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A comparative study of GVHD prophylaxis using low dose ATG versus PTCy for PBSCT based on two independent prospective cohorts

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Both anti-thymocyte globulin (ATG) and post-transplant cyclophosphamide (PTCy) have shown prophylactic effects on graft-versus-host disease (GVHD) in multiple phase III studies. We conducted a comparative study of for low dose ATG (thymoglobulin) versus PTCy for GVHD prophylaxis in peripheral blood stem cell transplantation (PBSCT). The ATG (n = 67) and PTCy (n = 40) groups included patients enrolled in multicenter phase II studies (JSCT-ATG15 and JSCT-PTCY19, respectively). The probability of GVHD-free and relapse-free survival at 2 years as the primary endpoint was not significantly different between these two groups (57.9% for ATG vs. 67.8% for PTCy, P = 0.49). Both neutrophil and platelet engraftments were both significantly shorter in the ATG group than in the PTCy group (neutrophils: median 13 days vs. 15 days, P = 0.007; platelets: median 20 days vs. 27 days, P = 0.007). The cumulative incidences of acute and chronic GVHD, relapse, non-relapse mortality, and off-immunosuppressant use were similar between these two groups. The probabilities of overall and progression-free survival were 83.4% and 70.0% in the ATG group and 76.5% and 75.2% in the PTCy group, respectively, with no significant differences. These data indicate that low dose ATG and PTCy are equivalent for GVHD prophylaxis for PBSCT.

Allogeneic hematopoietic stem cell transplantation (HSCT) is an established curative treatment for patients with severe hematological malignancies. Patients who received HSCT are prepared with a high-dose of chemotherapy and/or total body irradiation, followed by intravenous infusion of hematopoietic stem cells[1]. After infusion, hematopoietic stem cells will leave circulation and engraft in the bone marrow[2]. Graft-versus-host disease (GVHD) is one of the most severe complications, and its prophylaxis is essential for successful HSCT. Although calcineurin inhibitor and methotrexate has been used as conventional GVHD prophylaxis, more intensive GVHD prophylaxis is needed for HSCT associated with increased risk of GVHD, including peripheral blood stem cell transplantation (PBSCT)[3]. Anti-thymocyte globulin (ATG) has shown the GVHD prophylactic effects in a series of phase III randomized control trials[4–9], and post-transplant cyclophosphamide (PTCy) has also provided remarkable GVHD prophylactic effects on HLA haploidentical HSCT[10,11] and has been increasingly used for other types of HSCT. Our study group previously reported two prospective, multicenter, phase II studies, JSCT-ATG15 and JSCT-PTCY19, demonstrating that both low dose ATG and PTCy effectively prevented acute and chronic GVHD in PBSCT[12,13]. However, the position of the low dose ATG and the PTCy as GVHD prophylaxis in PBSCT other than haploidentical HSCT has not been elucidated.

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Materials and methods

Patients and study design

We conducted a comparative study to compare the clinical outcomes of patients with hematological malignancies (acute myeloid leukemia, acute lymphoblastic leukemia, myelodysplastic syndrome, and malignant lymphoma) who underwent PBSCT with low dose rabbit ATG (thymoglobulin) or PTCy as GVHD prophylaxis. Patients with "other leukemia" or "adult T-cell leukemia/lymphoma" were excluded. The ATG group included patients who were enrolled in the multicenter single-arm phase II study (JSCT-ATG15, jRCTs011180012), wherein a total of 2 mg/kg of thymoglobulin was administered on day −2 and −1 (1 mg/kg/day) in combination with calcineurin inhibitor (cyclosporine or tacrolimus) and short-term methotrexate for HLA-matched PBSCT using myeloablative conditioning (MAC)[12]. The PTCy group included patients enrolled in the multicenter single-arm phase II study (JSCT-PTCY19, jRCTs011190009), wherein 50 mg/kg of cyclophosphamide was administered on days 3 and 4 in combination with tacrolimus and mycophenolate mofetil for HLA-matched or HLA 1–2 allele mismatched PBSCT using MAC or reduced intensity conditioning (RIC)[13]. The brief summaries of both protocols were shown in the supplemental Table 1. The time intervals of patient's inclusion were from January 2016 to October 2018 in the JSCT-ATG15 study and from March 2020 to July 2021 in the JSCT-PTCY19 study, respectively.

