

# Impact of anti-thymocyte globulin on survival outcomes in female-to-male allogeneic hematopoietic stem cell transplantation

**Masaharu Tamaki** (✉ [m.tamaki.221@gmail.com](mailto:m.tamaki.221@gmail.com))

Jichi Medical University Saitama Medical Center <https://orcid.org/0000-0001-5899-7913>

**Yu Akahoshi**

Icahn School of Medicine at Mount Sinai <https://orcid.org/0000-0001-6825-9340>

**Masahiro Ashizawa**

Jichi Medical University

**Yukiko Misaki**

**Satoshi Koi**

<https://orcid.org/0000-0003-4969-6398>

**Sung-Won Kim**

National Cancer Center Hospital

**Yukiyasu Ozawa**

Japanese Red Cross Nagoya First Hospital

**Shin-ichiro Fujiwara**

Jichi Medical University

**Shinichi Kako**

Jichi Medical University Saitama Medical Center <https://orcid.org/0000-0002-2635-3395>

**Ken-ichi Matsuoka**

Okayama University Hospital <https://orcid.org/0000-0001-7955-8266>

**Masashi Sawa**

Anjo Kosei Hospital

**Yuta Katayama**

Hiroshima Red-Cross Hospital <https://orcid.org/0000-0001-5054-9104>

**Makoto Onizuka**

Tokai University School of Medicine <https://orcid.org/0000-0003-3864-0823>

**Yoshinobu Kanda**

Division of Hematology, Department of Medicine, Jichi Medical University, Tochigi, Japan

<https://orcid.org/0000-0002-4866-9307>

**Takahiro Fukuda**

National Cancer Center Hospital

**Yoshiko Atsuta**

Japanese Data Center for Hematopoietic Cell Transplantation/Nagoya University Graduate School of Medicine <https://orcid.org/0000-0003-4404-2870>

**Kimikazu Yakushijin**

Kobe University Hospital <https://orcid.org/0000-0003-2511-7857>

**Hideki Nakasone**

---

## Article

**Keywords:**

**Posted Date:** July 12th, 2022

**DOI:** <https://doi.org/10.21203/rs.3.rs-1826152/v1>

**License:**   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

# Abstract

Allogeneic hematopoietic cell transplantation between female donors and male recipients (female-to-male allo-HCT) is a well-established risk factor for inferior survival outcomes due to a higher incidence of graft-versus-host disease (GVHD). However, a clinical significance of anti-thymocyte globulin (ATG) in the female-to-male allo-HCT has not been elucidated. In this study, we retrospectively evaluated male patients who underwent allo-HCT between 2012 and 2019 in Japan. In the female-to-male allo-HCT cohort (n = 828), the use of ATG was not associated with a decreased risk of GVHD (HR of acute GVHD 0.691 [95% CI: 0.461 – 1.04], P = 0.074; HR of chronic GVHD 1.06 [95% CI: 0.738 – 1.52], P = 0.76), but was associated with favorable overall survival (OS) and a decreased risk of non-relapse mortality (NRM) (HR of OS 0.603 [95% CI: 0.400 – 0.909], P = 0.016; HR of NRM 0.506 [95% CI: 0.300 – 0.856], P = 0.011). The use of ATG in female-to-male allo-HCT resulted in survival outcomes that were almost equivalent to those in the male-to-male allo-HCT group. Therefore, GVHD prophylaxis with ATG might overcome the inferiority of survival outcomes in female-to-male allo-HCT.

## Introduction

Graft-versus-host disease (GVHD) is one of the most common complications after allogeneic hematopoietic cell transplantation (allo-HCT) and causes high morbidity and mortality<sup>1-3</sup>. Several efforts have been made to overcome the adverse impact of GVHD on survival outcomes<sup>2</sup>. In vivo T cell depletion with anti-thymocyte globulin (ATG) is a widely accepted procedure for GVHD prophylaxis. Several previous reports have shown a relationship between ATG and a reduced incidence of GVHD<sup>4-7</sup>. However, the immunosuppressive effect of ATG raises a concern about higher incidences of infectious complications and relapse, and the clinical impact of ATG on relapse, infection and overall survival is still a matter of debate.

Allo-HCT between female donors and male recipients (female-to-male allo-HCT) is a well-established risk factor for inferior survival outcomes<sup>8,9</sup>. Since male recipients have H-Y antigens, which are proteins encoded by the Y-chromosome and thus specific to males, these proteins are potential targets of GVHD in female-to-male allo-HCT, resulting in a higher incidence of chronic GVHD and non-relapse mortality (NRM)<sup>10,11</sup>. It may be reasonable to try to improve the survival inferiority of female-to-male allo-HCT by intensive GVHD prophylaxis. However, since previous reports have suggested a relationship between H-Y antigens and a graft-versus leukemia (GVL) effect<sup>10,12</sup>, an escalation of GVHD prophylaxis might induce an increased incidence of relapse in addition to infectious complications. We hypothesized that the use of ATG in female-to-male allo-HCT may contribute to a lower incidence of chronic GVHD and a higher incidence of relapse, and the prognosis in female-to-male allo-HCT may depend on which of these effects is predominant. Therefore, it is important to clarify an adequate GVHD prophylaxis for female-to-male allo-HCT. In this study, we evaluated the impact of ATG on survival outcomes in female-to-male allo-HCT.

## Methods

# Data source and patient selection

Clinical data were obtained from the Transplant Registry Unified Management Program (TRUMP) of the Japanese Society for Transplantation and Cellular Therapy (JSTCT) and the Japanese Data Center for Hematopoietic Cell Transplantation (JDCHCT) <sup>13,14</sup>.

This study included both adult and pediatric male recipients who were diagnosed as a standard risk disease status and who received their first allo-HCT from an unrelated human leukocyte antigen (HLA)-matched (8/8) or 1-locus mismatched (7/8) donor between January 2012 and December 2019 in Japan. Donor sources were limited to bone marrow (BM) or peripheral blood (PB). This study included acute myeloid leukemia (AML) in complete remission (CR 1, CR 2, CR 3-), acute lymphoblastic leukemia (ALL) in complete remission (CR 1, CR 2, CR 3-), myelodysplastic syndrome (MDS), chronic myeloid leukemia (CML) in chronic phase (CP 1, CP 2, CP 3-) and accelerated phase (AP), myeloproliferative neoplasm (MPN), malignant lymphoma (ML) in complete remission (CR 1, CR 2, CR 3-) including adult T cell leukemia/lymphoma (ATL). We excluded patients who were diagnosed with other diseases and for whom information on gender, survival and the last follow-up date were not available. This retrospective study was approved by the data management committee of JSTCT and by the Institutional Review Board of Jichi Medical University Saitama Medical Center.

