**Article**

**Title of the paper**

Low-dose antithymocyte globulin inhibits chronic graft-versus-host disease in peripheral blood stem cell transplantation from unrelated donors

**Running title**

Low-dose ATG in unrelated PBSCT

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**Conflict of interest**

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**Abstract**

Antithymocyte globulin (ATG) has been shown to reduce chronic graft-versus-host disease (GVHD) particularly in allogeneic peripheral blood stem cell transplantation (PBSCT) from unrelated donors; however, anti-GVHD effects of lower doses of ATG remains to be elucidated. We conducted a nationwide retrospective study to compare the outcomes of unrelated PBSCT with or without rabbit ATG (thymoglobulin) in 287 patients. A median ATG dose was 2.0 mg/kg. The primary endpoint, cumulative incidence of moderate - severe chronic GVHD at 2 years was 22.1% in the ATG group, which was significantly less than that in the non-ATG group (36.3%, $P$ = 0.025). The ATG group had higher incidence of immunosuppressant discontinuation, GVHD-free, relapse-free survival, and moderate - severe chronic GVHD-free, relapse-free survival at 2 years compared to the non-ATG group. The incidences of grade III - IV aGVHD and moderate - severe chronic GVHD were significantly higher in patients with high absolute lymphocyte count (ALC) before the administration of ATG, whereas relapse rate was significantly higher in patients with low ALC before ATG. In conclusion, low-dose ATG effectively suppresses chronic GVHD in unrelated PBSCT, and ALC before ATG may be a potential predictor for GVHD and relapse.

## Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is a curative treatment for hematological malignancies. With an increase in long-term survivors after HSCT in recent years, chronic graft-versus-host disease (cGVHD) is the major cause of poor quality-of-life after HSCT. Peripheral blood stem cell transplantation (PBSCT) from unrelated donors is a risk for cGVHD compared to bone marrow transplantation (BMT),[1,2] and PBSCT leads a lower GVHD-free, relapse-free survival (GRFS) compared to HSCT from other stem cell sources.[3-6] In Europe, prophylactic use of antithymocyte globulin (ATG) for GVHD is recommended in unrelated HSCT or PBSCT,[7] based on a series of randomized phase III studies.[8-12] In unrelated HSCT, anti-human-T-lymphocyte immunoglobulin (ATLG, equal to ATG-Fresenius) significantly decreased the incidence of severe acute GVHD (aGVHD) and cGVHD without an increase in relapse or non-relapse mortality, resulting in an improvement of GRFS and probability of immunosuppressive-free survival.[8,11] In related PBSCT, ATLG significantly reduced the incidence of cGVHD and improved cGVHD-free, relapse-free survival (CRFS), and showing the better quality-of-life with shorter immunosuppressive treatment.[11,12] However, data are limited specifically in unrelated PBSCT; a recent study showed that unrelated PBSCT with ATG results in better GRFS than BMT without ATG.[13]

Anti-GVHD effect of ATG is mediated by binding to donor T cells, but attenuated by the presence of other types of lymphocytes and host derived T cells. Recent studies reported conflicting results regarding the impacts of absolute lymphocyte count (ALC) before HSCT

on transplant outcomes. ALC before the administration of ATG or conditioning was associated with transplant outcomes in several studies,[14-18] while others failed to show any associations.[19,20]

We conducted a nationwide retrospective study to compare the clinical outcomes in patients with hematological malignancies who underwent unrelated PBSCT with or without rabbit ATG (thymoglobulin) as a GVHD prophylaxis, and an association between ALC before ATG and transplant outcomes.

## Materials and methods

### Patients and study design

We collected data of patients with hematological malignancies who underwent unrelated PBSCT between 2010 and 2017 from the Transplant Registry Unified Management Program (TRUMP) sponsored by the Japanese Society for Hematopoietic Cell Transplantation (JSHCT). and Japanese Data Center for Hematopoietic Cell Transplantation (JDCHCT).[21] Additional detailed data were collected, including refined disease risk index (DRI) at transplantation, ATG dose and schedule, ALC before ATG, grade of cGVHD, day of off-immunosuppressants, posttransplant lymphoproliferative disorder (PTLD), and transplant outcomes. The primary endpoint was cumulative incidence of moderate - severe (M/S) cGVHD at 2 years. The study was performed in accordance with institutional ethical guidelines, including the World Medical Association Declaration of Helsinki, and was

approved by the data management committee of the JSHCT/JDCHCT and the institutional review boards of the Hokkaido University (No. 018-0417).

