Randomized trial of anti-thymocyte globulin plus low-dose post-transplant cyclophosphamide to prevent graft-*versus*-host disease in haploidentical transplantation

Zheng-Li Xu,[1] Ting-Ting Han,[1] Xiao-Lu Zhu,[1] Jing Liu,[1] Meng Lv,[1] Yu-Qian Sun,[1] Xiao-Dong Mo,[1] Yi-Fei Cheng,[1] Lan-Ping Xu,[1] Xiao-Hui Zhang,[1] Xiao-Jun Huang[1,2] and Yu Wang[1]

[1]Peking University People's Hospital, Peking University Institute of Hematology, National Clinical Research Center for Hematologic Disease, Beijing Key Laboratory of Hematopoietic Stem Cell Transplantation, Collaborative Innovation Center of Hematology, Peking University and [2]Peking-Tsinghua Center for Life Sciences, Academy for Advanced Interdisciplinary Studies, Peking University, Beijing, China

Correspondence: Y. Wang
ywyw3172@sina.com

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Abstract

The combination of anti-thymocyte globulin (ATG) and posttransplant cyclophosphamide (PTCy) appears to be a potentially effective graft-*versus*-host disease (GVHD) prevention strategy for haploidentical transplantation. However, the majority of the evidence originated from retrospective studies without uniform protocols. Our previous findings indicated that 10 mg/kg ATG plus low-dose PTCy could decrease GVHD among high-risk populations transplanted from maternal or collateral relatives. We designed an open-label, phase III, randomized controlled trial to compare patients receiving granulocyte colony-stimulating factor (G-CSF)/ATG-based haploidentical transplantation with or without low-dose PTCy (14.5 mg/kg on days 3 and 4) in non-maternal, non-collateral haploidentical transplants from fathers, children or siblings. A total of 66 patients were randomly assigned to ATG-PTCy (N=44) or ATG (N=22) when the first interim analysis was performed. The interim analysis revealed that the 100-day cumulative incidences of grade 2-4 (18.2%, 95% confidence interval [CI]: 6.6-29.7 *vs*. 18.2%, 95% CI: 1.7-34.7; *P*=0.996) and 3-4 acute GVHD (2.3%, 95% CI: 0-6.7 *vs*. 0; *P*=0.480) were comparable between the ATG-PTCy and ATG cohorts, as was chronic GVHD at 1 year. The estimated 1-year disease-free survival (DFS) rates were also similar between ATG-PTCy and ATG cohorts (95.5%, 95% CI: 89.5–100 *vs*. 95.2%, 95% CI: 86.6–100; *P*=0.979). These results suggested that ATG/PTCy (low-dose) had no advantage over 10 mg/kg ATG-based prophylaxis in patients with haploidentical transplantation other than that of maternal donors or collateral relatives. Future work needs to focus on identifying which populations might benefit from the combined strategy in the context of G-CSF/ATG-based protocols (*clinicaltrials gov. Identifier: NCT 06108739*).

Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) from haploidentical donors has made significant progress over the past 20 years. Two mainstream haploidentical protocols without *in vitro* T-cell depletion include granulocyte colony-stimulating factor (G-CSF)/antithymocyte globulin (ATG)-based and posttransplant cyclophosphamide (PTCy)-based protocols.[1-3] Although both protocols overcome the HLA barrier and achieve acceptable incidences of severe graft-*versus*-host disease (GVHD), the optimization of GVHD prophylaxis, including the combination of the two protocols, is continuously being explored.[4,5] Previously published studies have demonstrated that PTCy plus ATG rather than PTCy alone limits the incidence of acute grade II-IV GVHD after reduced-intensity conditioning haploidentical transplantation, likely due to the combined effect of T and NK cell reconstitution.[6,7]

The G-CSF and ATG were two major elements in Beijing Protocol.[3,8-10] In the context of G-CSF/ATG-based haploidentical transplantation, maternal donor or collateral relatives have been proven to be associated with increased incidence of GVHD.[11] Previously, a novel regimen consisting of low-dose PTCy (14.5 mg/kg on days 3 and 4 after transplantation) in combination with 10 mg/kg ATG was designed. The addition of such a low dose PT-Cy was designed based on previous animal model and single-arm clinical cohort.[12] Published data have demonstrated that ATG plus low-dose PTCy could

significantly reduce both grade 3-4 acute GVHD (aGVHG) (5% *vs*. 18%) and 2-year chronic GVHD (cGVHD) (30% *vs*. 44%) compared with 10 mg/kg ATG-based prophylaxis among patients with maternal donor or collateral relatives at particularly high risk of GVHD.[13] With respect to the mechanism of action of low-dose PTCy in conjunction with ATG, *in vivo* immune reconstitution analysis revealed that a low dose of PTCy is sufficient for lymphopenia-induced regulatory T-cell (Treg) proliferation and that the effect on Treg becomes more obvious when low-dose PTCy is combined with ATG.[12]

Thus, the combination of ATG with low-dose PTCy has a synergistic mechanism to reduce GVHD through animal experiments, which has also been found to decrease GVHD among high-risk populations transplanted from maternal or collateral relatives. However, it is unclear whether the combination of ATG with low-dose PTCy could decrease GVHD in non-maternal, non-collateral haploidentical transplants. On the basis of these findings, we designed a prospective randomized controlled clinical study to explore this question.

Methods

Study design and participants

This study was an open-label, phase III, randomized controlled trial conducted at Peking University People's Hospital. The protocol was approved by the institutional review board of the participating centers. All of the patients or their legal guardians signed informed consent forms in accordance with the Declaration of Helsinki. This study was registered at (*clinicaltrials gov. Identifier: NCT 06108739*).

