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 hematologic 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 the G-CSF/ATG group 1 (N=230), and the PTCy+ATG^{low} group (N=123) was matched with the 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 non-relapse mortality (NRM; P<0.001), better 3-year overall survival (OS; P<0.001) and leukemia-free survival (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 leukemia-free survival (P=0.002) than the PTCy+ATG^{low} group. In conclusion, G-CSF/ATG-based haplo-HSCT may be a preferential choice for the Chinese population with hematologic malignancies. In the future, a randomized controlled study is needed for further confirmation.

Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is an effective option for curing a variety of hematologic malignancies.[1] In the last two decades, great advances have been made in haploidentical hematopoietic 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 (ATG^{low}) and G-CSF/ATG combined with low-

dose PTCy, have been attempted.[4-6] The rapid expansion of the above-mentioned 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 combined with ATG^{low} (PTCy+ATG^{low}) (N=42), the G-CSF/ATG protocol (N=110) had the most favorable neutrophil and platelet engraftment kinetics, the lowest non-relapse 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 leukemia (AML), patients treated with PTCy (N=193) had markedly lower NRM and better leukemia-free survival (LFS) than those treated with G-CSF/ATG (N=115), but patients in the PTCy cohort were transplanted earlier 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 leukemia (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-mentioned studies were limited by imbalanced characteristics before transplantation.

Considering the above-stated 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,[8] 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.[11]

Hence, we conducted the current study to compare the clinical outcomes of G-CSF/ATG, PTCy and 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 Figure 1. This work was a multicenter, retrospective trial based on the data from the CBMTRG. Informed consent was obtained from the patients or their families. The study was approved by the institutional review board of each center.

