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ORIGINAL ARTICLE

Impact of low-dose rabbit anti-thymocyte globulin in unrelated hematopoietic stem cell transplantation

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Abstract We retrospectively evaluated the outcome of administering low-dose rabbit anti-thymocyte globulin (thymoglobulin: ATG-T) to 219 patients (ATG-T group, $n = 30$; no-ATG-T group, $n = 189$) who received an initial unrelated hematopoietic stem cell transplantation (uHSCT). The median total dose of ATG-T was 1.5 mg/kg. There was no significant difference in the cumulative incidences of grade II–IV (42 vs. 38 %, $P = 0.87$) and grade III–IV (5 vs. 7 %, $P = 0.52$) acute GVHD. In patients who received uHSCT from a donor with at least one HLA allele mismatch, the cumulative incidence of extensive chronic GVHD was significantly lower in the ATG-T group than that in the no-ATG-T group (13 vs. 44 %, $P = 0.02$). No patient in the ATG-T group developed chronic lung dysfunction. The probabilities of 1-year, GVHD-free/relapse-free survival (GRFS) were 61 % in the ATG-T group and 35 % in the no-ATG-T group ($P = 0.02$). Patients in the ATG-T group discontinued immunosuppressive drugs significantly earlier than those in the no-ATG-T group ($P < 0.01$). The use of low-dose ATG-T did not increase the incidence of severe infectious disease. The use of low-dose ATG-T in patients who received uHSCT was associated with a superior GRFS, reflecting the reduced incidence of severe/persistent GVHD without compromising overall survival.

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Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) has become an integral part of treatment for various hematological disorders [1–3]. Although acute GVHD is still a major obstacle to success in allogeneic HSCT, chronic GVHD is also an important morbidity that is a major cause of treatment-related mortality in long-term survivors [4–6].

Although there is no established GVHD prophylaxis that can reduce the incidence of acute/chronic GVHD without increasing the risk of relapse, one promising agent is anti-thymocyte globulin (ATG). Finke et al. conducted a large, randomized controlled trial in patients who received an unrelated HSCT and found that ATG-Fresenius (ATG-F) as GVHD prophylaxis reduced the incidences of acute and chronic GVHD without compromising survival [7, 8]. A randomized trial by Bacigalupo et al. also showed that ATG-Thymoglobulin (ATG-T) reduced the incidence of chronic GVHD, especially chronic lung dysfunction, in patients who received an unrelated HSCT [9]. These studies suggested that ATG as GVHD prophylaxis reduced the incidence of GVHD without compromising survival. However, there is still controversy regarding the optimal dose of ATG.

The risk of GVHD might differ among different ethnicities and races [10–12]. Asian populations were reported to have a lower risk of GVHD compared to Caucasian populations [13, 14]. As excessive doses of ATG will attenuate the graft-versus-leukemia effect and increase viral infections, it is desirable to choose the minimum dose of ATG that is sufficient to control GVHD. In our previous single-institute retrospective

study of patients who received an unrelated bone marrow transplantation (BMT) using a reduced-intensity conditioning (RIC) regimen, the incidences of acute and chronic GVHD were promisingly low in patients who received low-dose ATG-F (5 or 10 mg/kg in total) [15]. In another retrospective study of Japanese nationwide transplant outcomes after unrelated BMT, the incidences of acute and chronic GVHD were again promisingly low considering the dose of ATG-F (median dose 10 mg/kg) [16]. Such low-dose ATG-F might be sufficient in Asian populations who receive unrelated BMT. However, no published data are available regarding the doses of different formulations of ATG-T.

Here, we retrospectively analyzed clinical outcomes in patients who received an unrelated HSCT at our institute to assess the impact of low-dose ATG-T.

Patients and methods

Study design

This was a single-center retrospective study that assessed the impact of ATG-T on clinical outcomes in consecutive patients who received an unrelated HSCT from 2009 to 2013 (ATG-T group, $n = 30$; no-ATG-T group, $n = 189$). In Japan, until September 2010 only BMT was approved for unrelated HSCT. This study was approved by the Institutional Review Board of the National Cancer Center, Tokyo, Japan.