The primary endpoint was the probability of GVHD-free and relapse-free survival (GRFS) at 2 years. The study was performed in accordance with the institutional ethical guidelines, including the World Medical Association Declaration of Helsinki, and was approved by the institutional review board of Hokkaido University (No. 024–0191). Informed consent was obtained from each patient for participation in the study. For patients who could not obtain informed consent, we disclosed the information about this clinical study on the website of our institution and guarantee the opportunity for refusal (opt-out). Since this study is a retrospective study, opt-out disclosure eliminates the need for patient consent.

Definitions

Neutrophil engraftment was defined as an absolute neutrophil count $> 0.5 \times 10^9$/L on the first three consecutive days, and platelet engraftment was defined as an absolute platelet count $> 2.0 \times 10^{10}$/L without transfusion support on the first seven preceding days. Acute GVHD was graded according to the consensus criteria[14], chronic GVHD was graded according to the criteria of the National Institutes of Health Consensus Development Project[15]. The refined disease risk index (DRI) was determined according to the scoring system as previously described[16]. Non-relapse mortality (NRM) was defined as death caused by any cause other than relapse. Relapse and causes of death were determined at the discretion of the clinician. Overall survival (OS) was calculated from the day of PBSCT, with patients alive at the time of the last follow-up censored. Progression-free survival (PFS) was calculated from the date of PBSCT until the date of disease recurrence, death from any cause, or the last follow-up for patients without these events being censored. GRFS was defined as the absence of grade III - IV acute or chronic GVHD requiring systemic therapy, relapse, or death[17].

Statistical analysis

Statistical analysis was performed using Fisher's exact test for categorical variables; Mann–Whitney U-test for continuous variables; Kaplan-Meier method and log-rank test for OS, PFS, and GRFS; and Gray's test for engraftment, acute and chronic GVHD, relapse, NRM, and off-immunosuppressants. We included age, sex, disease, donor, HLA compatibility, conditioning, performance status, DRI, disease status at transplant, and number of CD34 + cells were included as variables in univariate analysis for relapse, NRM, OS, PFS, and GRFS. $P < 0.05$ was used to determine statistical significance. All analyses were performed with EZR version 1.61 (Jichi Medical University, Saitama Medical Center; http://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmedEN.html)[18], a graphical user interface for R (The R Foundation for Statistical Computing; http://www.r-project.org) that extends the functionality of R Commander by adding statistical functions, and analyses were conducted using R version 4.3.1 and R Commander version 2.9-1.

Results

Patients and transplant characteristics

A total of 67 patients who were enrolled in the JSCT-ATG15 study were assigned to the ATG group, and 41 patients who were enrolled in the JSCT-PTCY19 study were assigned to the PTCy group (Supplemental Fig. 1). The median patient age was 46 years, ranging from 19 to 65 years. The PTCy group had higher numbers of DRI-high patients, unrelated donors, and higher number of CD34$^+$ cells than the ATG group. Patients who underwent RIC and HLA-mismatched transplantation were observed only in the PTCy group because of differences in the study protocol. Other factors did not differ between the groups (Table 1).