Patients were eligible if they had acute leukemia in complete remission and/or myelodysplastic syndrome and were indicated for allo-HSCT. Eligibility also included haploidentical donors other than maternal or collateral relatives, age between 12 and 55 years, an Eastern Cooperative Oncology Group (ECOG) score less than or equal to 3 and adequate basic organ function. The exclusion criteria were substantial vital organ function impairment, previous allo-HSCT, refractory malignant disease state, other malignant tumors requiring treatment, and active and non-controlled infectious diseases under treatment.

Randomization and protocols

The design was a superiority trial study. Randomization was performed with a central interactive response technology system. Patients were randomly assigned 2:1 to either the ATG-PTCy cohort or the ATG cohort. The conditioning regimen and GVHD prophylaxis were previously reported in detail.[14,15] All transplant recipients received busulfan-cyclophosphamide (Bu-Cy) myeloablative conditioning regimens.[11,16-18] In both cohorts, the Bu-Cy plus ATG conditioning regimen was applied as follows: cytarabine, 4 g/m^2/day intravenously (i.v.) on day -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 rabbit ATG (Sangstat-Genzyme), 2.5 mg/kg/day i.v. on days -5 to -2. The GVHD prophylaxis regimen consisted of cyclosporine A, mycophenolate mofetil (MMF), and short-term methotrexate (MTX).[16] CsA (1.5 mg/kg, every 12 hours, i.v.) was given from day -3 and the target concentration was adjusted to 200-250 ng/mL. Administration was oral once the patient's bowel function returned to normal. The target concentration of CsA was required for up to 100 days post HSCT, CsA was then gradually reduced and discontinued about 6-9 months post transplantation (balanced between the relapse risk and GVHD status). MMF (0.5 g every 12 hours) was administered orally from day -3 and discontinued upon myeloid engraftment. The dosage of MTX was 15 mg/m^2, administered i.v. on day +1, followed by 10 mg/m^2 on days 3, 6, and 11 after transplantation. In the ATG-PTCy cohort, two doses of 14.5 mg/kg Cy were given on days 3 and 4 posttransplantation. The G-CSF (5 ug/kg/day) were administered to donors from day -3 for consecutive 5 days, and the first apheresis was started on the morning of the fourth day after the start of the G-CSF administration.[19-22] All patients received peripheral blood as graft source. All patients enrolled in the study had donor-specific antibody (DSA) levels less than 2,000, and desensitization methods were not utilized.[23,24] Letermovir has been commonly used during transplant course from day +7 to day +100 in this study.

Outcome evaluation

The primary endpoint was the cumulative incidence of grade 2-4 aGVHD. The secondary endpoints included engraftment, the cumulative incidence of 3-4 aGVHD and cGVHD, infections, non-relapse mortality (NRM), disease relapse, overall survival (OS), and disease-free survival (DFS). Myeloid engraftment was defined as the first of 3 consecutive days with an ANC ≥0.5×10^9/L, and platelet engraftment was defined as the day the platelet count met or exceeded 20×10^9/L without transfusion for 7 days. aGVHD and cGVHD were assessed according to the international consensus, with Mount Sinai Acute GVHD International Consortium (MAGIC) criteria for aGVHD and the National Institutes of Health 2014 criteria for cGVHD.[25,26] Epstein-Barr virus (EBV) reactivation was defined based on titer ≥500 copies/mL. OS was calculated as the time from transplantation to death from any cause, and DFS was defined as the time from transplantation to relapse or death. GVHD and relapse-free survival (GRFS) events were defined as grade 3-4 aGVHD, intermediate-severe cGVHD, disease relapse, or death from any cause during follow-up after transplantation.[27]

Statistical analysis

Power calculations dictated that 196 subjects were necessary to detect a 20% difference between groups in grade

2-4 aGVHD on the basis of our previous results (from 45% to 25%), with a one-sided difference of 0.025 and a power of 0.80. The interim analysis was performed after one third, half and two thirds of the 196 cases was specified, and the conditional power for success was defined as 0.10. After the 66th patient was enrolled in this study, we performed the first interim analysis. As the conditional power was 0.025, which was less than the prespecified cutoff of 0.10, the Data and Safety Monitoring Board recommended halting the study owing to futility after the first interim analysis in August 2024. Data were collected on case report forms by medical record reviews.

The surviving patients were followed-up, and the results of the follow-up examinations were analyzed on December 31, 2024. Survival outcomes were estimated with the Kaplan-Meier method and compared using the log-rank test. The cumulative incidences of relapse, TRM, and GVHD were estimated while accounting for competing events and compared using Gray's test. SPSS 21.0 (Mathsoft, Seattle, WA, USA) and R version 3.4.4 (The R Foundation for Statistical Computing) were used for data analyses.

Results

Basic characteristics

Between November 30, 2023, and August 21, 2024, 66 patients were randomly assigned to the ATG-PTCy (N=44) or ATG (N=22) groups. The median follow-up was 264 days (range, 141-399) for the ATG-PTCy cohort and 257 days (range, 132-393) for the ATG cohort (*P*=0.915) post-transplantation. The baseline characteristics are presented in Table 1. The distribution of those patients was balanced in terms of patient age at transplantation, donor-recipient sex, disease type, and the kinship of haploidentical donors between the ATG-PTCy and ATG cohorts. The mean counts of lymphocytes pre-ATG were 1.07×10^9/L (range, 0.30-2.34) in the ATG-PTCy and 0.88×10^9/L (range, 0.18-2.26) in the ATG cohorts without significance (*P*=0.170). The median donor age was 34 (range, 16-59) *versus* 39.5 (range, 15-57) in the ATG-PTCy and ATG cohorts (*P*=0.414).