## Definitions

Disease risk index (DRI) was classified according to a previous report <sup>15</sup>. With regard to cytogenetics of DRI, for AML, t(8;21), inv(16) and t(15;17) were classified as favorable, complex ( $\geq 4$  abnormalities) was classified as adverse, and other karyotypes were classified as intermediate. For MDS, abnormal chromosome 7 and complex karyotype ( $\geq 4$  abnormalities) were classified as adverse and the other karyotypes were considered intermediate. ATL was classified as high risk in DRI. The hematopoietic cell transplantation comorbidity index (HCT-CI) was assessed based on a previous report <sup>16</sup>. Conditioning regimens were classified according to the criteria from the Center for International Blood and Marrow Transplantation Research <sup>17</sup>. Briefly, myeloablative conditioning regimen (MAC) was defined as total body irradiation (TBI)  $> 8$ Gy (fractionated) or  $> 5$  Gy (single dose), melphalan  $> 140$  mg/m<sup>2</sup>, intravenous busulfan  $> 7.2$  mg/kg, and all other regimens were classified as reduced-intensity conditioning (RIC). Chronic GVHD was diagnosed and graded based on standard criteria <sup>18</sup>.

## Statistical analysis

First, survival outcomes were compared between the female-to-male and male-to-male allo-HCT groups to validate the adverse impact of female-to-male allo-HCT in the entire cohort. Second, survival outcomes were compared between patients who received GVHD prophylaxis with and without ATG in the female-to-male allo-HCT cohort. Third, the impact of ATG was similarly analyzed in the male-to-male allo-HCT cohort.

Categorical and continuous variables were compared by Fisher's exact test and the Mann-Whitney U test, respectively. Overall survival (OS) was estimated by the Kaplan-Meier method and compared by the log-rank test. Cumulative incidences of relapse, non-relapse mortality (NRM), and acute and chronic GVHD were estimated by Gray's test. Relapse and NRM were treated as competing risks for each other. Death due to any cause was treated as a competing risk for acute and chronic GVHD. The cumulative incidence of chronic GVHD was evaluated among patients who survived without disease relapse for more than 100 days after allo-HCT. A Cox proportional hazard regression model was used for multivariate analyses of survival outcomes. The hazard ratios (HR) of types of sex-mismatched HCT or ATG were adjusted for the following pre-transplant clinical factors: age, disease type, DRI, HCT-CI, donor type and source, conditioning regimen, GVHD prophylaxis and allo-HCT year. A two-tailed P value < 0.05 was considered to be statistically significant. All analyses in this study were performed with EZR (<http://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmedEN.html>, Jichi Medical University Saitama Medical Center, Saitama, Japan), which is a graphical user interface for R version 3.6.3 (The Foundation for Statistical Computing, Vienna, Austria) <sup>19</sup>.

## Results

### Patient characteristics

According to the eligibility criteria, 828 female-to-male allo-HCT patients and 2431 male-to-male allo-HCT patients were identified. The median follow-up duration for survivors was 39.2 months (range, 0.1–105.5) in the entire cohort, and was shorter in the ATG group (26.2 months in the ATG group vs 41.2 months in the non-ATG group,  $P < 0.001$ ). The median age was 51 years (range, 0–75) in the female-to-male cohort, and 52 years (range, 0–74) in the male-to-male cohort. Patient characteristics are shown in Supplemental Table S1. The female-to-male cohort included more patients who were younger than 16 years old. The other characteristics were almost equivalent between these cohorts.

### Confirmation Of The Adverse Impact Of Female-to-male Allo-hct On Survival Outcomes

When survival outcomes were simply compared between the female-to-male and male-to-male allo-HCT groups, OS tended to be inferior to that for male-to-male allo-HCT (5-y OS 55.2% vs 59.4%,  $P = 0.11$ ), and NRM and chronic GVHD of female-to-male allo-HCT tended to be higher than those of male-to-male allo-HCT (5-y NRM 28.2% vs 24.2%,  $P = 0.066$ ; 5-y cGVHD 48.0 vs 42.8%,  $P = 0.059$ ). Multivariate analyses revealed that female-to-male allo-HCT was significantly associated with the risk of inferior OS (hazard ratio [HR] 1.16 [95% confidence interval {CI}: 1.02–1.33],  $P = 0.027$ ), and increased NRM (HR 1.20 [95% CI: 1.02–1.42],  $P = 0.030$ ) and chronic GVHD (HR 1.19 [95% CI: 1.04–1.36],  $P = 0.010$ ). On the other hand, the cumulative incidence of relapse (CIR) in female-to-male allo-HCT was not different from that in male-to-male allo-HCT (5-y CIR 19.3% vs 19.7%,  $P = 0.71$ ; HR 0.996 [95% CI: 0.821–1.21],  $P = 0.97$ ) (Supplemental Table S2). Regarding acute GVHD, grade II – IV acute GVHD (33.9% vs 40.8%,  $P < 0.001$ ) seemed to be

less common in female-to-male allo-HCT due to the greater mortality before the onset of acute GVHD in the current female-to-male allo-HCT cohort (27.8% vs 21.5%,  $P < 0.001$ ).

## **Clinical Impact Of Atg In The Female-to-male Allo-hct Cohort**

In the female-to-male allo-HCT cohort ( $n = 828$ ), 117 patients received ATG as GVHD prophylaxis. The ATG group included more patients who underwent allo-HCT from an HLA mismatched donor, more patients who used PB as a donor source, more patients who received RIC as a conditioning regimen, and more patients who underwent allo-HCT after 2015 (Table 1).

Table 1  
Patient characteristics.

		Female-to-male allo-HCT cohort			Male-to-male allo-HCT cohort		
		ATG (n = 117)	Non- ATG (n = 711)	P value	ATG (n = 314)	Non- ATG (n = 2117)	P value
Age	> 50 years	60 (51.3)	369 (51.9)	0.79	172 (54.8)	1119 (52.9)	0.048
	16–50 years	44 (37.6)	276 (38.8)		121 (38.5)	914 (43.2)	
	< 16 years	13 (11.1)	66 (9.3)		21 (6.7)	84 (4.0)	
Disease	AML	34 (29.1)	242 (34.0)	0.21	99 (31.5)	738 (34.9)	0.065
	ALL	24 (20.5)	168 (23.6)		58 (18.5)	490 (23.2)	
	MDS	43 (36.8)	201 (28.3)		97 (30.9)	586 (27.7)	
	CML and MPN	6 (5.1)	58 (8.2)		33 (10.5)	161 (7.6)	
	ML	10 (8.5)	42 (5.9)		27 (8.6)	142 (6.7)	
Disease risk index	Low	4 (3.4)	42 (5.9)	0.45	17 (5.4)	155 (7.3)	0.083
	Intermediate	94 (80.3)	545 (76.7)		248 (79.0)	1579 (74.7)	
	High	13 (11.1)	107 (15.0)		36 (11.5)	345 (16.3)	
	Very high	0 (0.0)	1 (0.1)		0 (0.0)	1 (0.1)	
HCT-CI	0–1	96 (82.1)	544 (76.5)	0.23	229 (72.9)	1590 (75.1)	0.33
	≥ 2	21 (17.9)	164 (23.1)		85 (27.1)	517 (24.4)	

Abbreviations: ATG, anti-thymocyte globulin; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; MDS, myelodysplastic syndrome; CML, chronic myeloid leukemia; MPN, myeloproliferative neoplasm; ML, malignant lymphoma; ATL, adult T cell leukemia/lymphoma; HCT-CI, hematopoietic cell transplantation comorbidity index; MAC, myeloablative conditioning regimen; RIC, reduced intensity conditioning regimen; GVHD, graft-versus-host disease; CsA, cyclosporin; TAC, tacrolimus; SCT, stem cell transplantation.