Engraftments

Neutrophil and platelet engraftments were both significantly higher and shorter, respectively, in the ATG group than in the PTCy group. Neutrophil engraftment was achieved in 100% and 97.6% of the ATG and PTCy groups, respectively ($P = 0.007$; Fig. 1a). The median time of platelet engraftment was 13 days, ranging from 12 to 27 days in the ATG group, and 15 days, ranging from 13 to 43 days in the PTCy group ($P < 0.001$; Fig. 1b). Platelet engraftment was achieved in 98.5% and 92.7% of the patients in the ATG and PTCy groups, respectively ($P = 0.007$, Fig. 1c). The median time of neutrophil engraftment was 20 days, ranging from 11 to 58 days, in the ATG group and 27 days, ranging from 11 to 117 days, in the PTCy group ($P = 0.003$, Fig. 1d).
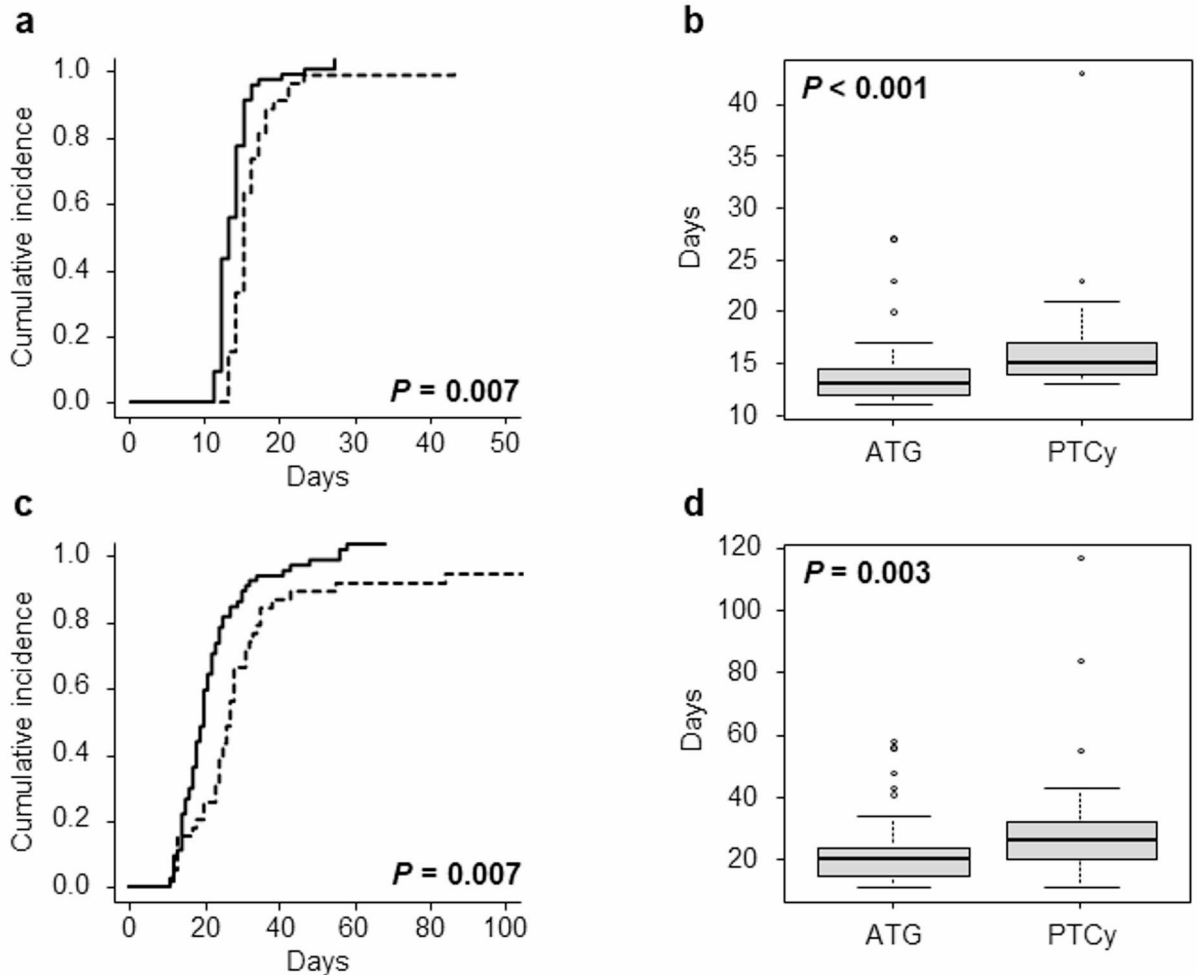
Fig. 1. Incidence of engraftment in the ATG and PTCy groups. The cumulative incidence of neutrophil engraftment in the ATG (*solid lines*) and PTCy (*dashed lines*) groups (**a**), and the median neutrophil engraftment days from the day of HSCT in the ATG and PTCy groups (**b**). The cumulative incidence of platelet engraftment in the ATG (*solid lines*) and PTCy (*dashed lines*) groups (**c**), and the median platelet engraftment days from the day of HSCT in the ATG and PTCy groups (**d**). The ends of the center box indicate the upper and lower quartile of the data, the line inside the rectangle indicates the median, the whiskers indicate the maximum and minimum values, and the dots outside the rectangle indicate outliers.