Engraftment and graft-*versus*-host disease

On day 28 after HSCT, 97.7% (95% CI: 92.1-100) of patients in the ATG-PTCy cohort and 100% of patients in the ATG cohort achieved neutrophil engraftment (*P*=0.206; Figure 1A). The median time to neutrophil engraftment was 14 (range, 12-28) *versus* 14 (range, 9-21) days posttransplantation in the ATG-PTCy and ATG cohorts, respectively (*P*=0.269). On day 30 after HSCT, 68.2% (95% CI: 54.1-82.3) of patients in the ATG-PTCy cohort and 81.8% (95% CI: 64.6-99.1) of patients in the ATG cohort achieved platelet engraftment (*P*=0.115). On day 100 after HSCT, 95.5% (95% CI: 88.1-100) of patients in the ATG-PTCy cohort and 100% of patients in the ATG cohort achieved platelet engraftment (*P*=0.084; Figure 1B). The median time to platelet engraftment was 20 (range, 12-267) *versus* 14.5 (range, 12-65) days posttransplantation in the ATG-PTCy and ATG cohorts, respectively (*P*=0.113). All of the patients achieved full donor chimerism during the follow-up.

The 100-day cumulative incidences of grade 2-4 (18.2%, 95% CI: 6.6-29.7 *vs*. 18.2%, 95% CI: 1.7-34.7; *P*=0.996; Figure 2A) and grade 3-4 aGVHD (2.3%, 95% CI: 0-6.7 *vs*. 0; *P*=0.480; Figure 2B) were comparable between the ATG-PTCy and ATG cohorts. Five patients (11.3%) in the ATG-PTCy cohort and one patient (4.5%) in the ATG cohort suffered steroid-refractory aGVHD (*P*=0.364). The 6-month cumulative incidences of cGVHD (34.4%, 95% CI: 19.4-49.3% *vs.* 23.5%, 95% CI: 4.9-42.2%) and moderate to severe cGVHD (20.0%, 95% CI: 7.3-32.6% *vs.* 9.1%, 95% CI: 0-21.4%) were similar between the ATG-PTCy and ATG cohorts. The estimated 1-year cumulative incidences of cGVHD (45.5%, 95% CI: 28.2-62.7 *vs*. 44.8%, 95% CI: 19.1-70.5; *P*=0.721; Figure 3A) and moderate to severe cGVHD (23.2%, 95% CI: 9.5-36.8 *vs*. 15.6%, 95% CI: 0-32.7; *P*=0.468; Figure 3B) were also similar between the ATG-PTCy and ATG cohorts.

Cytomegalovirus and Epstein-Barr virus infection

On day 100 after HSCT, a total of 9.1% (95% CI: 0.5-17.7) of the ATG-PTCy cohort and 9.1% (95% CI: 0-21.4) of the ATG cohort developed cytomegalovirus (CMV) viremia

Table 1. Patient characteristics between the two groups.

Characteristics	ATG + low-dose PTCy, N=44	ATG, N=22	*P*
Patient age at HSCT, years, median (range)	34 (16-59)	39.5 (15-57)	0.414
Patient-donor sex, N (%)			0.859
Matched	27 (61.4)	13 (59.1)	
Mismatched	17 (38.6)	9 (40.9)	
Disease types, N (%)			0.122
AML/MDS	27 (61.4)	13 (59.1)	
ALL	17 (38.6)	7 (31.8)	
Others	0	2 (9.1)	
Source of donor, N (%)			0.105
Parent	14 (31.8)	6 (27.3)	
Sibling	15 (34.1)	3 (13.6)	
Child	15 (34.1)	13 (59.1)	
N of HLA-A, -B, -C, -DR, -DQ mismatches, N (%)			0.854
0-4	15 (34.1)	7 (31.8)	
5	29 (65.9)	15 (68.2)	

AML: acute myeloid leukemia; ALL: acute lymphoblastic leukemia; MDS: myelodysplastic syndrome; HSCT: hematopoietic stem cell transplantation; ATG: anti-thymocyte globulin; PTCy: posttransplant cyclophosphamide.