Clinical outcomes

Endpoints included neutrophil recovery, overall survival (OS), relapse, non-relapse mortality (NRM), acute GVHD, chronic GVHD, infection, discontinuation of immunosuppressive drugs and GVHD-free relapse-free survival (GRFS). Neutrophil recovery was defined as an absolute neutrophil count (ANC) of 0.5×10^9/L for 3 consecutive days. OS was defined as time from HSCT to death from any cause or the time of the last follow-up. The incidences of grade II–IV or grade III–IV acute GVHD were based on standard criteria [17]. Chronic GVHD was defined according to previously published criteria [18]. Chronic lung dysfunction was defined according to Bacigalupo's report [9]. Discontinuation of immunosuppressive drugs was defined accordingly to previously studies [15, 19–21]. GRFS events were defined as grade III–IV acute GVHD, chronic GVHD requiring systemic immunosuppressive treatment, disease relapse, or death from any cause after HSCT [22].

Statistical analysis

Fisher's exact test was used to compare differences in the distribution of clinical features. The probability of OS was calculated by the Kaplan–Meier method. Comparison of survival curves was performed using the log-rank test. The cumulative incidences of engraftment, NRM, relapse, GVHD, cytomegalovirus (CMV) infection, and discontinuation of immunosuppressive drugs were evaluated using Gray's method. The cumulative incidence of extensive chronic GVHD was evaluated using the model by Fine and Gray for univariate and multivariate analyses. In the competing risk models for engraftment, GVHD and discontinuation of immunosuppressive drugs, relapse, and death before these events were defined as competing risks. In the competing risk models for NRM, relapse was defined as a competing risk. In the competing risk models for CMV infection, relapse and death without this event was defined as competing risks. The variables that were evaluated in these analyses were follows: patient's sex (male vs. female), patient's age at transplant (Age $\geq$ 60 vs. Age < 60), disease risk (low vs. intermediate vs. high risk), stem cell source (bone marrow vs. peripheral blood stem cells), intensity of the conditioning regimen (MAC vs. RIC), using of ATG-T (yes vs. no), HLA mismatch (none vs. 1 allele vs. more than 1 allele) and disease status (CR vs. non CR). Disease risk was based on the Center for International Blood and Marrow Transplant (CIBMTR) classifications [23]. For all analyses, $P < 0.05$ was considered as statistically significant. The statistical analyses were carried out using the EZR software package (Saitama Medical Center, Jichi Medical University), which is a graphical user interface for R (The R Foundation for Statistical Computing, version 2.13.0) [24].

Results

Patient characteristics

Patient characteristics are summarized in Table 1. The median age was 48 years (range 17–69). There was no statistically significant difference in the disease risk between the two groups. In the ATG-T group, most patients received a RIC regimen ($n = 23$, 77 %). The ATG-T group included more HSCT from an HLA mismatched donor than the no-ATG-T group (83 vs. 48 %, $P < 0.01$). As GVHD prophylaxis, tacrolimus and a short course of methotrexate was mainly used in both groups. The median total dose of ATG-T was 1.5 mg/kg (range 1.0–4.0 mg/kg). ATG-T was administered at a median of day −2 (range day −4 to −1; day-4, $n = 6$; day-3, $n = 1$; day-2, $n = 22$; day-1, $n = 1$). We used 2 mg/kg/day of methylprednisolone in total on the day of ATG-T administration to prevent the infusion reaction. The median follow-up period among survivors was 567 days (range 16–1562 days) after HSCT.