Donor type	Matched unrelated	29 (24.8)	440 (61.9)	< 0.001	89 (29.3)	1366 (63.2)	< 0.001
	Mismatched unrelated	88 (75.2)	271 (38.1)		225 (71.7)	751 (35.5)	
Donor source	Bone marrow	91 (77.8)	659 (92.7)	< 0.001	231 (73.6)	1956 (92.5)	< 0.001
	Peripheral blood	26 (22.2)	52 (7.3)		83 (26.4)	161 (7.6)	
Conditioning regimen	MAC	71 (60.7)	505 (71.0)	0.030	196 (62.4)	1496 (70.8)	0.0038
	RIC	46 (39.3)	206 (29.0)		118 (37.6)	621 (29.4)	
GVHD prophylaxis	CsA-based	8 (6.8)	68 (9.6)	0.17	21 (6.7)	167 (7.9)	0.70
	TAC-based	104 (88.9)	630 (88.6)		285 (90.8)	1905 (90.1)	
	Others	5 (4.3)	13 (1.8)		8 (2.5)	45 (2.1)	
SCT year	2012–2015	35 (29.9)	381 (53.6)	< 0.001	90 (28.7)	1107 (52.3)	< 0.001
	2016–2019	82 (70.1)	330 (46.4)		224 (71.3)	1010 (47.7)	

Abbreviations: ATG, anti-thymocyte globulin; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; MDS, myelodysplastic syndrome; CML, chronic myeloid leukemia; MPN, myeloproliferative neoplasm; ML, malignant lymphoma; ATL, adult T cell leukemia/lymphoma; HCT-CI, hematopoietic cell transplantation comorbidity index; MAC, myeloablative conditioning regimen; RIC, reduced intensity conditioning regimen; GVHD, graft-versus-host disease; CsA, cyclosporin; TAC, tacrolimus; SCT, stem cell transplantation.

The ATG group showed favorable OS compared with the non-ATG group (5-y OS 63.8% vs 53.9%,  $P = 0.035$ , Fig. 1A). A multivariate analysis also demonstrated that the use of ATG was significantly associated with superior OS (HR 0.603 [95% CI: 0.400–0.909],  $P = 0.016$ ) (Fig. 2, Supplemental Table S3). Similarly, NRM tended to be lower in the ATG group (5-y NRM 25.3% vs 28.9%,  $P = 0.11$ , Fig. 1B), although the difference was not significant. However, multivariate analysis revealed that the use of ATG was significantly associated with a reduced risk of NRM (HR 0.506 [95% CI: 0.300–0.856],  $P = 0.011$ ). There was no significant difference in CIR between the ATG and non-ATG groups (14.8% vs 19.9%,  $P = 0.38$ , Fig. 1C), and a multivariate analysis also showed no significant relationship between the use of ATG and CIR (HR 0.760 [95% CI: 0.429–1.34],  $P = 0.34$ ). Grade II – IV acute GVHD tended to be less common in the ATG group (26.7% vs 35.0%,  $P = 0.067$ , Fig. 1D), and the use of ATG seemed to be associated with a higher incidence of grade II – IV acute GVHD, but this difference was not statistically significant (HR 0.691 [95% CI: 0.461–1.04],  $P = 0.074$ ). The cumulative incidence of chronic GVHD was equivalent



between the ATG and non-ATG groups (5-y cumulative incidence of chronic GVHD 47.2% vs 48.2%,  $P = 0.99$ , Fig. 1E), and ATG was not associated with chronic GVHD in a multivariate analysis (HR 1.06 [95% CI: 0.738–1.52],  $P = 0.76$ ).

## Cause Of Death In The Female-to-male Allo-hct Cohort

The cause of death was mainly infection and disease progression (Fig. 3). The ATG group showed a significantly lower incidence of death due to progression and non-infectious pulmonary complications (progression 5 of 117 cases [4.3%] vs 79 of 711 cases [11.1%],  $P = 0.020$ ; non-infectious pulmonary complications 0 of 117 cases [0.0%] vs 30 of 711 cases [4.2%],  $P = 0.015$ ) (Supplemental Table S4). Although the number of deaths due GVHD was lower in the ATG group, there was no significant difference between the groups (1 of 117 cases [0.9%] vs 21 of 711 cases [3.0%],  $P = 0.35$ ). The other causes of death were comparable between the groups.

## Clinical Impact Of Atg In The Male-to-male Allo-hct Cohort

ATG was administered to 314 patients in the male-to-male cohort ( $n = 2431$ ). The ATG group included more patients who were younger than 16 years old, more patients who underwent allo-HCT from an HLA mismatched donor, more patients who used PB as a donor source, more patients who received RIC as a conditioning regimen and more patients who underwent allo-HCT after 2015 (Table 1).

OS was favorable in the ATG group (5-y OS 64.1% vs 58.8%,  $P = 0.039$ , Fig. 4A; HR of ATG 0.747 [95% CI: 0.587–0.951],  $P = 0.018$ , Fig. 2, Supplemental Table S5). The ATG group tended to show lower NRM (5-y NRM 18.9% vs 24.9%,  $P = 0.051$ , Fig. 4B), and ATG was significantly associated with a reduced risk of NRM (HR 0.680 [95% CI: 0.494–0.937],  $P = 0.018$ ). CIR was comparable between the ATG and non-ATG groups (5-y CIR 21.3% vs 19.4%,  $P = 0.65$ , Fig. 4C; HR 1.05 [95% CI: 0.775–1.41],  $P = 0.77$ ). The cumulative incidence of grade II – IV acute GVHD was significantly lower in the ATG group (34.2% vs 41.8%,  $P = 0.0065$ , Fig. 4D), and the use of ATG was associated with a reduced risk of grade II – IV acute GVHD (HR 0.721 [95% CI: 0.581–0.895],  $P = 0.0031$ ). The ATG group tended to show a lower incidence of chronic GVHD (5-y cumulative incidence of chronic GVHD 35.7% vs 43.8%,  $P = 0.059$ , Fig. 4E), and this tendency was also observed in a multivariate analysis (HR 0.802 [95% CI: 0.635–1.01],  $P = 0.065$ ).

In summary, the impact of ATG seemed to differ somewhat between the female-to-male and male-to-male allo-HCT groups (Fig. 2). The use ATG tended to be associated with a decreased risk of chronic GVHD only in the male-to-male allo-HCT cohort, whereas the impact of ATG on other survival outcomes was almost equivalent between the female-to-male and male-to-male allo-HCT cohorts.