GVHD

The cumulative incidences of grade II to IV acute GVHD on day 200 were 19.4% and 14.6% ($P = 0.54$, Fig. 2a), and those of grade III to IV acute GVHD on day 200 were 0.0% and 2.4% in the ATG and PTCy groups, respectively ($P = 0.201$, Fig. 2b). The cumulative incidence of all-grade chronic GVHD at 2 years was 21.0% and 14.6% ($P = 0.453$, Fig. 2c), and that of moderate to severe chronic GVHD at 2 years was 10.6% and 4.9% in the ATG and PTCy groups, respectively ($P = 0.356$, Fig. 2d), with no significant difference.

Relapse, NRM, off-immunosuppressants, and causes of death

The cumulative incidences of relapse and NRM at 2 years were 25.4% and 4.5% in the ATG group, and 17.7% and 7.3% in the PTCy group, respectively, with no significant differences (Fig. 3a, b). The cumulative incidence of immunosuppressant discontinuation without relapse at 2 years was 61.9% in the ATG group and 74.0% in the PTCy group ($P = 0.15$; Fig. 3c). The causes of death were similar between groups (Supplemental Table 2).

Survival outcomes

With a median follow-up of 721 days, the probability of OS at 2 year was 83.4% (95% CI, 72.0–90.4%) in the ATG group and 76.5% (95% CI, 59.1–87.2%) in the PTCy group ($P = 0.48$, Fig. 3d), PFS at 2 year was 70.0% (95% CI, 57.4–79.5%) in the ATG group and 75.2% (95% CI, 58.7–85.8%) in the PTCy group ($P = 0.66$, Fig. 3e), and GRFS at 2 year was 57.9% (95% CI, 45.2–68.7%) in the ATG group and 67.8% (95% CI, 51.0–79.9%) in PTCY group ($P = 0.48$, Fig. 3f), all with no significant difference.

|	ATG group ($n = 67$)	PTCY group ($n = 41$)	P-value
Sex			0.42
Male	36	26	
Female	31	15	
Age (median years (range))	46 (19–60)	45 (20–65)	0.82
Disease			0.22
AML	37	14	
ALL	20	11	
MDS	8	8	
ML	2	8	
Disease risk index			0.045
Low	8	3	
Intermediate	54	28	
High	5	10	
Disease status at transplantation			0.59
CR	58	34	
Not in CR	9	7	
Performance status			0.36
0	57	36	
≥ 1	10	5	
Conditioning			< 0.001
MAC	67	27	
RIC	0	14	
Donor relationship			< 0.001
Related	46	13	
Unrelated	21	28	
HLA compatibility			< 0.001
Match	67	20	
Mismatch	0	21	
CD34$^+$ cells (×10^6/kg) (median (range))	3.40 (1.90–7.17)	4.20 (1.30–14.0)	0.023

Table 1. Patient and transplant characteristics. Abbreviations: AML; acute myeloid leukemia, ALL; acute lymphoblastic leukemia, MDS; myelodysplastic syndrome, ML; malignant lymphoma, CR; complete remission, MAC; myeloablative conditioning, RIC; reduced-intensity conditioning, HLA; human leukocyte antigen.