Table 1 Patient characteristics

	ATG-T group	no-ATG-T group	*P* value
No. of patients	30	189	
Age, median (range), years	45 (23–68)	48 (17–69)	
Sex (male/female)	8/22	101/88	0.01
Diagnosis			
AML	9 (30)	73 (39)	0.05
ALL	5 (17)	34 (18)	
MDS	2 (7)	15 (8)	
ML	11 (36)	34 (18)	
ATL	1 (3)	29 (15)	
Others	2 (7)	4 (2)	
Disease risk			
Low	8 (27)	69 (36)	0.47
Intermediate	9 (30)	41 (22)	
High	13 (43)	79 (42)	
Disease status			
CR	16 (53)	91 (48)	0.70
non CR	14 (47)	98 (52)	
Conditioning regimen			
MAC	7 (23)	109 (58)	<0.01
CY + TBI	3 (10)	46 (24)	
CA + CY + TBI	1 (3)	4 (2)	
ETP + TBI	0 (0)	1 (1)	
ETP + CY + TBI	0 (0)	1 (1)	
BU + CY	3 (10)	44 (23)	
Flu + BU4	0 (0)	13 (7)	
RIC	23 (77)	80 (42)	
Flu + CY	1 (3)	0 (0)	
Flu + BU2	14 (47)	65 (34)	
Flu + MEL	8 (27)	15 (8)	
GVHD prophylaxis			
TAC + sMTX + ATG-T	30 (100)	0 (0)	
TAC + sMTX	0 (0)	189 (100)	
HLA mismatch			
None	5 (17)	99 (52)	<0.01
1 allele	13 (43)	49 (26)	
More than 1 allele	12 (40)	41 (22)	
Source of stem cells			
Peripheral blood	3 (10)	2 (1)	0.02
Bone marrow	27 (90)	187 (99)	
Performance status			
0–1	26 (87)	185 (98)	0.01
2–4	4 (13)	4 (2)	
HCT-CI			
0	15 (50)	115 (61)	0.47
1–2	9 (30)	48 (25)	
3-	6 (20)	26 (14)	
CMV status, donor/recipient			
Negative/negative	2 (7)	14 (7)	0.99
Negative/positive	13 (43)	73 (39)	
Positive/negative	2 (7)	14 (7)	
Positive/positive	13 (43)	85 (45)	
Unknown	0 (0)	3 (2)	

Values are *N* (%)

Others included aplastic anemia, plasma cell leukemia, myelofibrosis, and chronic active Epstein-Barr virus infection

AML acute myeloid leukemia, *ALL* acute lymphoblastic leukemia, *MDS* myelodysplastic syndrome, *ML* malignant lymphoma, *ATL* adult T cell leukemia/lymphoma, *CR* complete remission, *MAC* myeloablative conditioning, *RIC* reduced intensity conditioning, *CY* cyclophosphamide, *TBI* total body irradiation, *CA* cytarabine, *ETP* etoposide, *BU* busulfan, *Flu* fludarabine, *MEL* melphalan, *TAC* tacrolimus, *sMTX* short course of methotrexate, *HLA* human leukocyte antigen, *HCT-CI* Hematopoietic Cell Transplantation-specific Comorbidity Index, *CMV* cytomegalovirus

Engraftment

The cumulative incidences of neutrophil engraftment at day 28 were 100 and 93 % in the ATG-T and no-ATG-T groups ($P < 0.01$; Fig. 1a), respectively. The median times to neutrophil engraftment were 17 days (range 11–25 days) in the ATG-T group and 18 days (range 7–40 days) in the no-ATG-T group, respectively. In the ATG-T group, no graft failure was observed.

Acute and chronic GVHD

There was no statistically significant difference in the cumulative incidences of grade II–IV (42 vs. 38 %, $P = 0.87$; Fig. 1b) and grade III–IV (5 vs. 7 %, $P = 0.52$; Fig. 1c) acute GVHD. The cumulative incidence of extensive chronic GVHD in the ATG-T group tended to be lower than that in the no-ATG-T group (19 vs. 38 %, $P = 0.13$; Fig. 1d). In patients who received HSCT from a donor with at least one HLA allele mismatch, the cumulative incidence of extensive chronic GVHD was significantly lower in the ATG-T group than that in the no-ATG-T group (13 vs. 44 %, $P = 0.02$; Fig. 1e). In multivariate analysis of extensive chronic GVHD, The administration of ATG-T trended to be associated with a lower incidence of extensive chronic GVHD (Table 2). In all patients, there was a trend towards a lower incidence of chronic lung dysfunction in the ATG-T group than in the no-ATG-T group (0 vs. 24 %, $P = 0.07$; Fig. 1f).

Discontinuation of immunosuppressive drugs

At 2 years after HSCT, immunosuppressive drugs were discontinued in 60 and 25 % of the patients in the ATG-T and no-ATG groups, respectively. Patients in the ATG-T

group discontinued immunosuppressive drugs significantly earlier than those in the no-ATG-T group ($P < 0.01$; Fig. 1g).