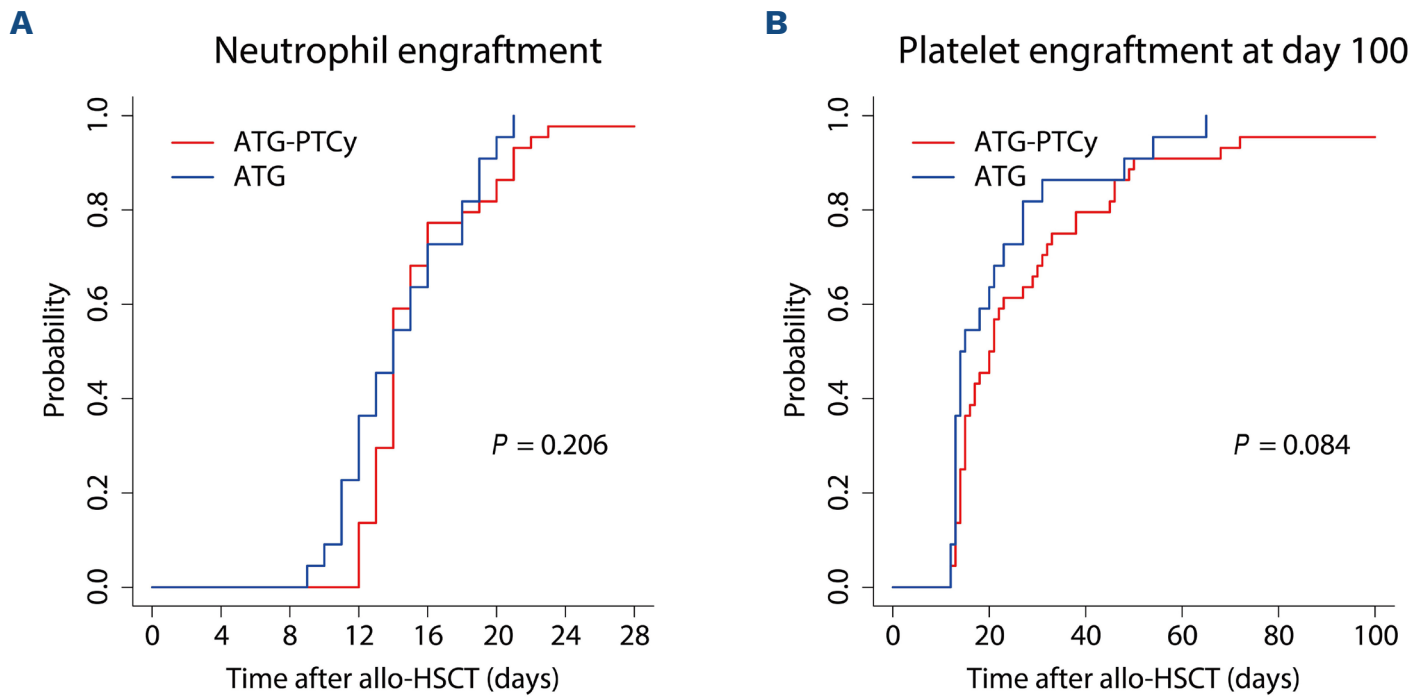

Figure 1. The cumulative incidence of engraftment between ATG-PTCy and ATG groups. (A) The cumulative incidence of neutrophil engraftment and (B) platelet engraftment. allo-HSCT: allogeneic hematopoietic stem cell transplantation; ATG: anti-thymocyte globulin; PTCy: posttransplant cyclophosphamide.

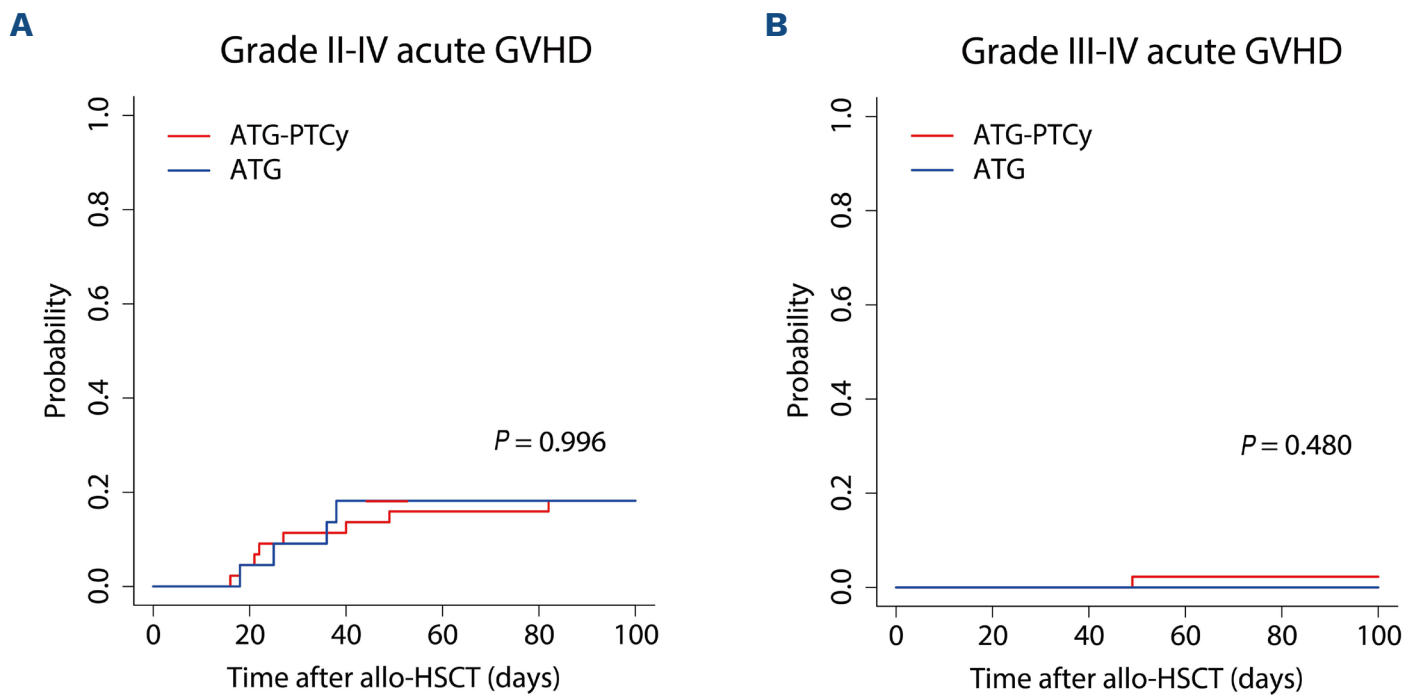

Figure 2. The 100-day cumulative incidences of acute graft-*versus*-host disease between ATG-PTCy and ATG groups. (A) The 100-day cumulative incidences of grade 2-4 and (B) grade 3-4 acute graft-*versus*-host disease (GVHD). allo-HSCT: allogeneic hematopoietic stem cell transplantation; ATG: anti-thymocyte globulin; PTCy: posttransplant cyclophosphamide.