Univariate analysis for relapse, NRM, OS, PFS and GRFS in pre-transplant parameters identified age (< 45 years) ($P = 0.040$) as a risk for relapse, disease status (not in CR) ($P = 0.012$) and CD34$^+$ cells (< 3.65×10^6/kg) ($P = 0.010$) as risks for NRM, DRI (high) as a risk for OS ($P = 0.020$), and performance status (≥ 1) as a risk for PFS ($P = 0.038$), respectively (Supplemental Table 3).

We also evaluated the association of donor (related or unrelated), HLA compatibility (matched or mismatched), and conditioning intensity (MAC or RIC) with survival outcomes between ATG and PTCy and found no significant difference in OS between all subgroups. The probabilities of OS at 2 years were 86.8%, 76.2%, 76.9%, and 76.0% ($P = 0.67$) in patients who received HSCT from related and unrelated donors in the ATG group and related and unrelated donors in the PTCy group (Fig. 4a); 83.4%, 78.5%, and 75.6% ($P = 0.77$) in the ATG group (all patients received from HLA-matched donor) and patients who received HSCT from HLA-matched and mismatched donors in the PTCy group (Fig. 4b); and 83.4%, 72.2%, and 85.7% ($P = 0.33$) in the ATG group (all patients received MAC) and patients who received MAC and RIC in the PTCy group (Fig. 4c), respectively. The probabilities of PFS (Supplemental Fig. 2a–c) and GRFS (Supplemental Fig. 2d–f) were also similar.