Infection

The incidence of CMV antigenemia in the ATG-T group was significantly higher compared to that in the no-ATG-T group (83 vs. 67 %, $P < 0.01$; Fig. 2a). However, there was no statistically significant difference in the cumulative incidence of CMV disease between the ATG-T and no-ATG-T groups (0 vs. 7 %, $P = 0.14$; Fig. 2b). There was no statistically significant difference in the incidence of Epstein-Barr virus disease (0 vs. 0.5 %), adenovirus disease (3 vs. 4 %), or BK virus disease (0 vs. 3 %) between the ATG-T and no-ATG-T groups. No patients developed post-transplant lymphoproliferative disorder (PTLD) in the ATG-T group.

NRM, OS, and GRFS

The cumulative incidences of 1-year NRM were 7 and 9 % in the ATG-T and no-ATG-T groups, respectively ($P = 0.65$; Fig. 3a). The use of low-dose ATG-T was not associated with an increased risk of relapse ($P = 0.20$; 18 vs. 29 % at 1 year, Fig. 3b). The probabilities of 1-year OS were 85 and 67 % in the ATG-T and no-ATG-T groups, respectively. There was no statistically significant difference between the 2 groups ($P = 0.14$; Fig. 3c). Grouped according to the pretransplant disease risk, the probabilities of OS at 1-year did not differ statistically between ATG-T and no-ATG-T groups in the low-, intermediate- and high-risk groups, respectively ($P = 0.40$; 88 vs. 82 % Fig. 3d, $P = 0.65$; 89 vs. 80 % Fig. 3e, $P = 0.22$; 84 vs. 62 % Fig. 3f). The probabilities of 1-year GRFS were 61 and 35 % in the ATG-T and no-ATG-T groups, respectively ($P = 0.02$; Fig. 3g).

Discussion

In this retrospective study of 219 patients who received unrelated HSCT, we evaluated the impact of low-dose ATG-T on clinical outcomes, focusing on the incidence of GVHD. The most important finding in this study was the significant impact of ATG-T on chronic GVHD as assessed by several clinical outcomes. Compared to the no-ATG-T group, the incidence of extensive chronic GVHD was significantly lower in the ATG-T group in patients who received an HSCT from a donor with at least one HLA allele mismatch. Patients in the ATG-T group did not suffer from chronic lung dysfunction during the study period, and discontinued immunosuppressive drugs significantly earlier than those in the no-ATG-T group. GRFS, a new clinical outcome proposed by Shernan et al. was significantly better in the ATG-T group than that in the no-ATG-T group [22].

