6%) and 62.9% (95% CI, 54.6–72.4%), respectively (P＜0.001, Fig. 2H).

G-CSF/ATG* vs. *PTCy+ATG^{low}

In addition, the 3-year OS rates were 81.9% (95% CI, 76.9–87.1%) in G-CSF/ATG Group 2 and 64.8% (95% CI, 56.8–73.9%) in the PTCy+ATG^{low} group (P<0.001, Fig. 3G). The 3-year LFS rates were 75.0% (95% CI, 69.3–81.3%) and 63.4% (95% CI, 55.4–72.5%), respectively (P=0.002, Fig. 3H).

Comparison between the PTCy and *PTCy+ATG^{low}* groups

The PTCy and PTCy+ATG^{low} groups were not matched due to the small sample size of these two groups. Nevertheless, we also compared the baseline data and clinical outcomes between the PTCy and PTCy+ATG^{low} groups among the patients enrolled in the study.

As shown in Supplemental Table 1, age at transplantation, sex, disease type, disease risk index, HCT-CI, number of HLA locus mismatches, donor□patient sex matches, donor□patient blood type matches, graft resources and infused mononuclear cells (MNCs) did not significantly differ between the PTCy (n=122) and PTCy+ATG^{low} (n=123) groups. The distribution of donor sources markedly differed, with a greater proportion of child donors in the PTCy+ATG^{low} group than in the PTCy group (40.7% vs. 27.9%, *P* = 0.047). In terms of clinical outcomes, the cumulative incidence of myeloid engraftment was greater in the PTCy+ATG^{low} group than in the PTCy group (100% vs. 97.5%, *P* = 0.003). The remaining results in both groups were similar, including platelet engraftment, acute GvHD, chronic GvHD,

relapse, nonrelapse mortality and survival outcomes.

Multivariate analysis

The clinical outcomes among the three groups based on all patients were presented in Supplemental Table 2. We combined the three groups of cases and included them in the multivariate analysis (Supplemental Tables 3 and 4). The multivariate analysis revealed that a low/intermediate-risk DRI and the G-CSF/ATG-based protocol predicted less NRM and better survival outcomes.

Discussion

To our knowledge, this study is the first to compare the clinical outcomes of patients receiving G-CSF/ATG, PTCy and PTCy plus low-dose ATG-based haplo-HSCT via PSM analysis to reduce confounding bias. Our findings suggested that the G-CSF/ATG-based protocol could reduce NRM and prolong OS and LFS than both the PTCy-based and PTCy plus low-dose ATG-based protocols. In addition, haplo-HSCT with G-CSF/ATG resulted in superior myeloid and platelet engraftment compared with haplo-HSCT with PTCy. The incidences of aGvHD, CMV viraemia, EBV viraemia or relapse did not significantly differ among the three groups in our current analysis.

Engraftment is an essential endpoint of observation when different haploidentical protocols are evaluated(16). The advantage of the G-CSF/ATG-

based protocol in engraftment has been reported in a series of studies. The incidence of myeloid engraftment reached approximately 99% with the G-CSF/ATG-based protocol(17-19) but ranged from 89% to 94% with the PTCy-based protocol(20-22). For direct comparison, Tsai et al. reported that 60-day neutrophil counts of 99.3%, 97.6% and 92.3% and 100-day platelet engraftment rates of 94.2%, 90.5% and 68.2% were achieved in the G-CSF/ATG, PTCy with ATG and PTCy groups, respectively (11). Similarly, our results revealed that patients in the G-CSF/ATG group had significantly greater neutrophil engraftment (98.3% vs. 97.5%) and platelet engraftment (93.5% vs. 84.4%) than patients in the PTCy group. However, myeloid or platelet engraftment did not differ between the G-CSF/ATG and PTCy plus ATG groups, suggesting that the addition of low-dose ATG would be beneficial for facilitating engraftment. Recently, another study from EBMT suggested that the addition of low-dose ATG to PTCy may also accelerate neutrophil recovery(23).

The incidences of acute GvHD did not significantly differ among the three strategies, with Grade II-IV aGvHDs of 25.2%-28.6%, 28.7%, 27.9%, and Grade III to IV aGvHDs of 10.2%-10.8%, 13.1%, and 14.8% in the G-CSF/ATG, PTCy and PTCy+ATG^{low} groups, respectively. Similarly, Nagler et al. reported that the cumulative incidences of Grade II-IV and Grade III-IV acute GvHD were 32.7% vs. 30.5% and 11.6% vs. 14.1%, respectively, in the G-CSF/ATG and PTCy groups, but these differences were not significant (10).

Whether the combination of PTCy with ATG further decreases the incidence of GvHD remains controversial and depends on the combination method used. Our previous study demonstrated that ATG combined with low-dose PTCy could exert synergistic effects on preventing GvHD by increasing the number of Treg cells, as indicated by clinical and preclinical data (24). Some studies have reported a significantly lower cumulative incidence of acute Grade II-IV GvHD with the combination of PTCy with low-dose ATG than with the PTCy protocol. In Makanga's study, PTCy+ATG led to an incidence of Grade II-IV aGvHD of 24%, compared with 59% for the PTCy protocol(25). In El-Cheikh's study, the PTCy+ATG and PTCy protocols resulted in incidences of Grade II-IV aGvHD of 12% and 22%, respectively(26). However, others have suggested that the addition of ATG does not provide any additional benefit in acute GvHD(23, 27, 28). In the present study, PTCy combined with low-dose ATG was not associated with a decreased incidence of GvHD.