## Survival Outcomes Stratified According To Sex-mismatch And Atg

Additionally, survival analyses were performed in the entire cohort according to the combination of sex-mismatch and ATG. When the female-to-male/ATG group was treated as a reference, the female-to-male/non-ATG group showed an increased risk of poor OS (HR 1.49 [95% CI: 1.03–2.18],  $P = 0.036$ ), whereas the risk of overall mortality was similar to those in both the male-to-male/ATG and male-to-male/non-ATG groups (Supplemental Table S6). An increased risk of NRM also tended to be observed in the female-to-male/non-ATG group (HR 1.51 [95% CI: 0.940–2.42],  $P = 0.089$ ), and the other groups showed a comparable risk of NRM. The risk of chronic GVHD was equivalent among these groups.

## Discussion

In this study, the clinical significance of ATG was evaluated in female-to-male and male-to-male allo-HCT cohorts. In the female-to-male cohort, ATG was significantly associated with favorable OS. Although ATG was not associated with reduced incidences of acute and chronic GVHD in the female-to-male cohort, there was a significant relationship between ATG and favorable NRM. In the male-to-male allo-HCT cohort, ATG was associated with OS and NRM, and also tended to be associated with chronic GVHD.

The sex-mismatched combination of a male recipient and a female donor is recognized as a risk factor for an inferior prognosis in allo-HCT. An unfavorable effect of female-to-male allo-HCT has been reported by several studies since 1989<sup>8,9,20,21</sup>. The sex-mismatched combination is also included in the EBMT score as an unfavorable risk factor<sup>22</sup>. Female-to-male allo-HCT is generally associated with increased incidences of NRM and chronic GVHD. On the other hand, some previous studies showed a reduced incidence of relapse which suggested the existence of a GVL effect due to sex-mismatch<sup>8,23–25</sup>. In fact, we previously reported that male recipients with deletion of Y-chromosome who underwent allo-HCT from a female donor showed a higher incidence of relapse<sup>12</sup>, suggesting attenuation of the GVL effect due to the loss of Y-chromosome. Thus, we initially hypothesized that the addition of ATG may contribute to a lower incidence of chronic GVHD and a higher incidence of relapse in female-to-male allo-HCT.

Based on the fact that female-to-male allo-HCT gives inferior survival outcomes due to a higher incidence of chronic GVHD, it might be reasonable to intensify GVHD prophylaxis in female-to-male allo-HCT. In vivo T cell depletion with ATG is a widely accepted strategy for GVHD prophylaxis. Several randomized control trials have shown that GVHD prophylaxis with ATG was associated with a decreased risk of GVHD and NRM,<sup>4–6</sup> and various studies have reported the clinical significance of GVHD prophylaxis with ATG<sup>7,26–29</sup>. However, the impact of ATG on OS remains controversial, since the intensification of GVHD prophylaxis with ATG was associated with an increased risk of infectious complications such as cytomegalovirus and Epstein-Barr virus reactivation<sup>30</sup>. Furthermore, intensive immunosuppression with ATG raises a concern about an increase of relapse, although a significant relationship between ATG and relapse has not been elucidated. The current study revealed an improvement of OS and NRM with ATG in female-to-male allo-HCT, even though ATG did not improve the incidence of chronic GVHD. While the incidence of death due to GVHD was also comparable between the ATG and non-ATG groups in the female-to-male allo-HCT cohort, death due to non-infectious pulmonary complication was significantly

rare in the ATG group. Although ATG could not reduce the incidence of GVHD in female-to-male allo-HCT, ATG might reduce the incidence of fatal allogeneic immune reaction. Moreover, CIR was equivalent between the ATG and non-ATG groups in the female-to-male allo-HCT cohort, and ATG was not adversely associated with relapse.

According to the result of the additional analysis based on a combination of sex-mismatch and ATG, the administration of ATG might reduce the adverse impact of female-to-male allo-HCT and improve OS and NRM, resulting in outcomes comparable to those with male-to-male allo-HCT. For the further improvement of survival outcomes, additional GVHD prophylaxis might be required in female-to-male allo-HCT. A promising approach to GVHD prophylaxis is posttransplant cyclophosphamide (PTCY). PTCY was reported as a highly effective GVHD prophylaxis in previous reports<sup>31–33</sup>. Several studies have shown that PTCY provides superior progression-free survival and GVHD/relapse-free survival compared with ATG<sup>34,35</sup>. Additionally, there have been several attempts to develop a combination therapy with ATG and PTCY<sup>36,37</sup>. Although we should be careful regarding a risk of infection and relapse, further investigation is also warranted to elucidate the clinical significance of PTCY in female-to-male allo-HCT.

The current study has several limitations due to its retrospective nature. First, the number of patients who underwent allo-HCT with ATG has recently increased. Therefore, the median follow-up duration in the ATG group was slightly shorter than that in the non-ATG group. A longer follow-up might reveal additional favorable and/or adverse impacts of ATG. Second, due to the limited sample size, we could not perform various subgroup analyses according to HLA-match/mismatch, donor source or disease type. Third, although the background condition was compensated by DRI and HCT-CI, the use of ATG might be avoided in patients who were at high risk of disease relapse or in poor general condition.

In conclusion, GVHD prophylaxis with ATG in female-to-male allo-HCT was significantly associated with superior OS and a reduced risk of NRM, despite no significant relationship with chronic GVHD. The use of ATG in female-to-male allo-HCT resulted in survival outcomes that were almost the same as those in the male-to-male allo-HCT group. GVHD prophylaxis with ATG might overcome the inferior survival outcomes in female-to-male allo-HCT.

## Declarations

### Acknowledgement:

The authors are grateful for the work of all of the physicians and data managers at the centers that contributed valuable data on transplantation to the JSTCT. We would also like to thank all of the members of the Transplant Registry Unified Management committees at JSTCT for their dedicated data management. H.N. received a grant for JSPS KAKENHI (JP21K07070).

### Author contributions:

M.T. and H.N. conceived the original idea. M.T. designed the study, analyzed data, and wrote the manuscript. Y.Akahoshi., M.A., M.Y. and K.Y. advised on methods and wrote the manuscript. S.K., SW.K., Y.O., SI.F., S.K., KI.M., M.S. and Y.Katayama. collected data and revised the manuscript. M.O, Y.Kanda, and T.F. collected data, revised the manuscript, and were responsible for data management at JSTCT. Y.Atsuta. managed the unified registry database and revised the manuscript. H.N. designed the study, advised on the methods, analyzed data, wrote and revised the manuscript, and was responsible for this project of JSTCT Transplant Complications Working Group. All authors approved the final version of the manuscript.