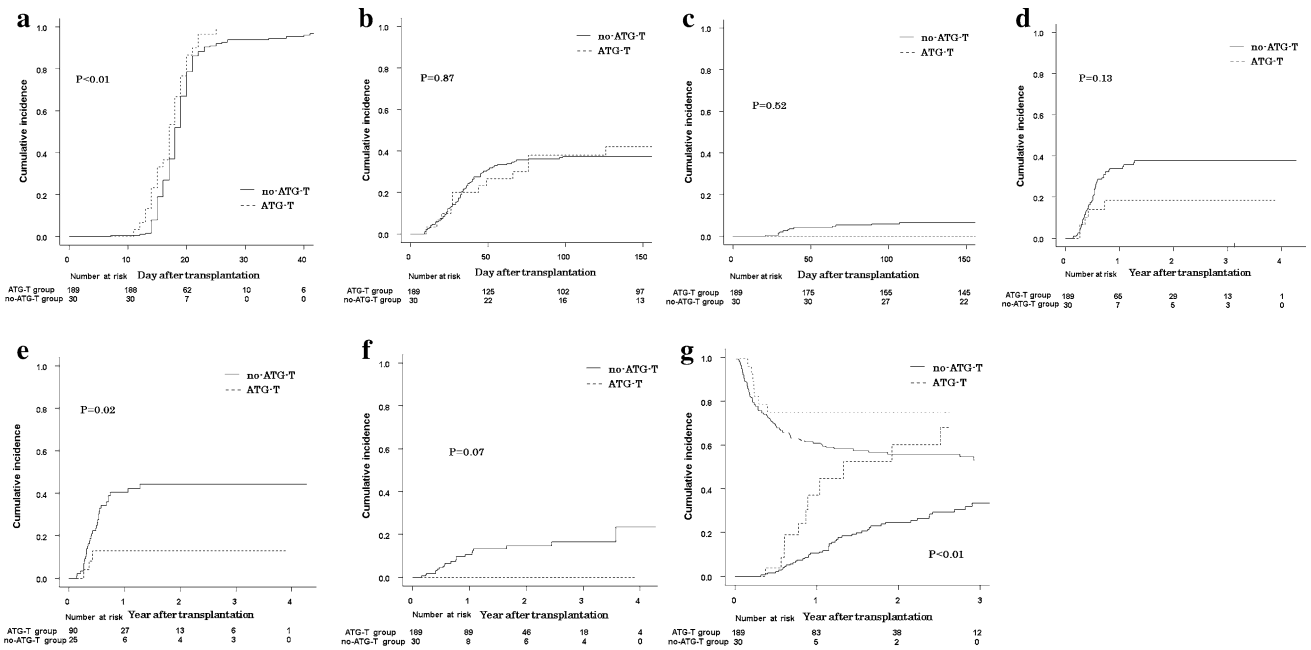

Fig. 1 **a** Engraftment, **b** grade II–IV acute GVHD, **c** grade III–IV acute GVHD, **d** extensive chronic GVHD, **e** extensive chronic GVHD in patients who received HSCT from a donor with at least one HLA allele mismatch, **f** chronic lung dysfunction, **g** discontinuation of immunosuppressive drugs shown by the *lower curve*. The competing risks of relapse and death are shown by the *upper curve*. Differences between *upper curves* and *lower curves* show the proportion of surviving patients who continued to receive immunosuppressive drugs

Table 2 Multivariate analysis for extensive chronic GVHD

Variable	HR (95 % CI)	*P* value
Disease status		
CR	Reference	
non CR	2.29 (1.15–4.57)	0.02
Disease risk		
Low	Reference	
Intermediate	0.94 (0.47–1.89)	0.86
High	0.37 (0.16–0.86)	0.02
Conditioning		
RIC	Reference	
MAC	0.92 (0.55–1.55)	0.76
ATG		
No	Reference	
Yes	0.42 (0.16–1.14)	0.09
HLA mismatch		
None	Reference	
1 allele	1.10 (0.58–2.09)	0.76
More than 1 allele	1.88 (1.02–3.46)	0.04
Source of stem cells		
Bone marrow	Reference	
Peripheral blood	1.38 (0.15–12.8)	0.78

HR hazard ratio, *CI* confidence interval

Therefore, our study suggests the possibility that even low-dose ATG-T could reduce the incidence of severe/refractory chronic GVHD in the setting of unrelated HSCT, thereby lowering the rate of chronic lung dysfunction and allowing for the discontinuation of immunosuppressive drugs.

Previous studies conducted in Western countries showed that the risks of acute and chronic GVHD were significantly reduced by the use of ATG [9, 25]. In practice, in Asian countries such as Korea and Japan we use a smaller dose of ATG than in Western countries, since the incidence of GVHD in Asian populations was reported to be lower than that in Caucasian populations [10–12]. Kim et al. reported that the use of low-dose ATG-T (2.5 mg/kg) was associated with a low incidence of acute GVHD in patients who received an HLA-mismatched unrelated HSCT [14]. In our previous single-institute retrospective study of patients who received an unrelated BMT using a RIC regimen, the incidences of acute and chronic GVHD were low in patients who received low-dose ATG-F (5 or 10 mg/kg in total) [15]. In the current study of low-dose ATG-T (median 1.5 mg/kg), the incidence of extensive chronic GVHD was low in patients who received an HLA-mismatched unrelated HSCT. However, there were some differences in patient characteristics between the ATG group and the no-ATG group. In multivariate analysis, there was a trend toward a lower incidence of extensive chronic GVHD. Although it was not a statistically significant difference, it could be due to the limited number of cases who received ATG-T. Although there have been no well-designed randomized controlled trials in Asia, low-dose ATG-T would contribute to the reduced incidence of GVHD. The reason why ATG reduces the risk of chronic GVHD independent of the impact on acute GVHD is still unclear as reviewed previously [26]. ATG might have immunomodulatory activity to induce immune tolerance, which should be further clarified in the future.