Currently, G-CSF/ATG is associated with lower NRM, which also prolongs LFS and OS compared with PTCy or PTCy with low-dose ATG, and no differences were observed in the relapse rates among the three haploidentical protocols. The superior engraftment of neutrophils and platelets in the G-CSF/ATG group may have reduced the risk of infection and bleeding, thus decreasing mortality and improving survival compared with PTCy. The addition of low-dose ATG to PTCy promoted engraftment but failed to translate to survival rates similar to those of G-CSF/ATG, which might be the

result of increased immunosuppression and increased susceptibility to infection. Similarly, Tsai et al. reported that patients receiving G-CSF/ATG had significantly lower NRM (18.5% vs. 30.5% vs. 39.1%) and longer OS (48.9% vs. 38.1% vs. 22.0%) than those receiving PTCy plus ATG and PTCy. In addition, patients receiving PTCy had a greater incidence of relapse (56.1%, n=26) than patients receiving G-CSF/ATG (34.5%, n=110) or PTCy plus ATG (38.5%, n=42)(8). Among adults with AML from the EBMT database, the LFS and OS rates were 56% versus 47.2% (P=0.26) and 58% versus 54.2% (P=0.37), respectively, for patients receiving PTCy (n=193) versus ATG (n=115). Multivariate analysis revealed that NRM was lower in the PTCy-based regimen group (22% vs. 30%), with no difference in relapse incidence. Notably, the follow-up period was markedly shorter for patients who received PTCy(9). Among adults with ALL from the EBMT database, the LFS was better with PTCy (n=98) than with the ATG protocol (n=336). The incidence of relapse was lower in the PTCy group, whereas the incidence of nonrelapse mortality was not different. However, more patients in the ATG group than in the PTCy group experienced relapsed/refractory ALL (30.6% vs. 16.4%) and underwent transplantation within an earlier period (median year of transplantation: 2011 vs. 2015) (10). Although both of these studies demonstrated the superior clinical outcomes of PTCy, they were limited by markedly imbalanced basic characteristics and selection bias.

The present study was limited by its retrospective nature, the relatively small number of patients treated with PTCy or PTCy combined with ATG, the inclusion of patients treated with various conditioning regimens and GvHD prophylaxis, and the transplant experience of different centres. The selection of conditioning regimens and GvHD prophylaxis is based on the routine clinical practice at each institute; thus, the conditioning protocols are heterogeneous. However, this study was the first to compare different protocols using PSM methods, reducing baseline bias across groups to the greatest extent possible. The PTCy and PTCy+ATG^{low} groups were not matched due to the small sample size of these two groups.

In conclusion, G-CSF/ATG-based haplo-HSCT may possess the advantages of engraftment and lower NRM for patients with haematologic malignancies based on data from the CBMTRG. However, data from a larger number of patients and prospective randomized controlled trials are necessary to clarify the clinical outcomes of different haplo-HSCT protocols. Furthermore, revealing the patterns and regulatory mechanisms involved in post-HSCT immune reconstitution is crucial for obtaining a deeper understanding of the prognosis among various HSCT protocols and for optimizing treatment strategies.

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Table 1 Comparison of baseline patient characteristics between the granulocyte colony-stimulating factor/antithymocyte globulin group 1 and the post-transplantation cyclophosphamide group after propensity score matching analysis.

Characteristics	**G-CSF/ATG group 1 (n=230)**	**PTCy group (n=122)**	**P value**
Median age at allo-HSCT, years (range)	32 (1–65)	31 (3–60)	0.462
Sex, n (%)			0.957
Male	103 (44.8%)	55 (45.1%)	
Female	127 (55.2%)	67 (54.9%)	
Diagnosis, n (%)			0.895
AML	124 (53.9%)	62 (50.8%)	
ALL	63 (27.4%)	35 (28.7%)	
MDS	23 (10.0%)	15 (12.3%)	
Others	20 (8.7%)	10 (8.2%)	
Disease risk index, n (%)			0.219
Low risk	9 (3.9%)	4 (3.3%)	
Intermediate + high risk	216 (93.9%)	111 (91.0%)	
Very high risk	5 (2.2%)	7 (5.7%)	
HCT-CI, n (%)			0.671
0	79 (34.3%)	37 (30.3%)	
1–2	141 (61.3%)	78 (63.9%)	
≥3	10 (4.3%)	7 (5.7%)	
Number of HLA-A/B/DRB1 mismatches, n (%)			**<0.001**
0–2	43 (18.7%)	52 (42.6%)	
3	187 (81.3%)	70 (57.4%)	
Donor-patient sex match, n (%)			0.386
Male-male	82 (35.7%)	37 (30.3%)	
Male-female	92 (40.0%)	47 (38.5%)	
Female-male	21 (9.1%)	15 (12.3%)	
Female-female	35 (15.2%)	23 (18.9%)	
Donor-recipient relation, n (%)			0.439

Parents-child	96 (41.7%)	43 (35.2%)	
Child-parents	68 (29.6%)	34 (27.9%)	
Sibling-sibling	65 (28.3%)	44 (36.1%)	
Others	1 (0.4%)	1 (0.8%)	
ABO match, n (%)			0.432
Match	123 (53.5%)	76 (62.3%)	
Minor mismatch	45 (19.6%)	21 (17.2%)	
Major mismatch	44 (19.1%)	17 (13.9%)	
Bidirectional mismatch	18 (7.8%)	8 (6.6%)	
MNCs ($\times10^8$/kg), median (range)	9.59 (1.00–18.67)	11.05 (3.58–34.63)	**<0.001**
CD34+ cells ($\times10^6$/kg), median (range)	3.50 (0.69–16.17)	6.07 (2.30–17.88)	**<0.001**
Graft resource, n (%)			**0.006**
BM+PB cell	18 (7.8%)	1 (0.8%)	
PB cell	212 (92.2%)	121 (99.2%)	

ALL, acute lymphocytic leukaemia; allo-HSCT, allogeneic haematopoietic stem cell transplantation; AML, acute myeloid leukaemia; ATG, antithymocyte globulin; BM, bone marrow; G-CSF, granulocyte colony-stimulating factor; GvHD, graft-versus-host disease; HCT-CI, haematopoietic cell transplantation-comorbidity index; MDS, myelodysplastic syndrome; MNCs, mononuclear cells; PB, peripheral blood; PTCy, posttransplantation cyclophosphamide.