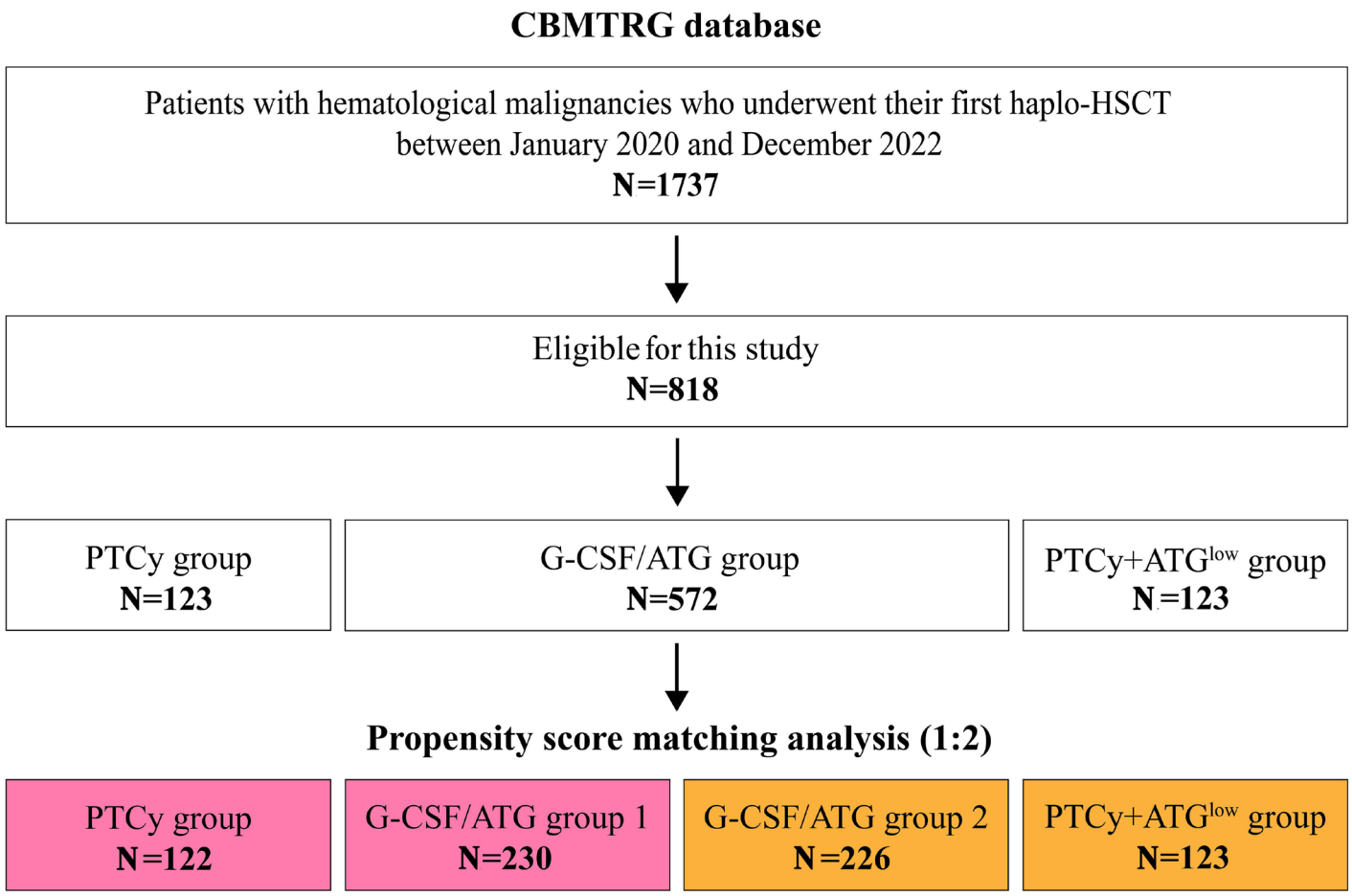

Figure 1. Flowchart of patient selection in different groups. haplo-HSCT: haploid hematopoietic stem cell transplantation; ATG: antithymocyte globulin; ATG^{low}: low-dose ATG; G-CSF: granulocyte colony-stimulating factor; PTCy: posttransplantation cyclophosphamide.

In order 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 hematopoietic cell transplantation-comorbidity index (HCT-CI).[12] The nearest-neighbor matching method using propensity scores was employed, with a caliper of 0.20. The balance was verified by assessing standardized mean differences between these groups.

Transplantation procedure

GCSF-ATG group

The modified busulfan-cyclophosphamide (Bu-Cy) plus ATG conditioning regimen included cytarabine 4 g/m^2/day intravenously (i.v.) on days -10 to -9, Bu 3.2 mg/kg/day i.v. on days -8 to -6, Cy 1.8 g/m^2/day i.v. on days -5 to -4, Me-CCNU 250 mg/m^2/day 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).[13]

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+ATG^{low} 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 to 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 i.v. busulfan (Bu) 3.2 mg/kg/day on days -6 to -3, fludarabine (Flu) 150 mg/m^2, and cytarabine (Ara-C) 1 g/m^2/day 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 χ^2 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 (CI) 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 hematologic 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 leukemia or myelodysplastic syndrome (MDS). A total of 818 patients with complete medical records were enrolled for PSM. After PSM, the G-CSF/ATG group 1 (N=230) was matched with the PTCy group (N=122), whereas the 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 the number of HLA mismatches, MNC, CD34$^+$ cells or graft resources, the baseline characteristics did not significantly differ between the two groups. The median follow-up periods for survivors were 1,057 days (range, 482-1,574 days), 970 days (range, 515-1,575 days), 1,015 days (range, 482-1,576 days), and 865 days (range, 497-1,512 days) in the G-CSF/ATG group 1, the PTCy group, the G-CSF/ATG group 2, and the PTCy+ATG^{low} group, respectively.

Engraftment

G-CSF/ATG versus PTCy

The CI rates of neutrophil engraftment on day 28 (98.3%, [95% confidence interval (CI): 96.5-100.0] *vs*. 97.5% [95% CI: 94.5-100], *P*=0.005; Figure 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; Figure 2B) in the 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 versus PTCy+ATG^{low}

The cumulative incidence of neutrophil engraftment on day 28 (98.7% [95% CI: 97.1-100] *vs*. 100%, P=0.784; Figure 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; Figure 3B) did not significantly differ between the 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).

Acute graft-*versus*-host disease

G-CSF/ATG versus PTCy

No significant difference in aGVHD was observed among the groups. The 100-day CI 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 the G-CSF/ATG group 1 and the PTCy group, respectively.