Discussion

The latest EBMT recommendation for GVHD prophylaxis states that ATG or PTCy should be preferred to prophylaxis with neither ATG nor PTCy for HSCT from HLA-matched unrelated donor, whereas PTCy should not be generally preferred to ATG HSCT from HLA-matched related donor because of the lack of high-quality evidence of direct comparative evidence[19]. Comparative studies of ATG and PTCy is increasing based on retrospective analysis except one prospective phase II study reported from Europe to evaluate the efficacy of 50 mg/kg for 2 days of PTCy or 2.5 mg/kg for 2 days of ATG for HLA-matched PBSCT after RIC. This study showed the comparable outcome of the primary endpoint, probability of GRFS at 2 years between the groups, except that the median time to neutrophil and platelet engraftment were significantly longer in the PTCy group than in the ATG group, which is consistent with the prospective phase II study[20]. Other transplant outcomes including NRM, relapse, PFS, and OS were also comparable between the groups, whereas detailed evaluation
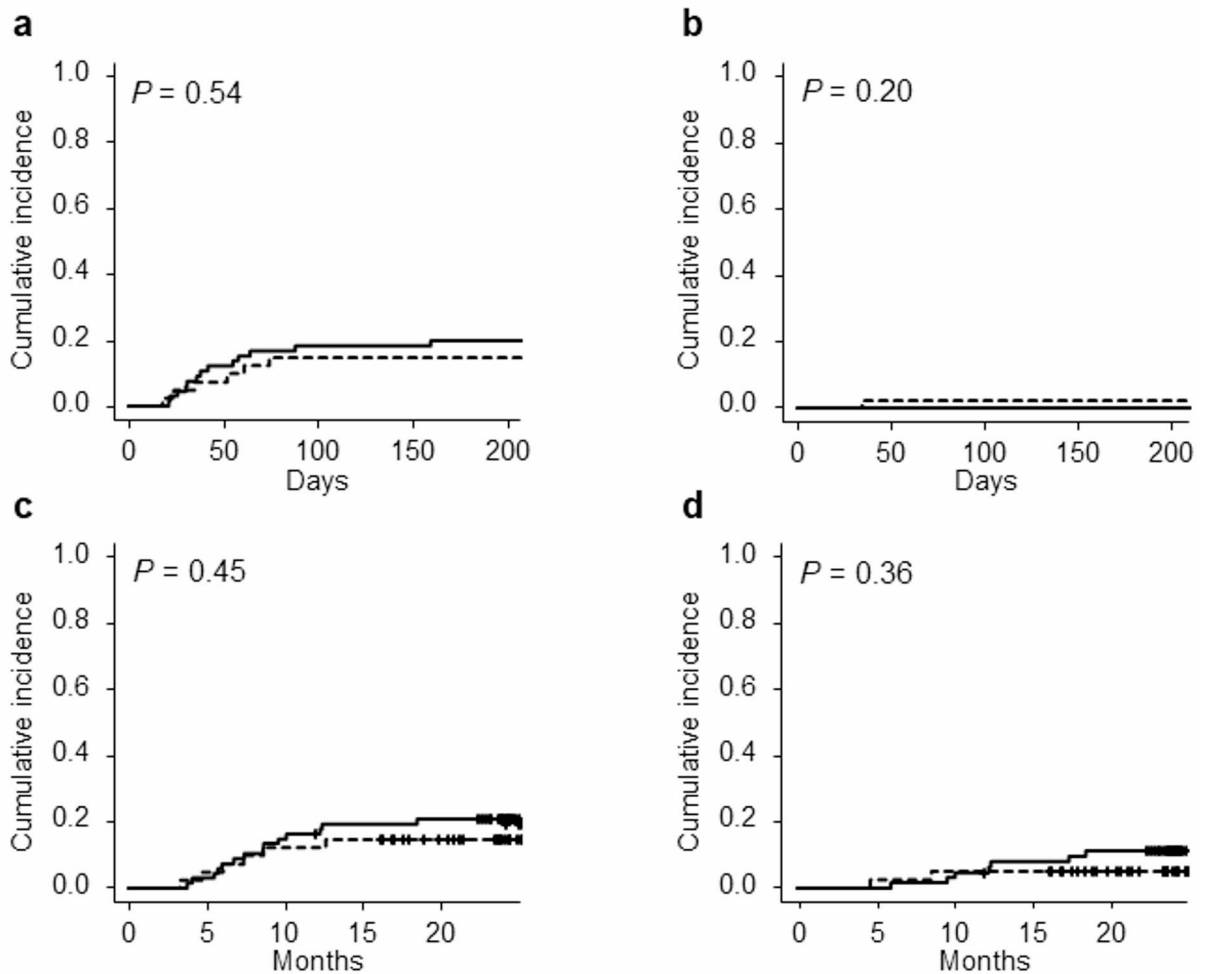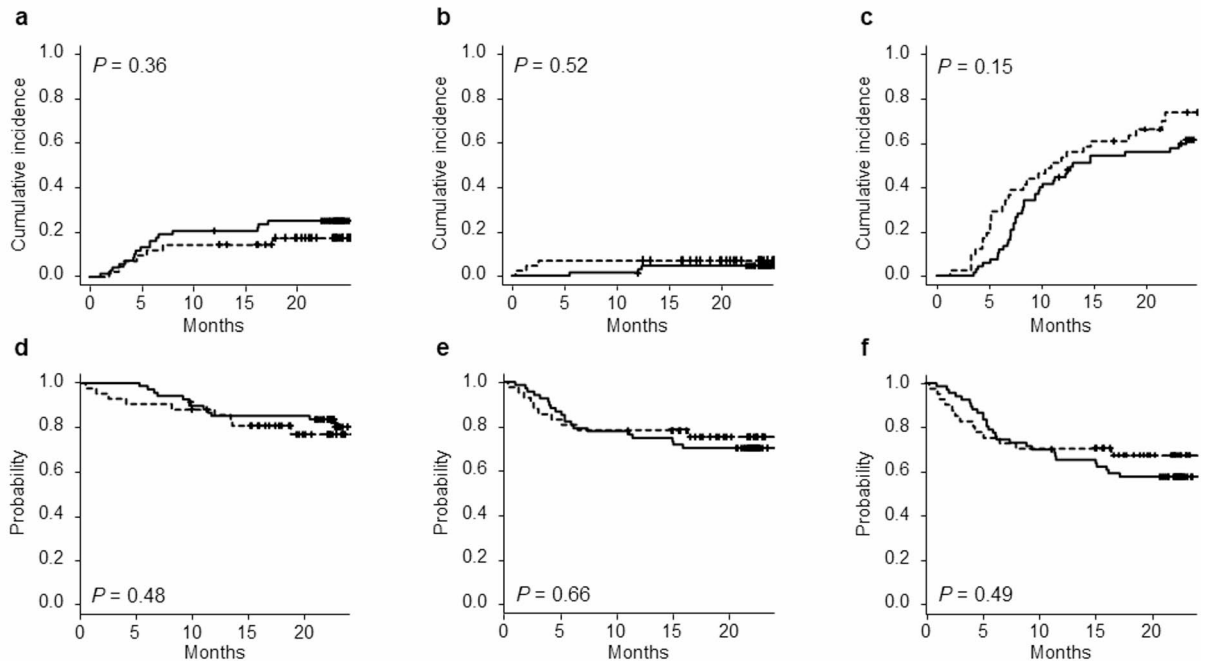
Fig. 2. Incidence of GVHD in the ATG and PTCy groups. The cumulative incidences of grade II-IV (**a**) and grade III-IV (**b**) acute GVHD, and overall cGVHD (**c**) and moderate to severe chronic GVHD (**d**) in the ATG (*solid lines*) and non-ATG (*dashed lines*) groups.