Table 2 Comparison of baseline patient characteristics between granulocyte colony-stimulating factor/antithymocyte globulin group 2 and the post-transplantation cyclophosphamide with low dose antithymocyte globulin group after propensity score matching analysis.

Characteristics	**G-CSF/ATG group 2 (n=226)**	**PTCy+ATG^{low} group (n=123)**	**P value**
Median age at allo-HSCT, years (range)	35 (6–65)	34 (2–60)	0.168
Sex, n (%)			0.818
Male	115 (50.9%)	61 (49.6%)	
Female	111 (49.1%)	62 (50.4%)	
Diagnosis, n (%)			0.843
AML	112 (49.6%)	61 (49.6%)	
ALL	80 (35.4%)	47 (38.2%)	
MDS	27 (11.9%)	11 (8.9%)	
Others	7 (3.1%)	4 (3.3%)	
Disease risk index, n (%)			0.598
Low risk	8 (3.5%)	2 (1.6%)	
Intermediate + high risk	214 (94.7%)	119 (96.7%)	
Very high risk	4 (1.8%)	2 (1.6%)	
HCT-CI, n (%)			0.875
0	52 (23.0%)	26 (21.1%)	
1–2	155 (68.9%)	88 (71.5%)	
≥3	18 (8.0%)	9 (7.3%)	
Number of HLA-A/B/DRB1 mismatches, n (%)			**<0.001**
0–2	48 (21.2%)	50 (40.7%)	
3	178 (78.8%)	73 (59.3%)	
Donor-patient sex match, n (%)			0.111
Male-male	88 (38.9%)	39 (31.7%)	
Male-female	76 (33.6%)	35 (28.5%)	
Female-male	27 (11.9%)	24 (19.5%)	
Female-female	35 (15.5%)	25 (20.3%)	

Donor-recipient relationship, n (%)			0.171
Parents-child	92 (40.7%)	42 (34.1%)	
Child-parents	76 (33.6%)	50 (40.7%)	
Sibling-sibling	57 (25.2%)	28 (22.8%)	
Others	1 (0.4%)	3 (2.4%)	
ABO match, n (%)			0.076
Match	125 (55.3%)	73 (59.3%)	
Minor mismatch	43 (19.0%)	20 (16.3%)	
Major mismatch	41 (18.1%)	28 (22.8%)	
Bidirectional mismatch	17 (7.5%)	2 (1.6%)	
MNCs ($\times 10^8$/kg), median (range)	9.60 (1.00–32.03)	11.91 (5.60–31.94)	**<0.001**
CD34+ cells ($\times 10^6$/kg), median (range)	3.41 (0.69–14.10)	5.60 (0.52–17.30)	**<0.001**
Graft resource, n (%)			**0.001**
BM+PB cell	17 (7.5%)	0	
PB cell	209 (92.5%)	123 (100%)	

ALL, acute lymphocytic leukaemia; allo-HSCT, allogeneic haematopoietic stem cell transplantation; AML, acute myeloid leukaemia; ATG, antithymocyte globulin; BM, bone marrow; G-CSF, granulocyte colony-stimulating factor; GvHD, graft-versus-host disease; HCT-CI, haematopoietic cell transplantation-comorbidity index; MDS, myelodysplastic syndrome; MNCs, mononuclear cells; PB, peripheral blood; PTCy, posttransplantation cyclophosphamide.

Table 3 Primary causes of death among patients.

Causes of death	G-CSF/ATG group 1 (n=37, %)	PTCy group (n=41, %)	G-CSF/ATG group 2 (n=41, %)	PTCy+ATG group (n=43, %)
Relapse	19 (51.4)	10 (24.4)	18 (43.9)	7 (16.3)
Infection	10 (27.0)	21 (51.2)	15 (36.6)	24 (55.8)
GvHD	3 (8.1)	6 (14.6)	4 (9.8)	5 (11.6)
Secondary poor graft function	2 (5.4)	2 (4.9)	2 (4.9)	1 (2.3)
Haemorrhage	0 (0.0)	2 (4.9)	0 (0.0)	3 (7.0)
TMA	2 (5.4)	0 (0.0)	0 (0.0)	0 (0.0)
Organ failure	1 (2.7)	0 (0.0)	2 (4.9)	2 (4.7)
PTLD	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.3)