G-CSF/ATG versus PTCy+ATG^{low}

The 100-day CI 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)

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*
Median age in years at allo-HSCT (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)	
MNC $\times 10^8$/kg, median (range)	9.59 (1.00-18.67)	11.05 (3.58-34.63)	<0.001
$CD34^+$ cells $\times 10^6$/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 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 syndrome; MNC: mononuclear cells; PB: peripheral blood; PTCy: posttransplantation cyclophosphamide.

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.

Chronic graft-*versus*-host disease

G-CSF/ATG versus PTCy

No significant difference in cGVHD was observed among the groups. The 3-year CI of cGVHD 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.

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*
Median age in years at allo-HSCT (range)	35 (6-65)	34 (260)	0.168
Sex, N (%) Male Female	 115 (50.9) 111 (49.1)	 61 (49.6) 62 (50.4)	0.818
Diagnosis, N (%) AML ALL MDS Others	 112 (49.6) 80 (35.4) 27 (11.9) 7 (3.1)	 61 (49.6) 47 (38.2) 11 (8.9) 4 (3.3)	0.843
Disease risk index, N (%) Low risk Intermediate + high risk Very high risk	 8 (3.5) 214 (94.7) 4 (1.8)	 2 (1.6) 119 (96.7) 2 (1.6)	0.598
HCT-CI, N (%) 0 1-2 ≥3	 52 (23.0) 155 (68.9) 18 (8.0)	 26 (21.1) 88 (71.5) 9 (7.3)	0.875
Number of HLA-A/B/DRB1 mismatches, N (%) 0-2 3	 48 (21.2) 178 (78.8)	 50 (40.7) 73 (59.3)	<0.001
Donor-patient sex match, N (%) Male-male Male-female Female-male Female-female	 88 (38.9) 76 (33.6) 27 (11.9) 35 (15.5)	 39 (31.7) 35 (28.5) 24 (19.5) 25 (20.3)	0.111
Donor-recipient relationship, N (%) Parents-child Child-parents Sibling-sibling Others	 92 (40.7) 76 (33.6) 57 (25.2) 1 (0.4)	 42 (34.1) 50 (40.7) 28 (22.8) 3 (2.4)	0.171
ABO match, N (%) Match Minor mismatch Major mismatch Bidirectional mismatch	 125 (55.3) 43 (19.0) 41 (18.1) 17 (7.5)	 73 (59.3) 20 (16.3) 28 (22.8) 2 (1.6)	0.076
MNC $\times10^8$/kg, median (range)	9.60 (1.00-32.03)	11.91 (5.60-31.94)	<0.001
CD34$^+$ cells $\times10^6$/kg, median (range)	3.41 (0.69-14.10)	5.60 (0.52-17.30)	<0.001
Graft resource, N (%) BM + PB cell PB cell	 17 (7.5) 209 (92.5)	 0 123 (100)	0.001

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 syndrome; MNC: mononuclear cells; PB: peripheral blood; PTCy: posttransplantation cyclophosphamide.

G-CSF/ATG versus PTCy+ATG^{low}

The 100-day CI 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 the G-CSF/ATG group 2 and the PTCy+ATG^{low} group, respectively.

Viremia

Cytomegalo virus (CMV) viremia (59.1% *vs*. 53.3%, *P*=0.291; 56.6% *vs*. 57.7%, *P*=0.845) or Epstein-Barr virus (EBV) viremia (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.