(*P*=1.000). A total of 20.5% (95% CI: 8.4-32.5) of the patients in the ATG-PTCy cohort and 27.3% (95% CI: 8.2-6.4) of the patients in the ATG cohort were diagnosed with EBV viremia (*P*=0.438). None of the patients in the two cohorts developed CMV disease. Two patients (4.5%) in the ATG-PTCy cohort and one patient (4.5%) in the ATG cohort had EBV-associated posttransplant lymphoproliferative disorders (*P*=1.000). Besides, the dynamic immune reconstitution between the two cohorts have been presented in Table 2 and Table 3. We have further analyzed the impact of CD3, CD4, CD19 and immunoglobulin at +1 month on clinical outcomes, and found no statistically significant differences.

Regimen related toxicity

All patients received the conditioning regimen according to a pre-defined schedule. Two patients (4.5%) in the ATG-PTCy group and two patients (9.1%) in the ATG group suffered grade 1-2 hemorrhagic cystitis (*P*=0.466). Four patients (9.1%) in the ATG-PTCy group and two patients (9.1%) in the ATG group suffered grade 1 cardiotoxicity (*P*=1.000). Besides, there were five patients and two patients who developed oral mucositis in the ATG-PTCy and ATG groups, respectively (*P*=0.777).

Survival and relapse

Two patients in the whole cohort died during our follow-up

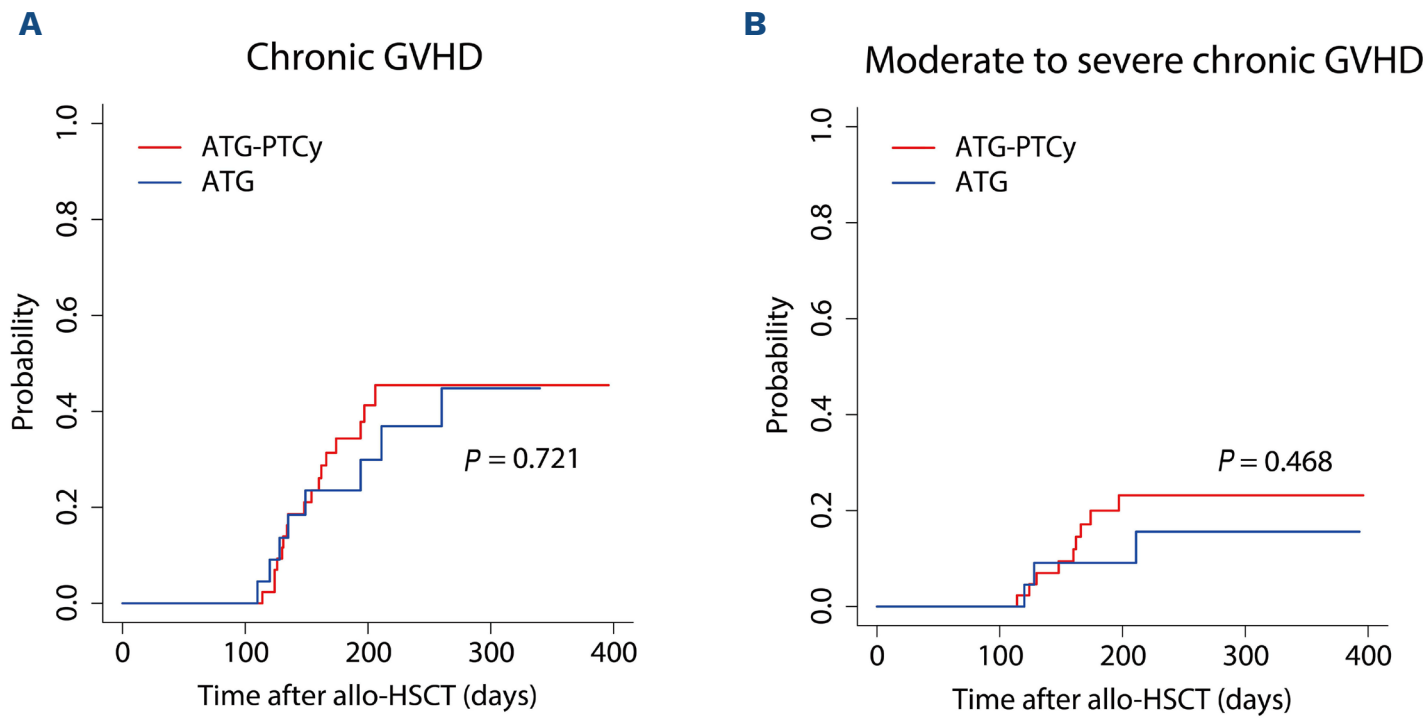

Figure 3. The cumulative incidences of chronic acute graft-*versus*-host disease between ATG-PTCy and ATG groups. (A) The cumulative incidences of chronic graft-*versus*-host disease (GVHD) and (B) moderate to severe chronic GVHD. allo-HSCT: allogeneic hematopoietic stem cell transplantation; ATG: anti-thymocyte globulin; PTCy: posttransplant cyclophosphamide.

due to relapse (1 in the ATG cohort) and thrombotic microangiopathy (1 in the ATG plus PTCy cohort). No significant difference in the cumulative incidence of relapse was observed between the ATG-PTCy (2.3%, 95% CI: 0-6.7%) and ATG cohorts (4.8%, 95% CI: 0-14.1%; *P*=0.582; Figure 4A). The cumulative incidence of transplantation-related mortality (TRM) was also similar between the ATG-PTCy (2.3%, 95% CI: 0-6.7%) and ATG (0%; *P*=0.480) cohorts (Figure 4B).