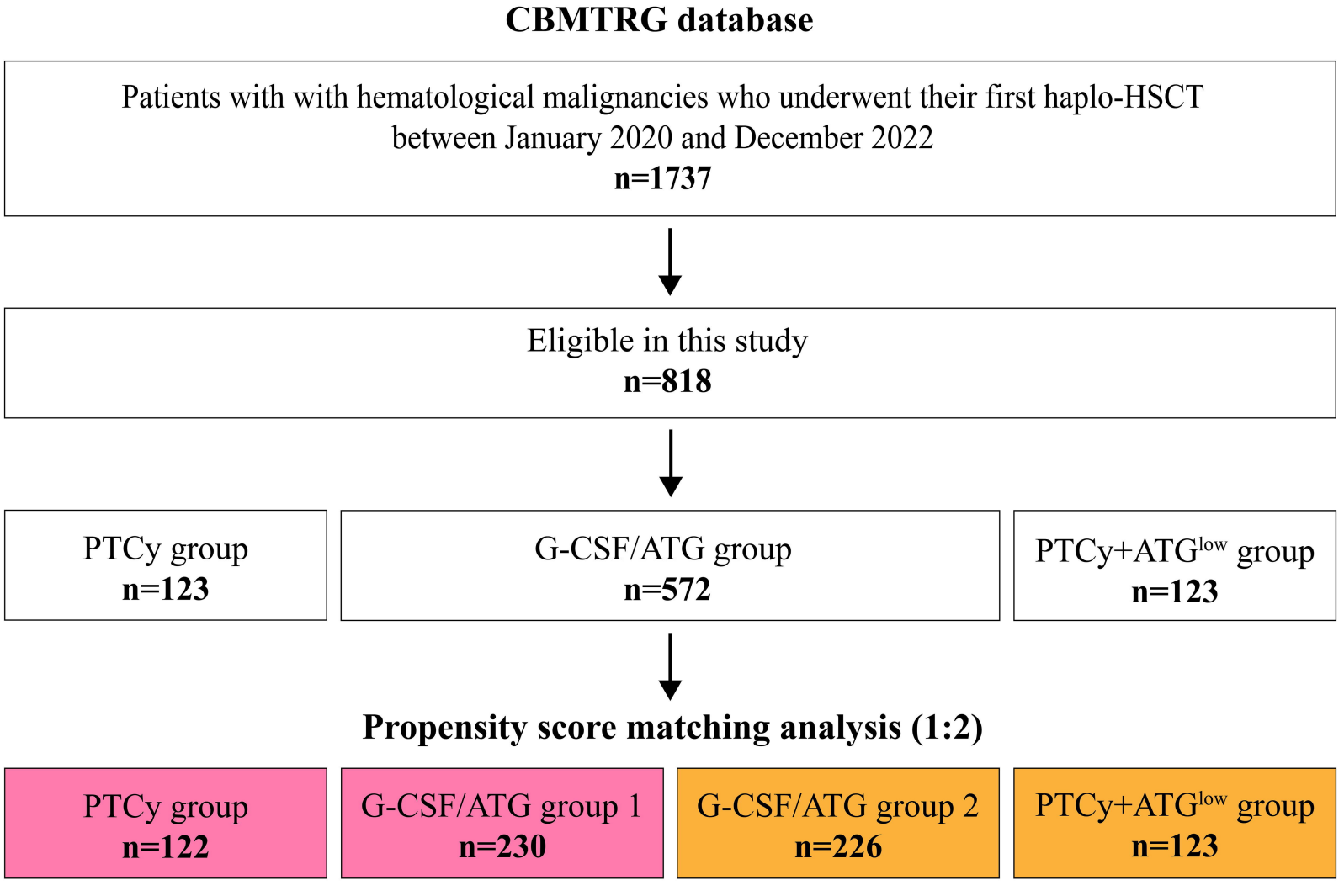
ATG, antithymocyte globulin; G-CSF, granulocyte colony-stimulating factor; GvHD, graft-versus-host disease; PTCy, posttransplantation cyclophosphamide. PTLD, posttransplant lymphoproliferative disorder; TMA, thrombotic microangiopathy