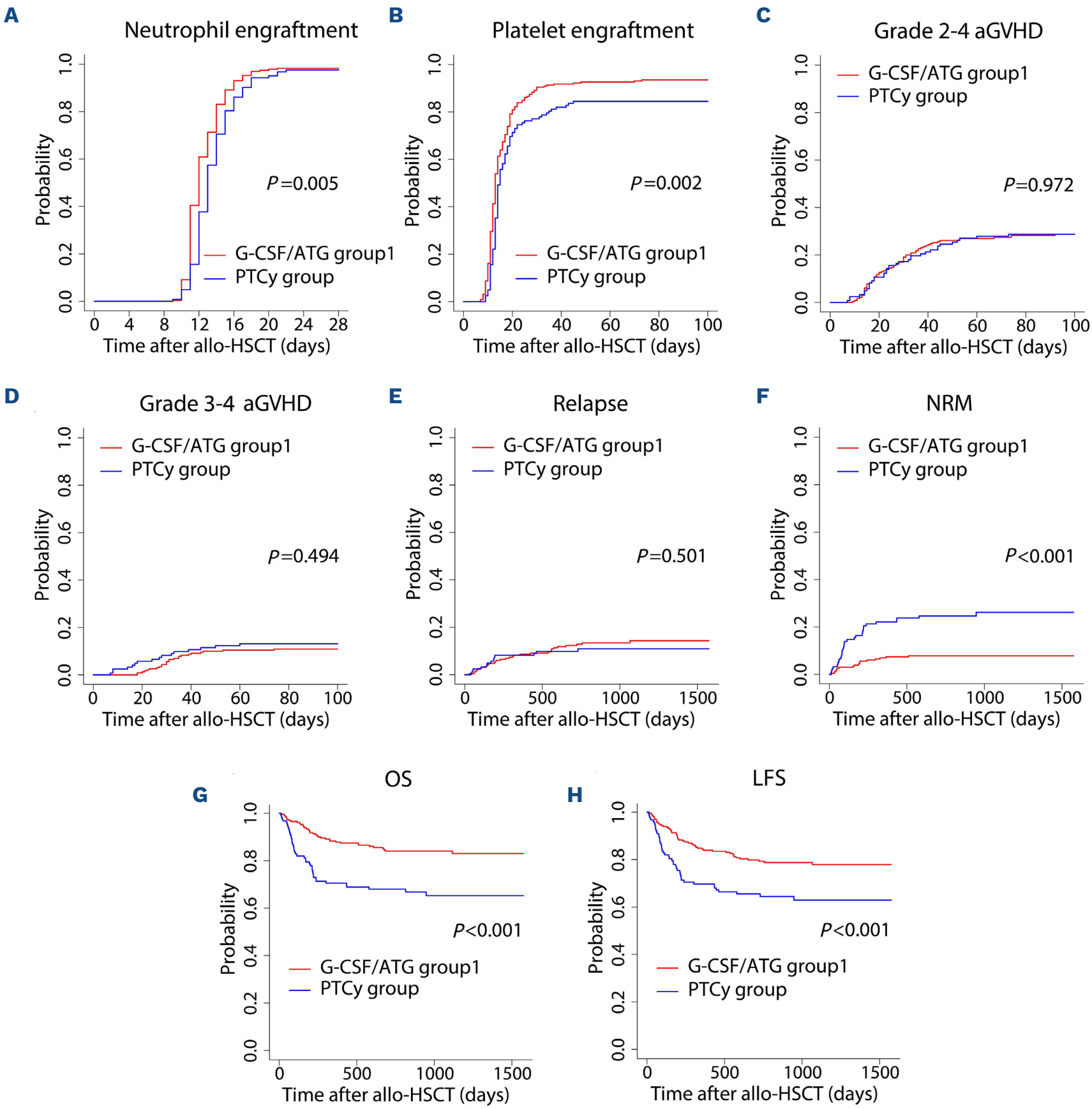

Figure 2. Comparison of baseline patient characteristics between the granulocyte colony-stimulating factor/antithymocyte globulin group 1 and the post-transplantation cyclophosphamide group after propensity score matching analysis. (A) Cumulative incidence (CI) of neutrophil engraftment. (B) CuI of platelet engraftment. (C) CI of grade 2-4 acute graft- *versus*-host (aGVHD) disease. (D) CI of grade 3-4 aGVHD. (E) CI of relapse. (F) CI of non-relapse mortality (NRM). (G) The overall survival (OS) probabilities. (H) Leukemia-free survival (LFS) probabilities. allo-HSCT: allogeneic hematopoietic stem cell transplantation; ATG: antithymocyte globulin; G-CSF: granulocyte colony-stimulating factor; PTCy: posttransplantation cyclophosphamide.

Relapse and non-relapse mortality

G-CSF/ATG versus 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 CI of relapse (CIR) 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; Figure 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; Figure 2F).