### Competing interests:

Y.Akashoshi received a grant for the Japan Society for the Promotion of Science Postdoctoral Fellowship for Research Abroad. Y.M. received honoraria from Abbvie. SI.F. received honoraria from Astellas Pharma, Chugai Pharmaceutical, Kyowa Kirin, Nippon Shinyaku, Novartis Pharma, Pfizer and Sanofi. S.K. received honoraria from Sanofi. M.S. received honoraria from Chugai Pharmaceutical, Pfizer, Astellas Pharma, Nippon Shinyaku, Ono Pharmaceutical, MSD, Bristol-Myers Squibb, Kyowa Kirin, Asahi Kasei Pharma, Novartis Pharma, Eisai, Otsuka Pharmaceutical, Daimippon Sumitomo Pharmaceutical, Sanofi, Takeda Pharmaceutical, Celgene, Mochida Pharmaceutical, Shire, Mundi Pharma, Abbvie, CSL Behring, Symbio, Janssen Pharmaceutical, Astra Zeneca, Daiichi Sankyo Pharmaceutical and Glaxo Smith Kline. Y.Atsuta. received honoraria from Novartis Pharma, Kyowa Kirin, Abbvie, Astellas Pharma, Mochida Pharmaceutical and Meiji Seika Pharma. H.N. received honoraria from Takeda Pharmaceutical, Sanofi, Otsuka Pharmaceutical, Bristol-Myers Squibb, Celgene, Pfizer, Novartis Pharma, Janssen Pharmaceutical, Eisai, Chugai Pharmaceutical and Nippon Shinyaku. The remaining authors declare no competing financial interests.

## References

1. Ferrara JLM, Chaudhry MS. GVHD: biology matters. *Blood Adv* 2018; **2**(22): 3411–3417. e-pub ahead of print 2018/11/30; doi: 10.1182/bloodadvances.2018020214
2. Hamilton BK. Current approaches to prevent and treat GVHD after allogeneic stem cell transplantation. *Hematology Am Soc Hematol Educ Program* 2018; **2018**(1): 228–235. e-pub ahead of print 2018/12/07; doi: 10.1182/asheducation-2018.1.228
3. Cutler CS, Koreth J, Ritz J. Mechanistic approaches for the prevention and treatment of chronic GVHD. *Blood* 2017; **129**(1): 22–29. e-pub ahead of print 2016/11/09; doi: 10.1182/blood-2016-08-686659
4. Kröger N, Solano C, Wolschke C, Bandini G, Patriarca F, Pini M *et al.* Antilymphocyte Globulin for Prevention of Chronic Graft-versus-Host Disease. *N Engl J Med* 2016; **374**(1): 43–53. e-pub ahead of print 2016/01/07; doi: 10.1056/NEJMoa1506002
5. Soiffer RJ, Kim HT, McGuirk J, Horwitz ME, Johnston L, Patnaik MM *et al.* Prospective, Randomized, Double-Blind, Phase III Clinical Trial of Anti-T-Lymphocyte Globulin to Assess Impact on Chronic

- Graft-Versus-Host Disease-Free Survival in Patients Undergoing HLA-Matched Unrelated Myeloablative Hematopoietic Cell Transplantation. *J Clin Oncol* 2017; **35**(36): 4003–4011. e-pub ahead of print 2017/10/19; doi: 10.1200/jco.2017.75.8177
6. Chang YJ, Wu DP, Lai YR, Liu QF, Sun YQ, Hu J *et al.* Antithymocyte Globulin for Matched Sibling Donor Transplantation in Patients With Hematologic Malignancies: A Multicenter, Open-Label, Randomized Controlled Study. *J Clin Oncol* 2020; **38**(29): 3367–3376. e-pub ahead of print 2020/07/12; doi: 10.1200/jco.20.00150
  7. Fuji S, Hirakawa T, Takano K, Doki N, Sawa M, Kanda Y *et al.* Disease-specific impact of anti-thymocyte globulin in allogeneic hematopoietic cell transplantation: a nationwide retrospective study on behalf of the JSTCT, transplant complications working group. *Bone Marrow Transplant* 2022; **57**(3): 479–486. e-pub ahead of print 2022/01/19; doi: 10.1038/s41409-022-01569-x
  8. Nakasone H, Remberger M, Tian L, Brodin P, Sahaf B, Wu F *et al.* Risks and benefits of sex-mismatched hematopoietic cell transplantation differ according to conditioning strategy. *Haematologica* 2015; **100**(11): 1477–1485. e-pub ahead of print 2015/08/08; doi: 10.3324/haematol.2015.125294
  9. Nannya Y, Kataoka K, Hangaishi A, Imai Y, Takahashi T, Kurokawa M. The negative impact of female donor/male recipient combination in allogeneic hematopoietic stem cell transplantation depends on disease risk. *Transpl Int* 2011; **24**(5): 469–476. e-pub ahead of print 2011/02/08; doi: 10.1111/j.1432-2277.2011.01229.x
  10. Nakasone H, Tian L, Sahaf B, Kawase T, Schoenrock K, Perloff S *et al.* Allogeneic HY antibodies detected 3 months after female-to-male HCT predict chronic GVHD and nonrelapse mortality in humans. *Blood* 2015; **125**(20): 3193–3201. e-pub ahead of print 2015/03/15; doi: 10.1182/blood-2014-11-613323
  11. Paul J, Nakasone H, Sahaf B, Wu F, Wang K, Ho V *et al.* A confirmation of chronic graft-versus-host disease prediction using allogeneic HY antibodies following sex-mismatched hematopoietic cell transplantation. *Haematologica* 2019; **104**(7): e314-e317. e-pub ahead of print 2019/01/19; doi: 10.3324/haematol.2018.199646
  12. Tamaki M, Kameda K, Kimura SI, Harada N, Uchida N, Doki N *et al.* Deletion of Y chromosome before allogeneic hematopoietic stem cell transplantation in male recipients with female donors. *Blood Adv* 2022; **6**(6): 1895–1903. e-pub ahead of print 2022/02/03; doi: 10.1182/bloodadvances.2021006456
  13. Atsuta Y. Introduction of Transplant Registry Unified Management Program 2 (TRUMP2): scripts for TRUMP data analyses, part I (variables other than HLA-related data). *Int J Hematol* 2016; **103**(1): 3–10. e-pub ahead of print 2015/11/09; doi: 10.1007/s12185-015-1894-x
  14. Kanda J. Scripts for TRUMP data analyses. Part II (HLA-related data): statistical analyses specific for hematopoietic stem cell transplantation. *Int J Hematol* 2016; **103**(1): 11–19. e-pub ahead of print 2015/11/22; doi: 10.1007/s12185-015-1907-9
  15. Armand P, Kim HT, Logan BR, Wang Z, Alyea EP, Kalaycio ME *et al.* Validation and refinement of the Disease Risk Index for allogeneic stem cell transplantation. *Blood* 2014; **123**(23): 3664–3671. e-pub