of these outcomes is difficult because of the diversity of primary diseases and disease states at transplant. Other retrospective studies almost showed the comparable transplant outcomes between ATG and PTCy for HLA-matched HSCT[21–24], whereas a recent study indicates the favorable transplant outcomes in the PTCy group compared to that in the ATG group[25].

In our study, PTCy had comparable efficacy for GVHD prevention to low dose ATG, although the patient backgrounds of JSCT-PTCY19, wherein an HLA-mismatched donor or RIC as a conditioning was included, were heterogeneous compared to those of JSCT-ATG15. Notably, patients who received HSCT from an HLA-mismatched donor or RIC as conditioning in the PTCy group had similar outcomes to the others. Almost all phase III trials that demonstrated the efficacy of ATG used MAC[4–9], whereas phase III trials that demonstrated the efficacy of PYCy used RIC[10,11], suggesting that conditioning intensity may affect the transplant outcomes between ATG and PTCy. A previous matched-pair analysis between ATG and PTCy in HLA-mismatched HSCT showed that the incidence of grade III-IV acute GVHD was significantly lower in patients with PTCy than in those with ATG, and leukemia-free survival, OS, GRFS, and NRM were favorable outcomes in the PTCy group[26]. Other studies on HSCT from HLA-mismatched unrelated donors also reported favorable outcomes for PTCy compared to those for ATG[27–31].

Another notable complication with PTCy is cardiac toxicity[32,33]. In the PTCy group, one patient died of cardiomyopathy. Although adverse effects were not evaluated in this study, both phase II studies investigated grade 3–4 non-hematologic toxicity within 100 days after HSCT. Major grade 3–4 adverse effects within 30 days after HSCT were as follows: lung: 3%, nervous system: 1%, liver: 6%, SOS: 3%, renal and urinary: 5%, and infection: 13% in JSCT-ATG15; cardiac: 4%, lung: 2%, liver: 2%, SOS: 2%, renal and urinary: 5%, and infection: 19% in JSCT-PTCY19, and from 30 to 100 days after HSCT were as follows: lung: 1%, liver: 5%, renal and urinary: 4%, and infection: 13% in JSCT-ATG15; cardiac: 3%, lung: 6%, liver: 3%, and infection: 12% in JSCT-PTCY19, with no significant difference between the studies, although cardiac complication was prominent in