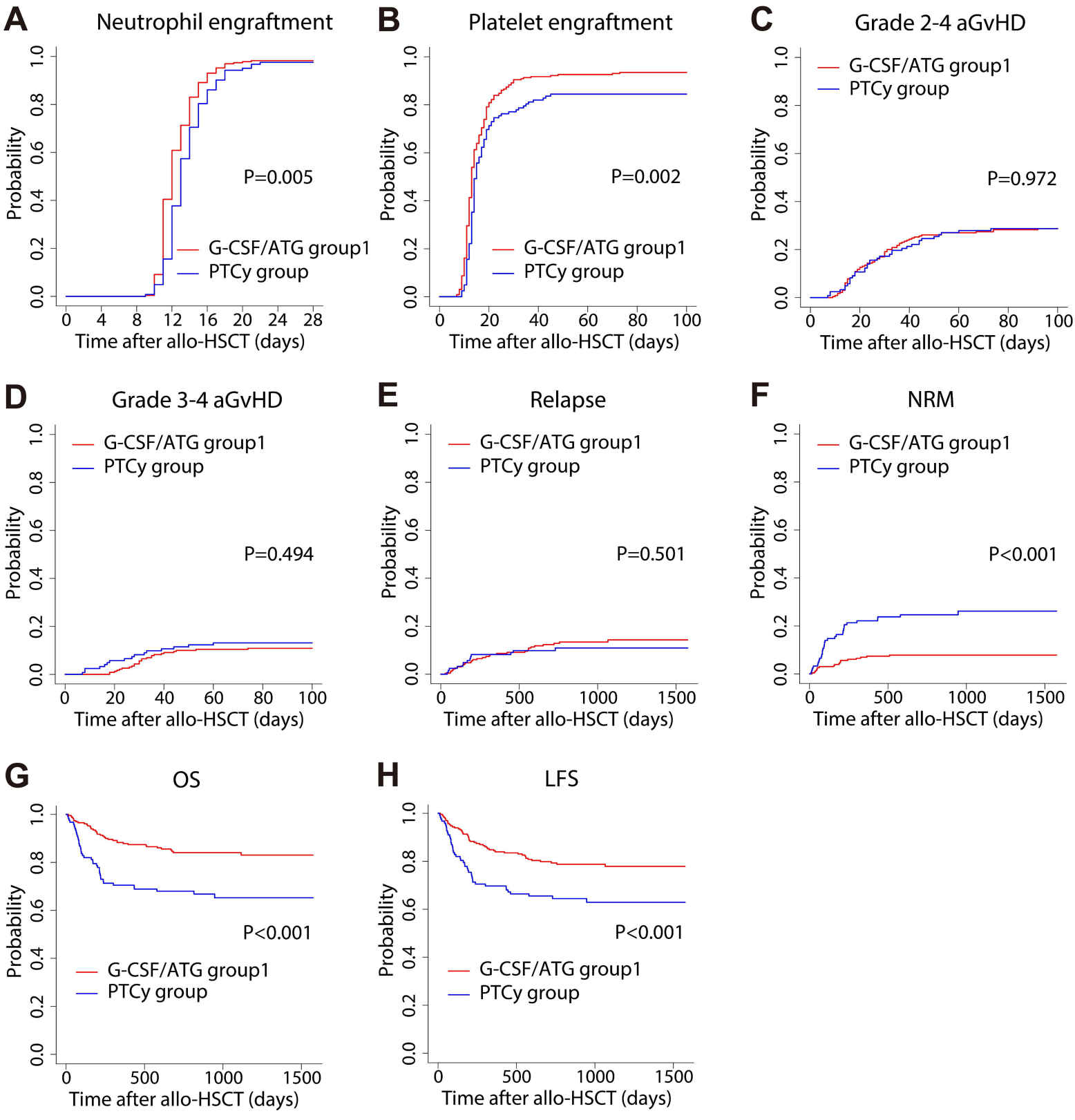
Figure legends

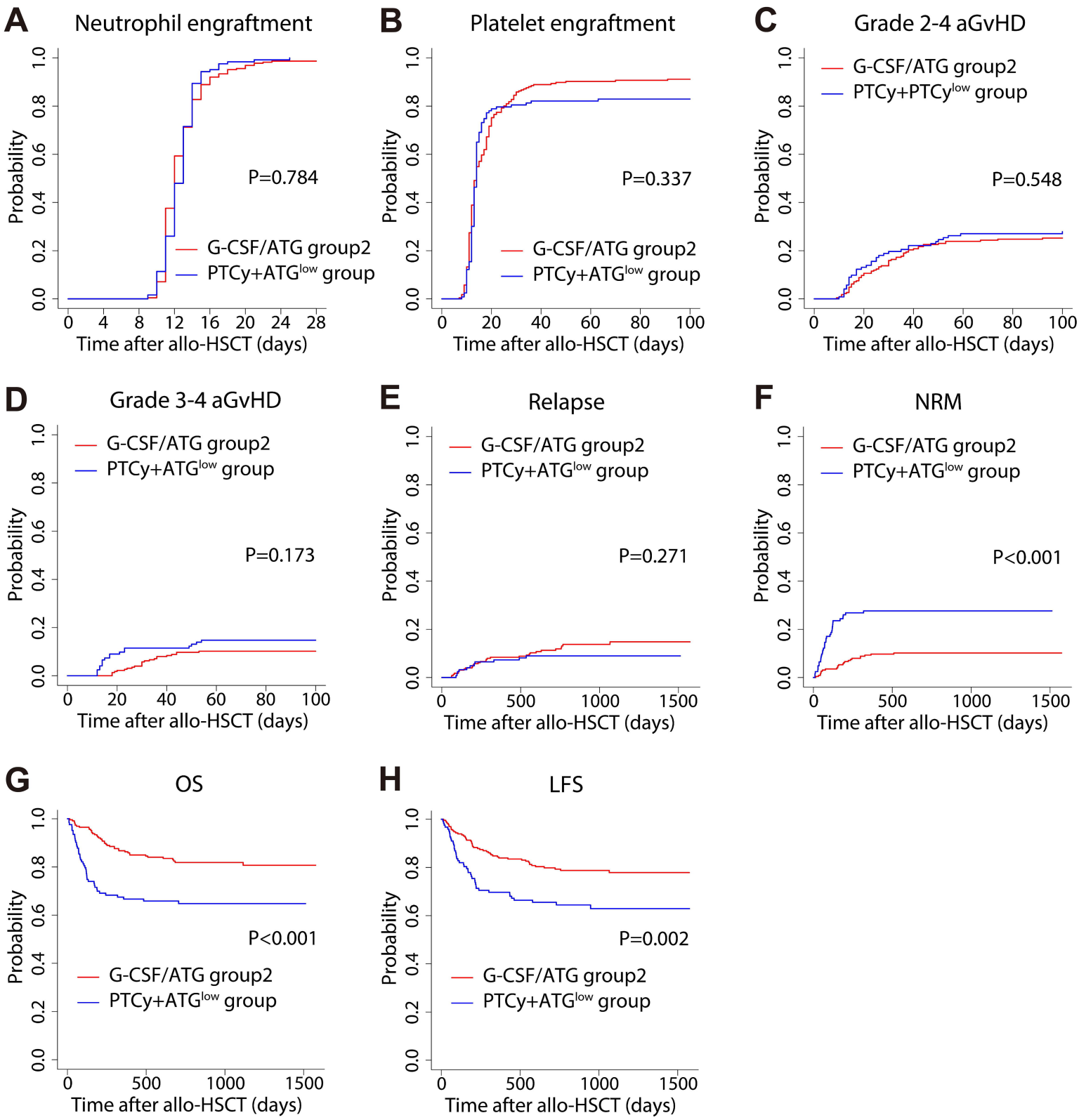
Figure 1. Flowchart of patient selection in different groups.