ahead of print 2014/04/20; doi: 10.1182/blood-2014-01-552984

16. Sorror ML, Maris MB, Storb R, Baron F, Sandmaier BM, Maloney DG *et al.* Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. *Blood* 2005; **106**(8): 2912–2919. e-pub ahead of print 2005/07/05; doi: 10.1182/blood-2005-05-2004
17. Giralt S, Ballen K, Rizzo D, Bacigalupo A, Horowitz M, Pasquini M *et al.* Reduced-intensity conditioning regimen workshop: defining the dose spectrum. Report of a workshop convened by the center for international blood and marrow transplant research. *Biol Blood Marrow Transplant* 2009; **15**(3): 367–369. e-pub ahead of print 2009/02/11; doi: 10.1016/j.bbmt.2008.12.497
18. Sullivan KM, Agura E, Anasetti C, Appelbaum F, Badger C, Bearman S *et al.* Chronic graft-versus-host disease and other late complications of bone marrow transplantation. *Semin Hematol* 1991; **28**(3): 250–259. e-pub ahead of print 1991/07/01;
19. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant* 2013; **48**(3): 452–458. e-pub ahead of print 2012/12/05; doi: 10.1038/bmt.2012.244
20. Gahrton G. Risk assessment in haematopoietic stem cell transplantation: impact of donor-recipient sex combination in allogeneic transplantation. *Best Pract Res Clin Haematol* 2007; **20**(2): 219–229. e-pub ahead of print 2007/04/24; doi: 10.1016/j.beha.2006.09.007
21. Schetelig J, de Wreede LC, van Gelder M, Andersen NS, Moreno C, Vitek A *et al.* Risk factors for treatment failure after allogeneic transplantation of patients with CLL: a report from the European Society for Blood and Marrow Transplantation. *Bone Marrow Transplant* 2017; **52**(4): 552–560. e-pub ahead of print 2017/01/24; doi: 10.1038/bmt.2016.329
22. Gratwohl A. The EBMT risk score. *Bone Marrow Transplant* 2012; **47**(6): 749–756. e-pub ahead of print 2011/06/07; doi: 10.1038/bmt.2011.110
23. Randolph SS, Gooley TA, Warren EH, Appelbaum FR, Riddell SR. Female donors contribute to a selective graft-versus-leukemia effect in male recipients of HLA-matched, related hematopoietic stem cell transplants. *Blood* 2004; **103**(1): 347–352. e-pub ahead of print 2003/09/13; doi: 10.1182/blood-2003-07-2603
24. Gratwohl A, Hermans J, Niederwieser D, van Biezen A, van Houwelingen HC, Apperley J. Female donors influence transplant-related mortality and relapse incidence in male recipients of sibling blood and marrow transplants. *Hematol J* 2001; **2**(6): 363–370. e-pub ahead of print 2002/03/29; doi: 10.1038/sj.thj.6200117
25. Gahrton G, Iacobelli S, Apperley J, Bandini G, Björkstrand B, Bladé J *et al.* The impact of donor gender on outcome of allogeneic hematopoietic stem cell transplantation for multiple myeloma: reduced relapse risk in female to male transplants. *Bone Marrow Transplant* 2005; **35**(6): 609–617. e-pub ahead of print 2005/02/08; doi: 10.1038/sj.bmt.1704861
26. Shiratori S, Sugita J, Ota S, Kasahara S, Ishikawa J, Tachibana T *et al.* Low-dose anti-thymocyte globulin for GVHD prophylaxis in HLA-matched allogeneic peripheral blood stem cell transplantation.

- Bone Marrow Transplant* 2021; **56**(1): 129–136. e-pub ahead of print 2020/07/07; doi: 10.1038/s41409-020-0985-3
27. Shiratori S, Kurata M, Sugita J, Ota S, Kasahara S, Ishikawa J *et al.* Graft-Versus-Host Disease Prophylaxis Using Low-Dose Antithymocyte Globulin in Peripheral Blood Stem Cell Transplantation-A Matched-Pair Analysis. *Transplant Cell Ther* 2021; **27**(12): 995.e991-995.e996. e-pub ahead of print 2021/09/10; doi: 10.1016/j.jtct.2021.08.029
  28. Arai Y, Jo T, Matsui H, Kondo T, Takaori-Kondo A. Efficacy of antithymocyte globulin for allogeneic hematopoietic cell transplantation: a systematic review and meta-analysis. *Leuk Lymphoma* 2017; **58**(8): 1840–1848. e-pub ahead of print 2016/12/14; doi: 10.1080/10428194.2016.1266624
  29. Ratanatharathorn V, Deol A, Ayash L, Cronin S, Bhutani D, Lum LG *et al.* Low-dose antithymocyte globulin enhanced the efficacy of tacrolimus and mycophenolate for GVHD prophylaxis in recipients of unrelated SCT. *Bone Marrow Transplant* 2015; **50**(1): 106–112. e-pub ahead of print 2014/10/07; doi: 10.1038/bmt.2014.203
  30. Kang HM, Kim SK, Lee JW, Chung NG, Cho B. Efficacy of low dose antithymocyte globulin on overall survival, relapse rate, and infectious complications following allogeneic peripheral blood stem cell transplantation for leukemia in children. *Bone Marrow Transplant* 2021; **56**(4): 890–899. e-pub ahead of print 2020/11/18; doi: 10.1038/s41409-020-01121-9
  31. Luznik L, O'Donnell PV, Symons HJ, Chen AR, Leffell MS, Zahurak M *et al.* HLA-haploidentical bone marrow transplantation for hematologic malignancies using nonmyeloablative conditioning and high-dose, posttransplantation cyclophosphamide. *Biol Blood Marrow Transplant* 2008; **14**(6): 641–650. e-pub ahead of print 2008/05/21; doi: 10.1016/j.bbmt.2008.03.005
  32. Kanakry CG, O'Donnell PV, Furlong T, de Lima MJ, Wei W, Medeot M *et al.* Multi-institutional study of post-transplantation cyclophosphamide as single-agent graft-versus-host disease prophylaxis after allogeneic bone marrow transplantation using myeloablative busulfan and fludarabine conditioning. *J Clin Oncol* 2014; **32**(31): 3497–3505. e-pub ahead of print 2014/10/01; doi: 10.1200/jco.2013.54.0625
  33. Mielcarek M, Furlong T, O'Donnell PV, Storer BE, McCune JS, Storb R *et al.* Posttransplantation cyclophosphamide for prevention of graft-versus-host disease after HLA-matched mobilized blood cell transplantation. *Blood* 2016; **127**(11): 1502–1508. e-pub ahead of print 2016/01/15; doi: 10.1182/blood-2015-10-672071
  34. Nykolyszyn C, Granata A, Pagliardini T, Castagna L, Harbi S, Bouabdallah R *et al.* Posttransplantation cyclophosphamide vs. antithymocyte globulin as GVHD prophylaxis for mismatched unrelated hematopoietic stem cell transplantation. *Bone Marrow Transplant* 2020; **55**(2): 349–355. e-pub ahead of print 2019/09/20; doi: 10.1038/s41409-019-0682-2
  35. Battipaglia G, Labopin M, Kröger N, Vitek A, Afanasyev B, Hilgendorf I *et al.* Posttransplant cyclophosphamide vs antithymocyte globulin in HLA-mismatched unrelated donor transplantation. *Blood* 2019; **134**(11): 892–899. e-pub ahead of print 2019/07/05; doi: 10.1182/blood.2019000487

36. Law AD, Salas MQ, Lam W, Michelis FV, Thyagu S, Kim DDH *et al.* Reduced-Intensity Conditioning and Dual T Lymphocyte Suppression with Antithymocyte Globulin and Post-Transplant Cyclophosphamide as Graft-versus-Host Disease Prophylaxis in Haploidentical Hematopoietic Stem Cell Transplants for Hematological Malignancies. *Biol Blood Marrow Transplant* 2018; **24**(11): 2259–2264. e-pub ahead of print 2018/07/17; doi: 10.1016/j.bbmt.2018.07.008
37. Sun X, Yang J, Cai Y, Wan L, Huang C, Qiu H *et al.* Low-dose antithymocyte globulin plus low-dose posttransplant cyclophosphamide combined with cyclosporine and mycophenolate mofetil for prevention of graft-versus-host disease after HLA-matched unrelated donor peripheral blood stem cell transplantation. *Bone Marrow Transplant* 2021; **56**(10): 2423–2431. e-pub ahead of print 2021/05/27; doi: 10.1038/s41409-021-01358-y

## Figures

Figure 1.