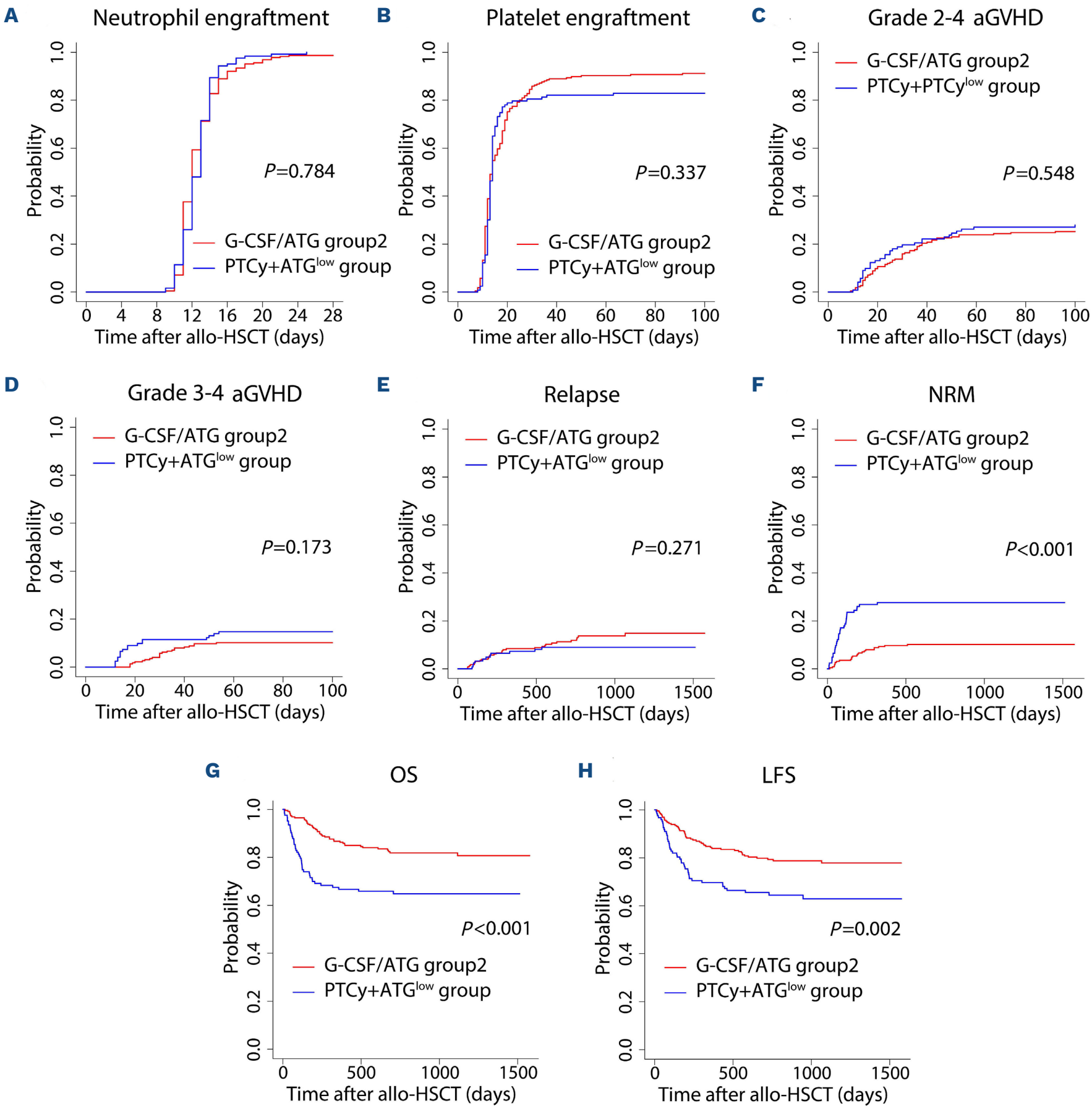

Figure 3. Comparison of baseline patient characteristics between granulocyte colony-stimulating factor/antithymocyte globulin group 2 and the post-transplantation cyclophosphamide with low-dose antithymocyte globulin group after propensity score matching analysis. (A) Cumulative incidence (CI) of neutrophil engraftment. (B) CI of platelet engraftment. (C) CI of grade 2-4 acute graft-*versus*-host disease (aGVHD). (D) CI of grade 3-4 aGVHD. (E) Cumulative incidence of relapse. (F) CI of non-relapse mortality (NRM). (G) The overall survival (OS) probabilities. (H) Leukemia-free survival (LFS) probabilities. allo-HSCT: allogeneic hematopoietic stem cell transplantation; ATG: antithymocyte globulin; ATG^{low}: low-dose ATG; G-CSF: granulocyte colony-stimulating factor; PTCy: posttransplantation cyclophosphamide; $PTCy^{low}$: low-dose PTCy.