Previous studies found that RIC regimens, including those with ATG-T (5 mg/kg), were associated with low mortality and high long-term disease-free survival without GVHD [27], while the use of higher-dose ATG-T (8 mg/kg) increased the risk of relapse after unrelated HSCT [28]. Previous studies demonstrated no impact of ATG on OS [7, 9, 25, 29]. However the use of ATG improved survival free of immunosuppressive therapy for chronic GVHD [8]. Similarly in our study, while the use of low-dose ATG-T did not improve OS, patients in the ATG-T group discontinued immunosuppressive drugs significantly earlier than in the no-ATG-T group. Focusing on

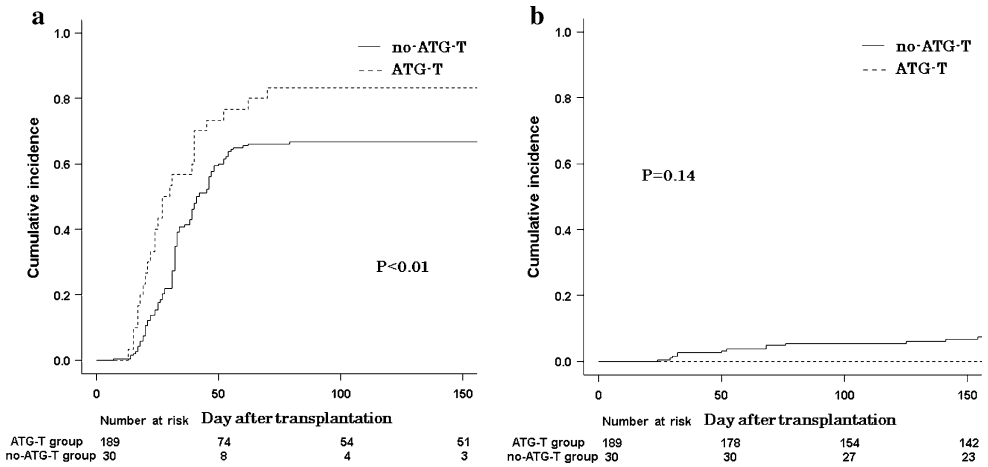

Fig. 2 **a** CMV antigenemia, **b** CMV disease

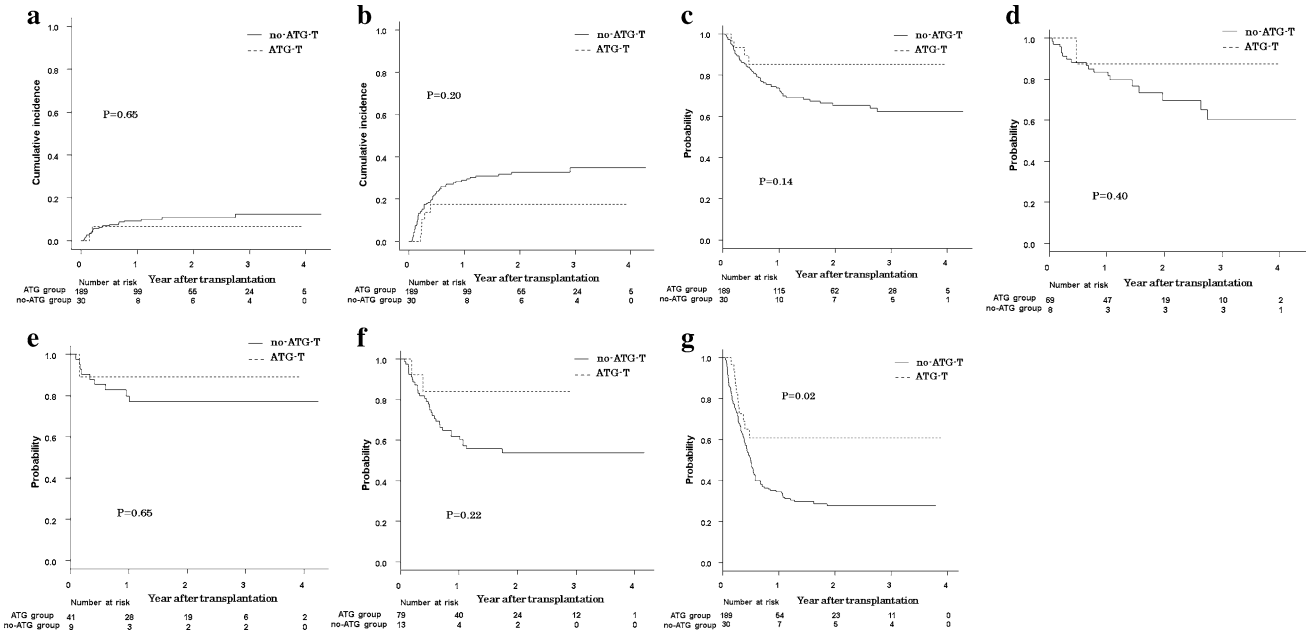

Fig. 3 **a** Non-relapse mortality, **b** relapse, **c** overall survival, **d**, **e**, **f** overall survival grouped according to disease risk; **d** low risk, **e** intermediate risk, **f** high risk, **g** GVHD-free/relapse-free survival

chronic lung dysfunction, recent studies demonstrated that the use of ATG-T in HSCT reduced the incidence of this condition. Bacigalupo et al. reported that ATG-T reduced the incidence of chronic lung dysfunction in patients who received an unrelated HSCT [9]. Dirou et al. also reported a low rate of pulmonary complications and lung function impairment in patients who received RIC regimens that included low-dose ATG-T (5 mg/kg), [30] although Milano et al. reported that the use of ATG-T (range 4.5–6 mg/kg) did not decrease the incidence of pulmonary complications at 1 year after HSCT [31]. In our study, the incidence of chronic lung dysfunction in the ATG-T group tended to be lower than that in the no-ATG-T group. However, the follow-up period might be insufficient to evaluate the incidence of chronic lung dysfunction. Further study incorporating routine serial monitoring of lung function is needed to confirm the beneficial impact of ATG on the incidence of chronic lung dysfunction. A recent study proposed the use of GRFS as a novel valuable composite endpoint comprising grade III–IV acute GVHD, chronic GVHD requiring systemic therapy, relapse, and death after HSCT [22]. GRFS is considered to measure the probability of OS with a good QoL after allogeneic HSCT. In our study, the probability of GRFS in the ATG-T group was significantly higher than that in the no-ATG-T group. Our results are consistent with results from previous studies, and suggest that the use of low-dose ATG-T might lead to a better QoL after unrelated HSCT.