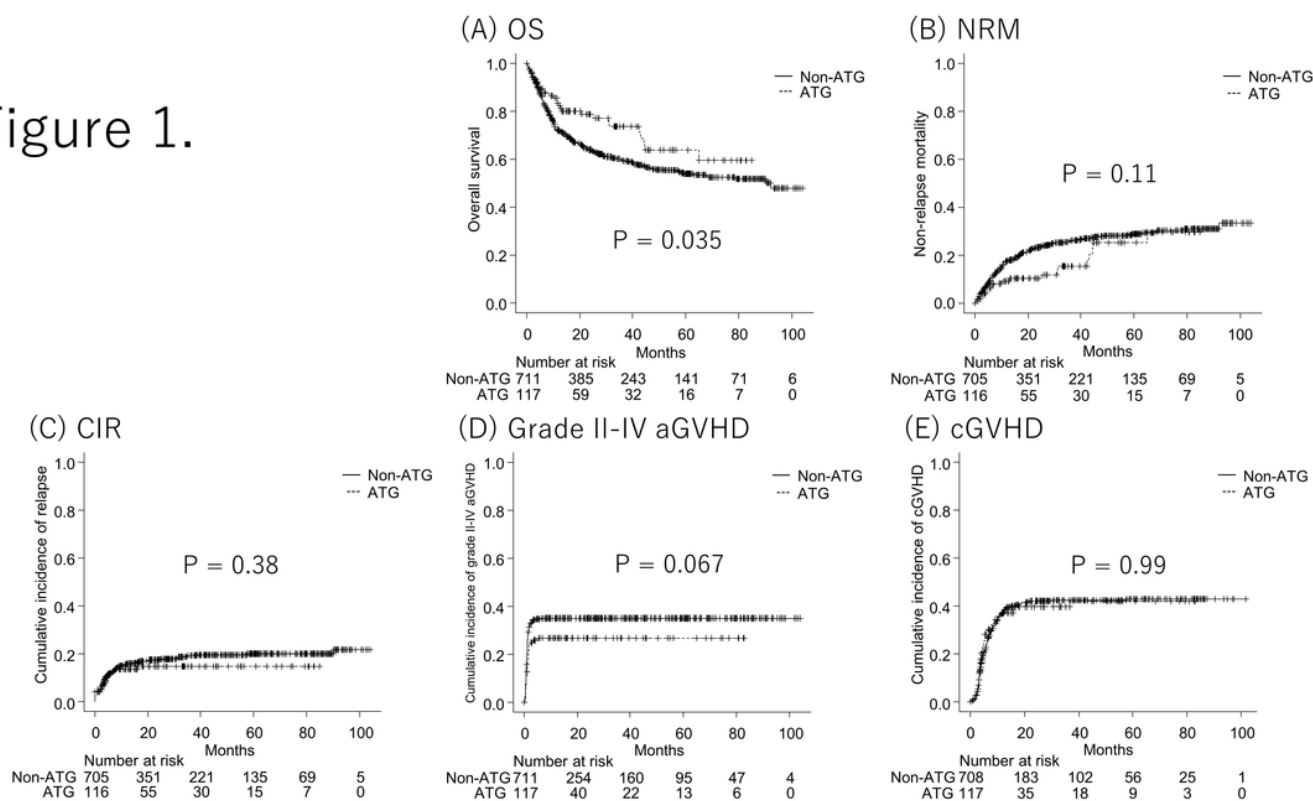


Figure 1

Survival outcomes according to administration of anti-thymocyte globulin in the female-to-male cohort. (A) Overall survival. (B) Cumulative incidence of relapse. (C) Non-relapse mortality. (D) Cumulative incidence of acute graft-versus-host disease. (E) Cumulative incidence of chronic graft-versus-host disease.



Figure 2.

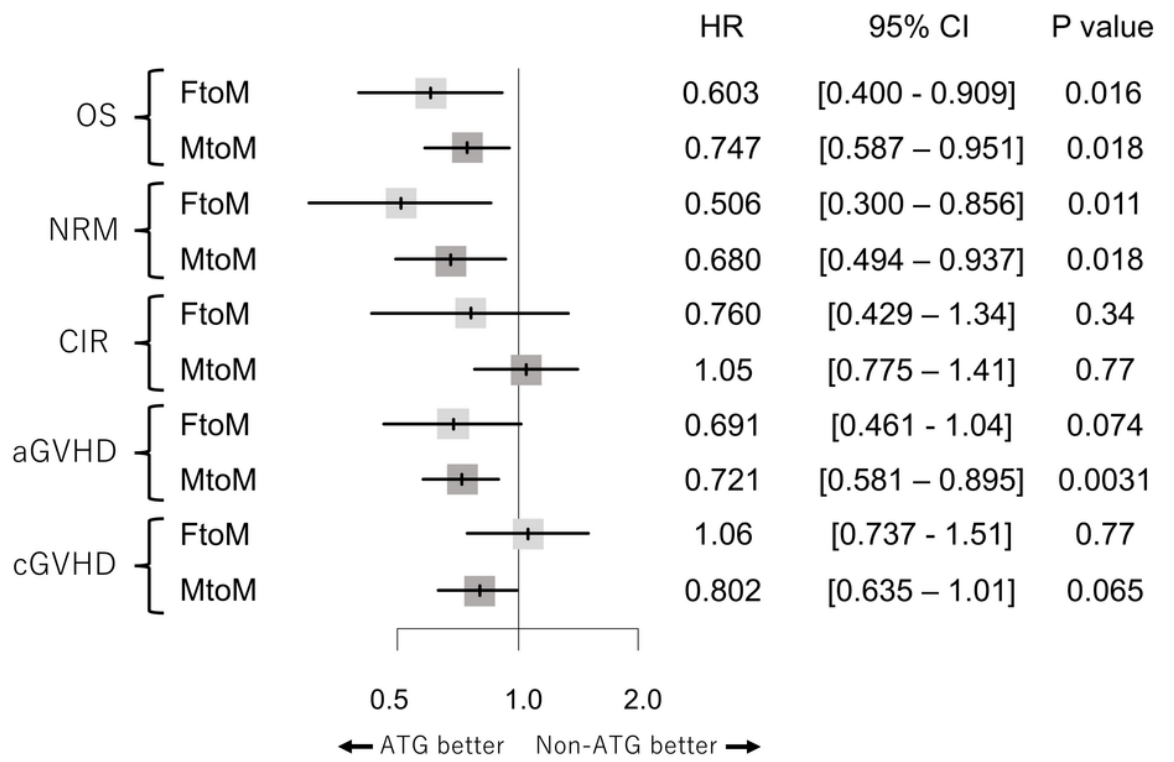


Figure 2

Impact of anti-thymocyte globulin on survival outcomes in the female-to-male (FtoM) and male-to-male (MtoM) allo-HCT cohorts.