Fig. 3. Incidences of relapse, NRM, and off-immunosuppressants, and probabilities of OS, PFS and GRFS in the ATG and PTCy groups. The cumulative incidences of relapse (**a**), NRM (**b**), and off-immunosuppressants (**c**), and the probabilities of OS (**d**), PFS (**e**), and GRFS (**f**) in the ATG (*solid lines*) and non-ATG (*dashed lines*) groups.
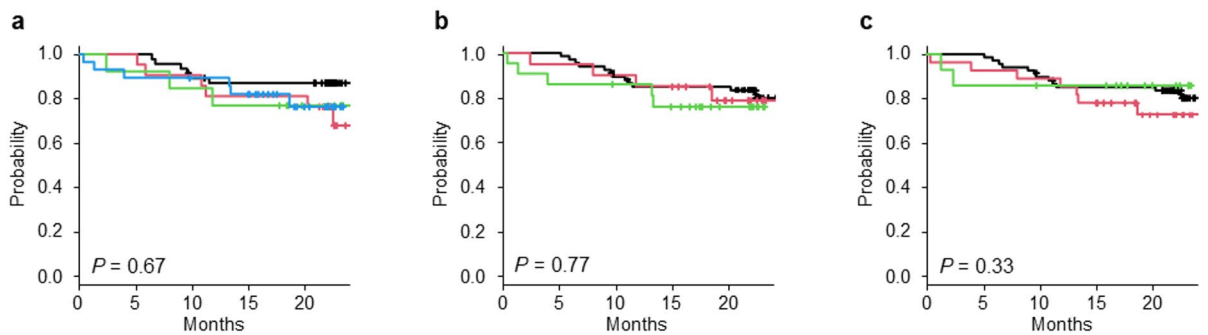
Fig. 4. Probability of OS in the ATG and PTCy groups according to donor, HLA compatibility, and conditioning intensity. The probability of OS in the ATG and non-ATG groups according to donor (**a**), HLA compatibility (**b**), and conditioning intensity (**c**). (**a**); related donor (black line) and unrelated donor (red line) in the ATG group, and related donor (green line) and unrelated donor (blue line) in the PTCy group, (**b**); HLA-matched donor (black line) in the ATG group, and HLA-matched donor (red line) and HLA-mismatched donor (green line) in the PTCy group, (**c**); MAC (black line) in the ATG group, and MAC (red line) and RIC (green line) in the PTCy group.

JSCT-PTCY19. A recent study evaluating early cardiac toxicity also showed that the incidence of cardiac toxicity until day 180 after HSCT was significantly higher in patients with PTCy than in those without PTCy, and total body irradiation and pre-transplant cardiac morbidity were identified as risk factors for early cardiac toxicity[33]. Detailed evaluation of pre-transplant cardiac function and risk assessment may be essential for using PTCy.

Our study is the first to compare the clinical outcomes of fixed low dose ATG and PTCy for PBSCT. Recent studies tend to adopt lower dose of ATG because higher dose of ATG is a risk for relapse or infection related mortality[34–38]. EBMT recommendation also states that the recommended dose of ATG ranges from 2.5 to 5 mg/kg in HSCT from HLA-matched related donor to 4.5–6 mg/kg in HSCT from HLA-matched unrelated donor with the caution that higher doses are associated with a higher risk of infectious complications[39]. Our previous

pilot study showed that only a total of 2 mg/kg of ATG can reduce naïve T cells at day 28 after PBSCT[40], leading to the evidence of JSCT-ATG15 study[12].

Our study has some limitations, particularly for the evaluation of OS or PFS, that should be considered when reviewing the results, including its retrospective design, the comparison of two prospective studies with a limited number of patients, and diversity of primary disease and disease status at transplant. Nevertheless, our data indicated the efficacy and safety of GVHD prophylaxis using both ATG and PTCy. Larger prospective studies are needed to establish the roles of ATG and PTCy in GVHD prophylaxis.

Data availability
The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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Declarations

Competing interests
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Additional information
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