Figure 2. Comparison of baseline patient characteristics between the granulocyte colony-stimulating factor/antithymocyte globulin group 1 and the post-transplantation cyclophosphamide group after propensity score matching analysis. (A) Cumulative incidence of neutrophil engraftment. (B) Cumulative incidence of platelet engraftment. (C) Cumulative incidence of Grade 2-4 acute graft versus host disease. (D) Cumulative incidence of Grade 3-4 acute graft versus host disease. (E) Cumulative incidence of relapse. (F) Cumulative incidence of nonrelapse mortality. (G) The overall survival probabilities. (H) Leukaemia-free survival probabilities.

Figure 3. Comparison of baseline patient characteristics between granulocyte colony-stimulating factor/antithymocyte globulin group 2 and the post-transplantation cyclophosphamide with low dose antithymocyte globulin group after propensity score matching analysis. (A) Cumulative incidence of neutrophil engraftment. (B) Cumulative incidence of platelet engraftment. (C) Cumulative incidence of Grade 2-4 acute graft versus host disease. (D) Cumulative incidence of grade 3-4 acute graft versus host disease. (E) Cumulative incidence of relapse. (F) Cumulative incidence of nonrelapse mortality. (G) The overall survival probabilities. (H) Leukaemia-free survival probabilities.


CBMTRG database
Patients with with hematological malignancies who underwent their first haplo-HSCT between January 2020 and December 2022
n=1737
Eligible in this study
n=818
PTCy group
n=123
G-CSF/ATG group
n=572
PTCy+ATGlow group
n=123
Propensity score matching analysis (1:2)
PTCy group
n=122
G-CSF/ATG group 1
n=230
G-CSF/ATG group 2
n=226
PTCy+ATGlow group
n=123


A
Neutrophil engraftment
Probability
P=0.005
G-CSF/ATG group1
PTCy group
Time after allo-HSCT (days)
B
Platelet engraftment
P=0.002
C
Grade 2-4 aGvHD
P=0.972
D
Grade 3-4 aGvHD
P=0.494
E
Relapse
P=0.501
F
NRM
P<0.001
G
OS
P<0.001
H
LFS
P<0.001


A
Neutrophil engraftment
Probability
0.0
0.2
0.4
0.6
0.8
1.0
P=0.784
G-CSF/ATG group2
PTCy+ATG^low group
0
4
8
12
16
20
24
28
Time after allo-HSCT (days)
B
Platelet engraftment
Probability
P=0.337
G-CSF/ATG group2
PTCy+ATG^low group
0
20
40
60
80
100
Time after allo-HSCT (days)
C
Grade 2-4 aGvHD
Probability
G-CSF/ATG group2
PTCy+PTCy^low group
P=0.548
0
20
40
60
80
100
Time after allo-HSCT (days)
D
Grade 3-4 aGvHD
Probability
G-CSF/ATG group2
PTCy+ATG^low group
P=0.173
0
20
40
60
80
100
Time after allo-HSCT (days)
E
Relapse
Probability
G-CSF/ATG group2
PTCy+ATG^low group
P=0.271
0
500
1000
1500
Time after allo-HSCT (days)
F
NRM
Probability
G-CSF/ATG group2
PTCy+ATG^low group
P<0.001
0
500
1000
1500
Time after allo-HSCT (days)
G
OS
Probability
P<0.001
G-CSF/ATG group2
PTCy+ATG^low group
0
500
1000
1500
Time after allo-HSCT (days)
H
LFS
Probability
P=0.002
G-CSF/ATG group2
PTCy+ATG^low group
0
500
1000
1500
Time after allo-HSCT (days)

Clinical outcomes of three haploidentical transplantation protocols for hematologic malignancies based on data from the Chinese Bone Marrow Transplantation Registry Group

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# ZL X, J J, SB W contributed equally to this work.

Methods

Study endpoints and definitions

The primary study endpoint was leukaemia-free survival (LFS). The secondary study endpoints included engraftment, acute GvHD (aGvHD), cytomegalovirus (CMV) viraemia, Epstein–Barr virus (EBV) viraemia, relapse, NRM and OS. LFS was defined as the duration from transplantation to either death or relapse, depending on which occurred first. Neutrophil engraftment was defined as an absolute neutrophil count (ANC) in peripheral blood (PB) of ≥ 0.5×10^9/L for three consecutive days, and platelet engraftment was defined as a platelet count of ≥ 20×10^9/L for seven consecutive days in the absence of platelet transfusion. aGVHD was defined and graded according to the modified Seattle–Glucksberg criteria. Using real-time quantitative PCR to detect the copy numbers of CMV-DNA and EBV-DNA in PB, a CMV-DNA count exceeding 5×10^2/L was diagnosed as CMV viraemia, and an EBV-DNA count exceeding 1×10^3/L was diagnosed as EBV viraemia. Relapse was defined as the presence of ≥5% bone marrow (BM)

blasts or the reappearance of extramedullary leukaemia after complete remission (CR). NRM was defined as the incidence of death due to causes other than relapse or disease progression. OS was defined as the duration from transplantation to death due to any cause or to the time at which survival was confirmed.

Statistical analysis

The data were updated until April 30, 2024. Death was considered the competing risk for engraftment and GvHD, whereas relapse and NRM were competing risks for each other. Hazard ratios (HRs) for OS and LFS were estimated from univariate and multivariate Cox regression analyses. HRs for engraftment, aGvHD, relapse, and NRM were estimated from univariate and multivariate competing risk regression analyses. The factors included in the regression model were patient age, sex, disease type, disease risk index (DRI), HCT-CI score, donor–recipient relationship, donor–recipient sex match, donor–recipient ABO match status, source of stem cells, mononuclear cell (MNC) count, CD34+ cell count, and transplant protocol. All of the factors with $P < 0.1$ in the univariate analysis were included in the multivariate regression.

Supplementary Table 1. Baseline patient characteristics and clinical outcomes between PTCy and PTCy with ATG group.