At 1 year after HSCT, the estimated overall survival rate was 97.7% (95% CI: 93.4-100) for the ATG-PTCy cohort and 83.3% (95% CI: 58.3-100) for the ATG cohort (*P*=0.666; Figure 5A). The estimated 1-year DFS rates were also similar between the ATG-PTCy and ATG cohorts (95.5% CI: 89.5-100 *vs*. 95.2%, 95% CI: 86.6-100; *P*=0.979; Figure 5B). The estimated GRFS was 70.5% (95% CI: 61.0-81.4) for the ATG-PTCy cohort and 79.5±9.4% (95% CI: 66.0-95.5) for the ATG cohort (*P*=0.422).

Discussion

The optimization of GVHD prevention strategies in haploidentical transplantation has been under continuous exploration. To the best of our knowledge, this is the first prospective randomized controlled study to compare the efficacy of ATG-PTCy *versus* 10 mg/kg ATG protocols for GVHD prophylaxis. The current interim analysis revealed no statistically significant differences in terms of aGVHD, cGVHD, CMV, EBV, TRM, relapse or survival between the two cohorts.

A series of previous studies failed to reach a consensus on whether PTCy plus low-dose ATG can decrease the incidence of GVHD and improve prognosis compared with PTCy alone. Retrospective comparative studies partially

Table 2. The CD3, CD4, and CD19 cell immune reconstitution between ATG-PTCy and ATG groups.

Time, months	Lymphocyte subset, ×10⁶/L	ATG + PTCy, mean±SE	ATG, mean±SE	*P*
+1	CD3	110.66±35.93	185.13±34.66	0.006
	CD4	25.83±7.64	46.52±9.26	0.021
	CD19	3.62±0.97	9.46±2.14	0.004
+2	CD3	469.20±151.32	422.63±78.63	0.185
	CD4	90.07±26.68	83.66±12.89	0.185
	CD19	37.87±14.99	64.47±27.84	0.265
+3	CD3	558.88±108.86	487.02±74.59	0.557
	CD4	109.35±22.03	105.50±14.28	0.222
	CD19	47.84±17.30	76.30±24.92	0.070
+4.5	CD3	447.85±86.22	730.92±191.92	0.099
	CD4	82.95±17.20	110.47±24.14	0.150
	CD19	35.79±9.45	181.77±95.79	0.011
+6	CD3	974.43±146.21	1,150.36±310.70	0.504
	CD4	109.89±15.97	149.84±25.69	0.048
	CD19	50.69±11.61	170.32±73.24	0.078
+9	CD3	823.15±127.51	960.19±236.48	0.550
	CD4	130.73±19.62	146.06±28.28	0.630
	CD19	85.41±21.57	302.33±136.36	0.104
+12	CD3	522.58±74.18	589.99±44.21	0.492
	CD4	99.54±18.23	126.08±24.29	0.310
	CD19	60.20±19.64	121.79±30.99	0.062

SE: standard error; ATG: anti-thymocyte globulin; PTCy: posttransplant cyclophosphamide.

support the notion that PTCy combined with low-dose ATG can reduce acute or cGVHD compared with PTCy alone,[6,28-32] but other studies do not support this finding.[4] The latest published report from the European Society for Blood and Marrow Transplantation (EBMT) Acute Leukemia Working Party enrolled 3,649 adult AML patients who underwent haplo-HSCT and who received either PTCy (N=2999), ATG (N=358), or combination prophylaxis (N=292). The combination of PTCy and ATG, however, led to significantly decreased rates of grade 2-4 (19.6% *vs*. 27.5%) and grade 3-4 (6.8% *vs*. 9.6%) aGVHD but did not affect survival or relapse outcomes compared with PTCy alone.[7] Another recent report from the Chinese Bone Marrow Transplantation Registry Group (CBMTRG) compared the clinical outcomes of G-CSF/ATG (N=572), PTCy (N=123) and PTCy combined with low-dose ATG (N=123). Compared with PTCy alone, PTCy plus ATG was not associated with fewer cases of 2-4 (28.7% *vs*. 27.9%) or 3-4 aGVHD (13.1% *vs*. 14.8%). Compared with the PTCy or PTCy plus ATG group, the G-CSF/ATG group led to faster engraftment, lower TRM and greater survival.[4] The inconsistency in conclusions from these studies may be related to the differences in the enrolled populations; the various combinations with other drugs, such as calcineurin inhibitors, methotrexate or mycophenolate mofetil; and the retrospective nature of these studies.

Similarly, there are no consistent and definite outcomes concerning whether the addition of PTCy can reduce the incidence of GVHD in the context of an ATG-based protocol. Several studies have indicated that the combination of reduced-dose ATG (5-7.5 mg/kg) and intermediate-dose PTCy (50-80 mg/kg) can reduce the incidence of aGVHD, with some potentially translating into a survival advantage.[33-36] Previously, our team published a nationwide mul-

Table 3. Humoral immune reconstitution between ATG-PTCy and ATG groups.

Time, months	Ig type g/L	ATG + PTCy, mean±SE	ATG, mean±SE	*P*
+1	IgA	0.78±0.08	0.88±0.12	0.540
	IgG	10.13±0.52	9.65±0.49	0.609
	IgM	0.43±0.04	0.42±0.05	0.714
+2	IgA	0.55±0.08	0.73±0.11	0.191
	IgG	8.51±1.09	7.49±0.74	0.860
	IgM	0.74±0.39	0.45±0.08	0.385
+3	IgA	0.57±0.11	1.16±0.38	0.021
	IgG	7.04±0.81	7.15±0.98	0.776
	IgM	1.13±0.66	1.00±0.13	0.001
+4.5	IgA	0.46±0.09	1.09±0.16	<0.001
	IgG	6.47±0.66	6.88±0.85	0.594
	IgM	0.47±0.06	1.26±0.29	0.002
+6	IgA	0.63±0.08	0.89±0.15	0.111
	IgG	9.28±0.79	10.80±1.21	0.281
	IgM	0.65±0.10	1.20±0.22	0.009
+9	IgA	0.77±0.13	1.11±0.21	0.115
	IgG	9.26±0.84	12.73±2.07	0.052
	IgM	0.75±0.14	1.95±1.00	0.224
+12	IgA	0.48±0.14	1.45±0.47	0.028
	IgG	10.07±1.20	12.72±0.83	0.083
	IgM	0.87±0.32	0.77±0.11	0.645

SE: standard error; ATG: anti-thymocyte globulin; PTCy: posttransplant cyclophosphamide; Ig: immunoglobulin.