A concern associated with the use of ATG is the increased risk of infectious diseases. Several studies suggested that ATG did not increase the risk of infection-related mortality [7, 32–34]. Bacigalupo et al. reported higher infection-related mortality using high-dose ATG-T (15 mg/kg), but not using low-dose ATG-T (7.5 mg/kg) [9]. In our study, the use of low-dose ATG-T did not increase the incidence of severe infectious disease. Furthermore, there were no cases of PTLD in the ATG-T group, suggesting that low-dose ATG-T did not cause marked immunosuppression. As the incidence of PTLD was previously reported to be rather low even in patients who received high-dose ATG [35], the incidence of PTLD should be confirmed in larger studies.

In previous randomized studies, ATG-F was shown to have a negative effect on both neutrophil and platelet engraftment [7, 29]. Other reports, however, found that ATG-T had no negative effect on neutrophil engraftment [36]. In our previous study, the use of low-dose ATG-F (5 or 10 mg/kg in total) increased the incidence of graft failure [15]. In contrast, our current study showed that low-dose ATG-T did not increase graft failure. The reason for this discrepancy is unclear, but 2 factors in this study might have contributed to the avoidance of graft failure: the formulation of ATG, and our practice of using melphalan or adding low-dose total body irradiation in patients at high-risk of graft failure, such as those with untreated myelodysplastic syndrome.

The limitations of this study should be clarified. We retrospectively analyzed the data at our center. The major limitation of the study was the limited number of cases who received ATG-T and significant difference in patient characteristics between the ATG-T and no-ATG-T groups. We consider that the benefit of low-dose ATG-T should be re-evaluated in prospective studies and larger retrospective clinical studies like a study using a registry data.

In conclusion, the use of low-dose ATG-T in patients who received unrelated HSCT was associated with a superior GRFS, reflecting the reduced incidence of severe/persistent GVHD without compromising OS. The results of this study support further investigation of ATG as prophylaxis for GVHD. The clinical role of low-dose ATG-T as prophylaxis for GVHD should be assessed in prospective clinical trials.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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