Characteristics	PTCy group (n=122)	PTCy+ATG^{low} group (n=123)	P value
Median age at allo-HSCT, years (range)	31 (3–60)	34 (2–60)	0.478
Sex, n (%)			0.479
Male	55 (45.1%)	61 (49.6%)	
Female	67 (54.9%)	62 (50.4%)	
Diagnosis, n (%)			0.176
AML	62 (50.8%)	61 (49.6%)	
ALL	35 (28.7%)	47 (38.2%)	
MDS	15 (12.3%)	11 (8.9%)	
Others	10 (8.2%)	4 (3.3%)	
Disease risk index, n (%)			0.132
Low risk	4 (3.3%)	2 (1.6%)	
Intermediate + high risk	111 (91.0%)	119 (96.7%)	
Very high risk	7 (5.7%)	2 (1.6%)	
HCT-CI, n (%)			0.250
0	37 (30.3%)	26 (21.1%)	
1–2	78 (63.9%)	88 (71.5%)	
≥3	7 (5.7%)	9 (7.3%)	
Number of HLA-A/B/DRB1 mismatches, n (%)			

0–2			
3			
Donor-patient sex match, n (%)			0.265
Male-male	37 (30.3%)	39 (31.7%)	
Male-female	47 (38.5%)	35 (28.5%)	
Female-male	15 (12.3%)	24 (19.5%)	
Female-female	23 (18.9%)	25 (20.3%)	
Donor-recipient relationship, n (%)			**0.047**
Parents-child	43 (35.2%)	42 (34.1%)	
Child-parents	34 (27.9%)	50 (40.7%)	
Sibling-sibling	44 (36.1%)	28 (22.8%)	
Others	1 (0.8%)	3 (2.4%)	
ABO match, n (%)			0.098
Match	76 (62.3%)	73 (59.3%)	
Minor mismatch	21 (17.2%)	20 (16.3%)	
Major mismatch	17 (13.9%)	28 (22.8%)	
Bidirectional mismatch	8 (6.6%)	2 (1.6%)	
MNCs ($\times 10^8$/kg), median (range)	11.05 (3.58–34.63)	11.91 (5.60–31.94)	0.103
$CD34^+$ cells ($\times 10^6$/kg), median (range)	6.07 (2.30–17.88)	5.60 (0.52–17.30)	0.139
Graft resource, n (%)			0.233

BM+PB cell	1 (0.8%)	0	
PB cell	121 (99.2%)	123 (100%)	
Neutrophil engraftment	97.5% (94.5–100%)	100%	**0.003**
Platelet engraftment	84.4% (77.9–90.9%)	82.9% (76.2–89.7%)	0.281
aGvHD24	28.7% (20.7–36.8%)	27.9% (19.9–35.9%)	0.928
aGvHD34	13.1% (7.1–19.2%)	14.8% (8.4–21.1%)	0.694
3-year cGvHD	28.3% (18.9–37.6%)	24.5% (15.2–33.8%)	0.611
3-year moderate and severe cGvHD	10.6% (3.7–17.5%)	8.1% (2.7–134.4%)	0.900
CMV viremia	65 (53.3%)	71 (57.7%)	0.484
EBV viremia	17 (13.9%)	24 (19.5%)	0.242
3-year CIR	10.9% (5.3–16.6%)	9.0% (3.9-14.0%)	0.644
3-year NRM	26.2% (18.1-34.3%)	27.6% (19.7-35.6%)	0.546
3-year OS	65.2% (57.0-74.7%)	64.8% (56.8-73.9%)	0.661
3-year LFS	62.9% (54.6-72.4%)	63.4% (55.4-72.5%)	0.741

ALL, acute lymphocytic leukemia; allo-HSCT, allogeneic hematopoietic stem cell transplantation; AML, acute myeloid leukemia; ATG, antithymocyte globulin; BM, bone marrow; G-CSF, granulocyte colony-stimulating factor; GvHD, graft-versus-host disease; HCT-CI, hematopoietic cell transplantation-comorbidity index; MDS, myelodysplastic syndromes; MNCs, mononuclear cells; PB, peripheral blood; PTCy, posttransplantation cyclophosphamide.

Supplementary Table 2. The clinical outcomes among the three groups based on all patients enrolled in the study.

	G-CSF/ATG (n=309)	PTCy (n=122)	PTCy+ATG^{low} (n=123)	P value
3-year NRM	8.7% (5.6–11.9%)	26.2% (18.1–34.3%)	27.6% (19.7–35.6%)	<0.001
3-year CIR	14.5% (10.4–18.7%)	10.9% (5.3–16.6%)	9.0% (3.9–14.0%)	0.426
3-year OS	83.6% (79.5–87.8%)	65.2% (57.0-74.7%)	64.8% (56.8–73.9%)	<0.001
3-year LFS	76.7% (72.0–81.8%)	62.9% (54.6–72.4%)	63.4% (55.4–72.5%)	<0.001
Neutrophil engraftment at day28	98.7% (97.4–100%)	97.5% (94.5–100%)	100%	0.026
Platelet engraftment at day100	92.9% (90.0–95.8%)	84.4% (77.9–90.9%)	82.9% (76.2–89.7%)	0.009
Grades II-IV aGvHD at day100	27.8% (22.9–32.8%)	28.7% (20.7–36.8%)	27.9% (19.9–35.9%)	0.989
Grades III-IV aGvHD at day100	11.7% (8.1–15.2%)	13.1% (7.1–19.2%)	14.8% (8.4–21.1%)	0.604
3-year cGvHD	34.4% (28.8–40.1%)	28.3% (18.9–37.6%)	24.5% (15.2–33.8%)	0.159
3-year moderate and severe	15.6% (11.3–20.0%)	10.6% (3.7–17.5%)	8.1% (2.7–134.4%)	0.136
PTLD	6 (1.9%)	2 (1.6%)	1 (0.8%)	0.828
CMV	177 (57.3%)	65 (53.3%)	71 (57.7%)	0.716
EBV	44 (14.2%)	17 (13.9%)	24 (19.5%)	0.346

ATG, antithymocyte globulin; aGvHD, acute graft-versus-host disease; cGvHD, chronic graft-versus-host disease; G-CSF, granulocyte colony-stimulating factor; LFS, leukemia-free survival; NRM, non-relapse mortality; OS, overall survival; PTLD, Posttransplant lymphoproliferative disorders; PTCy, posttransplantation cyclophosphamide.