G-CSF/ATG versus PTCy+ATG^{low}

Thirty patients (13.3%) and 11 patients (9.0%) in the 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; Figure 3E). Compared with that in the PTCy+ATG group, the 3-year NRM in the 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; Figure 3F).

Overall survival and leukemia-free survival

G-CSF/ATG versus 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 the G-CSF/ATG group 1 and 65.2% (95% CI: 57.0-74.7) in the PTCy group (*P*<0.001; Figure 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; Figure 2H).

G-CSF/ATG versus PTCy+ATG^{low}

In addition, the 3-year OS rates were 81.9% (95% CI: 76.9-87.1) in the G-CSF/ATG group 2 and 64.8% (95% CI: 56.8-73.9) in the PTCy+ATG^{low} group (*P*<0.001; Figure 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; Figure 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 the *Online Supplementary Table S1*, 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 (MNC) 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 CI 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, aGVHD, cGVHD, relapse, NRM and survival outcomes.

Multivariate analysis

The clinical outcomes among the three groups based on all patients were presented in the *Online Supplementary Table S2*. We combined the three groups of cases and included them in the multivariate analysis (*Online Supplementary Tables S3, S4*). 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+ATG^{low}-based haplo-HSCT via PSM analysis to reduce confounding bias. Our findings suggest that the G-CSF/ATG-based protocol can reduce NRM and prolong OS and LFS than both the PTCy-based and PTCy+ATG^{low}-based protocols. In addition, haplo-HSCT with G-CSF/ATG results in superior myeloid and platelet engraftment compared with haplo-HSCT with PTCy. The incidences of aGVHD, CMV

Table 3. Primary causes of death among patients.

Causes of death, N (%)	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)
Hemorrhage	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.

viremia, EBV viremia or relapse do not differ significantly among the three groups in our current analysis.

Engraftment is an essential endpoint of observation when different haploidentical protocols are evaluated.[15] 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[16-18] but ranged from 89% to 94% with the PTCy-based protocol.[19-21] 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+ATG and PTCy groups, respectively.[8] 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+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 ATG^{low} to PTCy may also accelerate neutrophil recovery.[22]

The incidences of aGVHD did not significantly differ among the three strategies, with grade 2-4 aGVHD of 25.2-28.6%, 28.7%, 27.9%, and grade 3-4 aGVHD 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 CI of grade 2-4 and grade 3-4 aGVHD were 32.7% *versus* 30.5% and 11.6% *versus* 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.[23] Some studies have reported a significantly lower CI of acute grade 2-4 GVHD with the combination of PTCy+ATG^{low} than with the PTCy protocol. In Makanga's study, PTCy+ATG led to an incidence of grade 2-4 aGVHD of 24%, compared with 59% for the PTCy protocol.[24] In El-Cheikh's study, the PTCy+ATG and PTCy protocols resulted in incidences of grade 2-4 aGVHD of 12% and 22%, respectively.[25] However, others have suggested that the addition of ATG does not provide any additional benefit in aGVHD.[22,26,27] In the present study, PTCy+ATG^{low} 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+ATG^{low}, 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 ATG^{low} 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 NRM 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+ATG, the inclusion of patients treated with various conditioning regimens and GVHD prophylaxis, and the transplant experience of different centers. 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 hematologic malignancies based on data from the CB-MTRG. 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.

Disclosures

No conflicts of interest to disclose.

Contributions

X-JH designed the research. Z-LX, M-HL and X-JH analyzed the data and wrote the manuscript. All authors provided patient data and gave final approval of the manuscript.

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Data-sharing statement

The data that support the findings of this study are available upon reasonable request from the corresponding author.

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