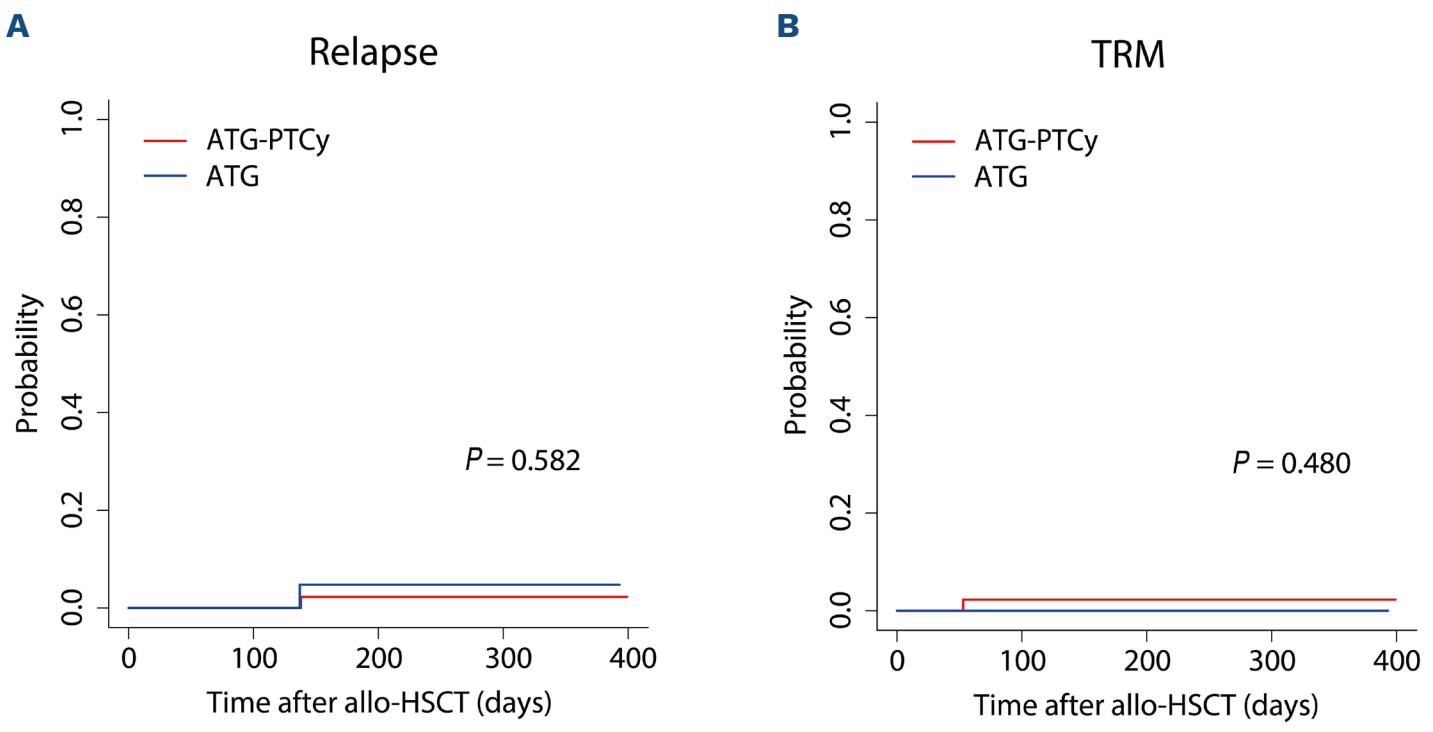

Figure 4. The comparisons of relapse and transplantation-related mortality between ATG-PTCy and ATG groups. (A) The cumulative incidence of relapse and (B) transplantation-related mortality (TRM). allo-HSCT: allogeneic hematopoietic stem cell transplantation; ATG: anti-thymocyte globulin; PTCy: posttransplant cyclophosphamide.