Figure 3.

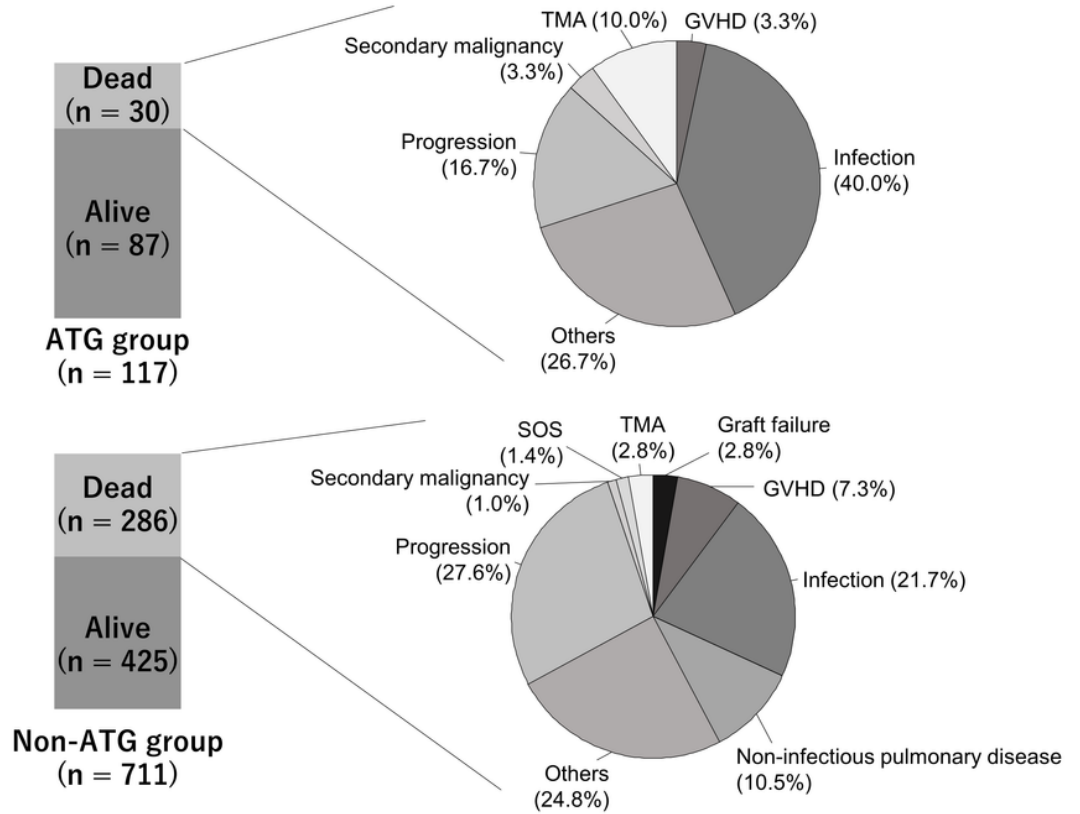
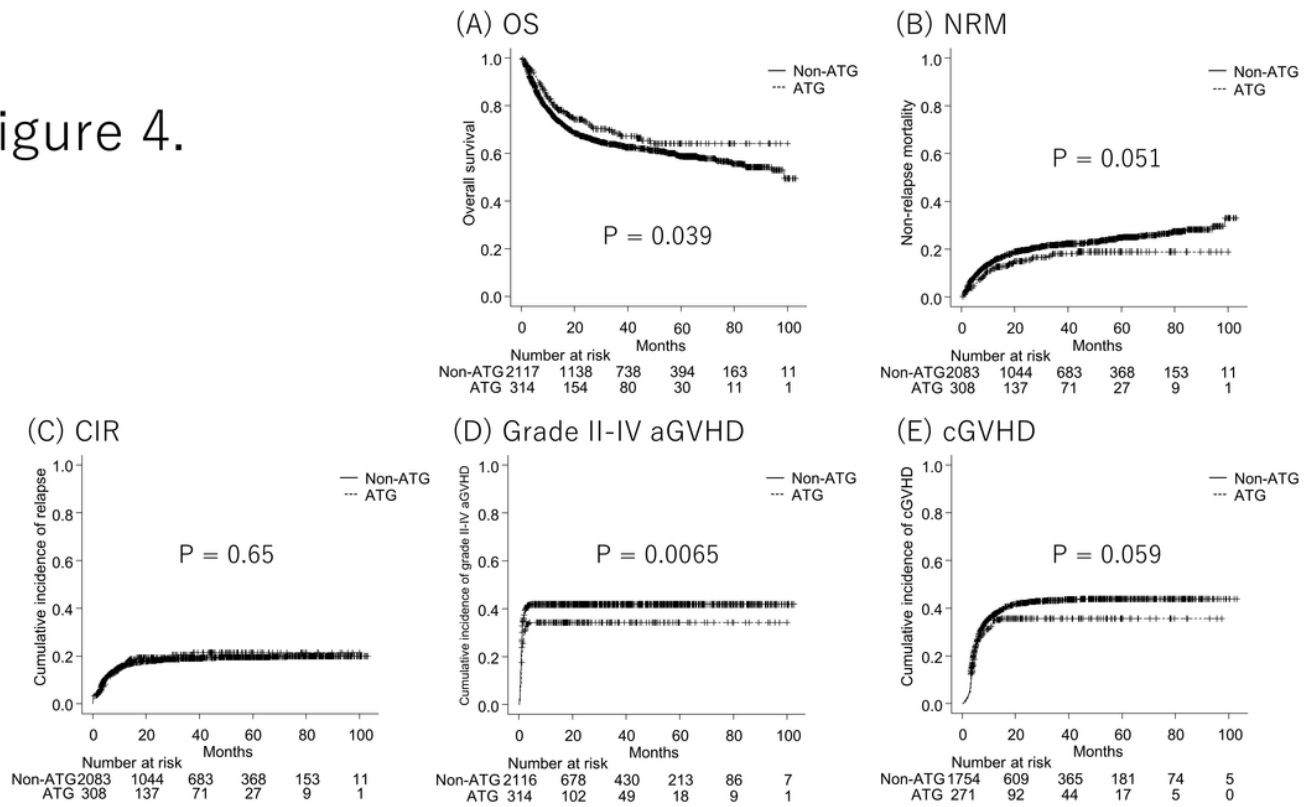


Figure 3

Cause of death in the female-to-male allo-HCT cohort.

Figure 4.



## Figure 4

Survival outcomes according to administration of anti-thymocyte globulin in the male-to-male cohort. (A) Overall survival. (B) Cumulative incidence of relapse. (C) Non-relapse mortality. (D) Cumulative incidence of acute graft-versus-host disease. (E) Cumulative incidence of chronic graft-versus-host disease.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SexMismatchATGSupplementalfiles.pdf](#)