Supplementary Table 3. Multivariate analysis of risk factors for relapse, NRM, OS and LFS in G-CSF/ATG group, PTCy group and PTCy with ATG group.

Variables	Relapse		NRM		OS		LFS	
	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value
Patient age	/	/	1.02 (1.00-1.04)	0.058	1.01 (1.00–1.02)	0.22	/	/
High/very high vs.	2.55 (1.45–4.49)	**0.001**	3.04 (1.75–5.32)	**<0.001**	2.16 (1.50–3.11)	**<0.001**	2.39 (1.72–3.31)	**<0.001**
HCT-CI>0 vs. HCT-CI=0	/	/	1.25 (0.66–2.37)	0.49	1.40 (0.89–2.21)	0.141	11.29 (0.90–1.85)	0.160
Others vs. parent-child	0.62 (0.35-1.09)	0.10	/	/	/	/	/	/
≥$10*10^8$ vs. <$10*10^8$ MNCs	/	/	/	/	1.07 (0.74–1.55)	0.728	/	/
≥$4*10^6$ vs. <$4*10^6$ $CD34^+$ cells	0.55 (0.31–0.96)	**0.037**	0.86 (0.47–1.55)	0.49	/	/	/	/
PTCy-based vs. G-CSF/ATG-	/	/	2.59 (1.30–5.12)	**0.007**	1.94 (1.24–3.04)	**0.004**	1.46 (0.99–2.15)	0.057
PTCy+ATG^{low} based vs. G-			2.56 (1.24–5.26)	**0.011**	2.12 (1.36-3.30)	**<0.001**	1.56 (1.06–2.30)	**0.024**

ATG, antithymocyte globulin; CI, confidence interval; DRI, disease risk index, G-CSF, granulocyte colony-stimulating factor; HCT-CI, hematopoietic cell transplantation-comorbidity index, HR, hazard ratio; LFS, leukemia-free survival; MNCs, mononuclear cells; NRM, non-relapse mortality; OS, overall survival; PTCy, posttransplantation cyclophosphamide.

Supplementary Table 4. Multivariate analysis of risk factors for engraftment, aGvHD and cGvHD in G-CSF/ATG group, PTCy group and PTCy with ATG group.

Variables	Neutrophil engraftment		Platelet engraftment		Grade 2-4 aGvHD		Grade 3-4 aGvHD	
	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value
Female vs. male for patient	/	/	1.14 (0.96–1.35)	0.14	/	/	0.69 (0.41–1.16)	0.16
ALL vs. AML	/	/	0.69 (0.57–0.84)	**<0.001**	/	/	1.14 (0.64–2.03)	0.66
MDS vs. AML	/	/	0.73 (0.55–0.98)	**0.038**	/	/	2.17 (1.11–4.24)	**0.023**
Others vs. AML	/	/	0.68 (0.48–0.95)	**0.025**	/	/	2.58 (1.21–5.48)	**0.014**
High/very high vs.	0.78 (0.64–0.95)	**0.013**	0.79 (0.66–0.95)	**0.011**	1.62 (1.18–2.24)	**0.003**	1.90 (1.18–3.06)	**0.008**
HCT-CI>0 vs. HCT-CI=0	/	/	0.88 (0.74–1.05)	**0.015**	/	/	/	/
Others vs female-male. in	/	/	1.25 (0.94–1.66)	0.13	/	/	0.76 (0.40–1.47)	0.42
≥4*10^6 vs. <4*10^6 $CD34^+$ cells	1.56 (1.28–1.90)	**<0.001**	/	/	/	/	/	/
PTCy-based vs. G-CSF/ATG-	0.54 (0.42–0.69)	**<0.001**	0.76 (0.62–0.93)	**0.009**	/	/	/	/
PTCy+ATG^{low} based vs. G-	0.81 (0.64–1.02)	0.068	0.87 (0.69–1.10)	0.24	/	/	/	/

Variables	cGvHD		Moderate and severe cGvHD	
	HR (95% CI)	P value	HR (95% CI)	P value
Patient age	1.01 (0.99–1.02)	0.34	/	/
High/very high vs. low/intermediate risk of DRI	1.54 (1.10–2.15)	**0.013**	1.70 (1.01–2.84)	**0.046**

HCT-CI>0 vs. HCT-CI=0	1.29 (0.88–1.89)	0.18	/	/
PTCy-based vs. G-CSF/ATG-based protocol	0.71 (0.46–1.10)	0.13	/	/
PTCy+ATG^{low} based vs. G-CSF/ATG-based	0.60 (0.38–0.95)	**0.030**	/	/

aGvHD, acute graft-versus-host disease; ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia; ATG, antithymocyte globulin; cGvHD, chronic graft-versus-host disease; CI, confidence interval; DRI, disease risk index; G-CSF, granulocyte colony-stimulating factor; HCT-CI, hematopoietic cell transplantation-comorbidity index; HR, hazard ratio; MDS, myelodysplastic syndromes; PTCy, posttransplantation cyclophosphamide.