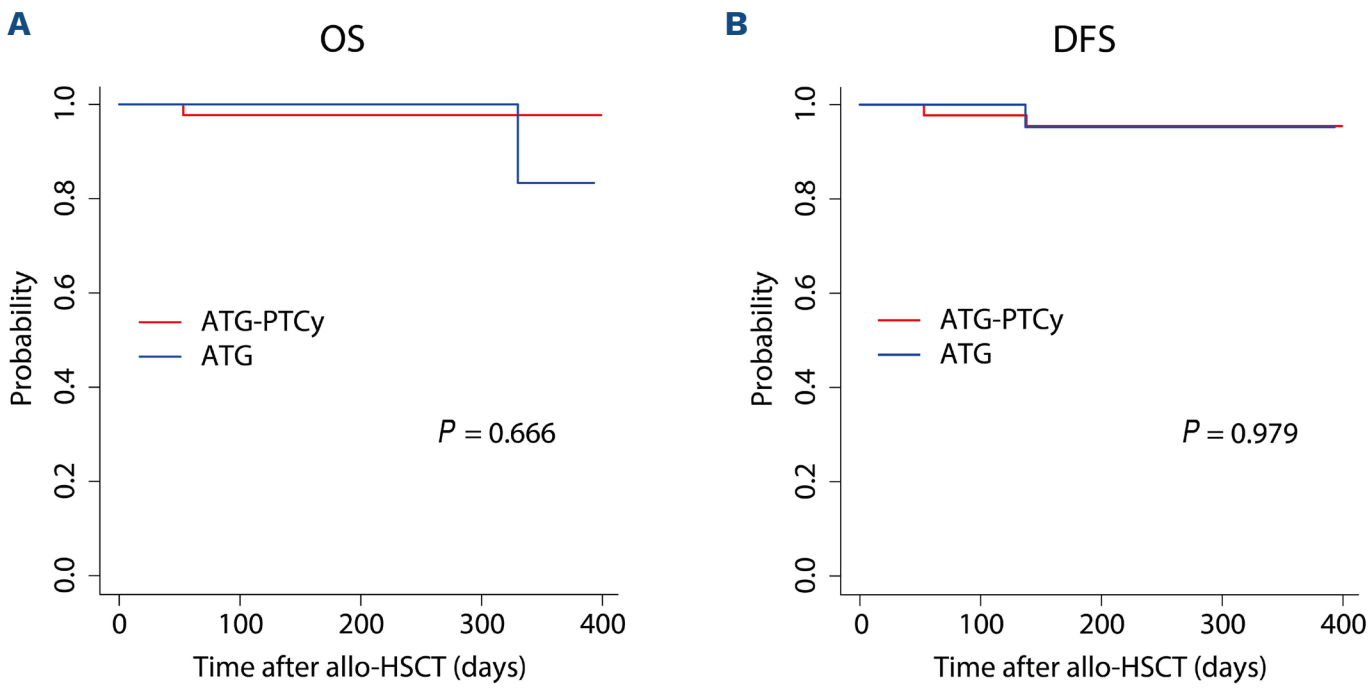

Figure 5. The survival outcomes between ATG-PTCy and ATG groups. The estimated rates of (A) overall survival (OS) and (B) disease-free survival (DFS). allo-HSCT: allogeneic hematopoietic stem cell transplantation; ATG: anti-thymocyte globulin; PTCy: posttransplant cyclophosphamide.

ticenter prospective clinical study that revealed that the combination of 10 mg/kg rabbit ATG (r-ATG) plus low-dose PTCy (14.5 mg/kg, +3/+4) could significantly reduce GVHD among patients transplanted from maternal donors or collateral relatives who are considered to be at high risk of developing aGVHD.[13] The cumulative incidence of 100-day grade 3-4 aGVHD (5% *vs*. 18%) and TRM (6% *vs*. 15%) in the prospective ATG-PTCy cohort was significantly lower than that in the 10 mg/kg ATG group from the external control cohort. With respect to the mechanism of low-dose PTCy in combination with ATG, *in vivo* experiments have suggested that low-dose PTCy is sufficient to decrease aGVHD in a mouse model, partly because of the enhancement of rapid Treg reconstitution.[12]

Notably, none of the aforementioned studies were prospective randomized controlled trials. Whether the combined strategy is more optimized for GVHD prophylaxis requires higher-level evidence-based medical evidence. In the current prospective randomized controlled study, ATG plus low-dose PTCy failed to decrease aGVHD or cGVHD among patients transplanted from haploidentical donors other than maternal donors or collateral relatives; thus, the trial stopped recruiting at the interim analysis. The reason why ATG-PTCy could reduce GVHD in maternal donors or collateral relatives but not in other haploidentical donors remains unclear. It is speculated that the combination strategy of ATG plus low-dose PTCy might be beneficial for patients at increased risk of developing GVHD. Most likely, transplants from maternal donors or collateral relatives might inherently be different from those from other donors in the reconstruction of T-cell subsets. In addition to defining populations that could benefit, perhaps modulation of the regimens or doses could also have benefit in some settings, which needed further research. Nowadays, the combinations of different doses of ATG and PTCy are being tested, including 6 mg/kg ATG plus 40 mg/kg PTCy as reported.[37] Perhaps these different dose combinations are suitable for different populations and transplant scenarios. Whether the combined strategy with double T-cell depletion might delay immune reconstitution and increase the risk of viral infection should be considered. The current study revealed that the combined strategy did not increase the risk of CMV or EBV infection or affect survival outcomes. It is worth noting that 4.5% of patients in the ATG-PTCy cohort had EBV-associated PTLD. The latest published research indicates that letermovir prophylaxis for CMV may increase the risk of EBV-associated PTLD. The incidences of PTLD in the letermovir and the non-letermovir group were 9.9% and 2.6% (P=0.001).[38] In the current study, patients routinely received letermovir, which appears to result in a somewhat higher incidence compared to previous observations.

The interim analysis outcomes of the control group are extremely good. It is therefore not possible to expect to design a study with outcomes that would be better than this control group. This limits the interpretation of the study. However, the examination of outcomes at later time points might reveal differences between ATG and ATG with low-dose PT-Cy in addition to the need for randomized studies and studies of dose variation.

In summary, the interim analysis of this prospective multicenter randomized controlled study indicated that the ATG plus low-dose PTCy and ATG cohorts had similar GVHD incidences and survival outcomes, with no additional adverse events. Future work needs to focus on identifying which populations can benefit from the combined strategy, aiming to improve the efficacy of haploidentical donor transplantation among the specific population.

Disclosures
No conflicts of interest to disclose.

Contributions
YW X-JH designed the research. Z-LX and YW analyzed the data and wrote the manuscript. All authors provided patient data and gave final approval for 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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