**Definitions**

Neutrophil engraftment was defined as an absolute neutrophil count > 0.5 × $10^9$/L on the first of 3 consecutive days, and platelet engraftment was defined as an absolute platelet count > 2.0×$10^{10}$/L without transfusion support on the first of 7 preceding days. aGVHD was graded according to the consensus criteria,[22] and cGVHD was graded according to the criteria of the National Institutes of Health (NIH) consensus development project.[23] Hematopoietic cell transplantation-specific comorbidity index (HCT-CI) and DRI were determined according to the scoring systems, as previously described.[24,25] The choice of giving or not ATG was at the discretion of each physician. ALC was calculated on the first day of ATG or the day before ATG. Overall survival (OS) was calculated from the day of PBSCT, with patients alive at the time of last follow-up censored. Progression-free survival (PFS) was calculated from the date of PBSCT until the date of disease recurrence or death from any cause, or last follow-up for patients without these events censored. GRFS was defined as the absence of grade III - IV aGVHD, cGVHD requiring systemic therapy, relapse, or death,[3] and M/S CRFS was defined as the absence of M/S cGVHD, relapse, or death.[15] Non-relapse mortality (NRM) was defined as death due to any cause other than relapse. Relapse and causes of death were determined based on the decision of each clinician.

## Statistical analysis

Statistical analysis was performed using Fisher's exact test for categorical variables, Mann–Whitney *U*-test or Kruskal-Wallis test for continuous variables, Kaplan-Meier method and Log-rank test for OS, PFS, GRFS, and M/S CRFS, Gray's test for engraftment, aGVHD and cGVHD, relapse, NRM and off-immunosuppressants. In multivariate analysis, Fine and Gray competing risk regression model were performed for cGVHD, relapse, and NRM and Cox proportional hazards model were performed for GRFS, M/S CRFS, OS, and PFS with a threshold *P*-value < 0.1 in each univariate analysis using pre-transplant parameters. The cutoff value of recipient and donor age, $CD34^+$ cells, and ALC were determined based on ROC-curve analysis. Results were expressed as hazard ratio (HR) with the 95% confidence interval (95% CI). A value of $P < 0.05$ was used to determine statistical significance, and all analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Japan), which is a graphic user interface for R (The R Foundation for Statistical Computing, Vienna, Austria).[26]

## Results

### Patients and transplant characteristics

A total of 358 patients in 74 institutes who underwent unrelated PBSCT between 2010 and 2017 were identified from the TRUMP database and additional detailed data were

collected from 290 patients (81.0%) in 46 institutes. A total of 287 patients (97 in the ATG group and 190 in the non-ATG group) were finally analyzed in this study after excluding 3 patients because of the types of underlying diseases (Supplemental Fig 1).

The median age was 53 years, ranging from 17 to 71 years. The ATG group had higher rate of human leukocyte antigen (HLA) mismatched transplantation compared to the non-ATG group. In myeloablative conditioning (MAC) regimen, fludarabine + busulfan based regimen was more frequently used in the ATG group, whereas, busulfan + cyclophosphamide was more frequently used in the non-ATG group. The other factors were not different between the groups. The median dose of ATG was 2.0 mg/kg, ranging from 1.0 to 3.0 mg/kg. Details of ATG dose and schedule were shown in Supplemental Table 1. The median ALC before ATG was 0.061 × $10^9$/L, ranging from 0 to 1.30 × $10^9$/L and ALC before conditioning was 0.69 × $10^9$/L, ranging from 0.090 to 2.06 × $10^9$/L (Table 1).

**Engraftment and acute GVHD**

There were no statistical significances in the incidence of both neutrophil and platelet engraftment between the ATG and non-ATG groups (Supplemental Fig 2a, b). The cumulative incidence of grade II - IV and grade III - IV aGVHD at day 100 were 34.2% (95% CI, 24.7 - 43.8%) and 5.5% (95% CI, 2.0 - 11.5%) in the ATG group and 39.4% (95% CI, 32.1 - 46.6%) and 10.1% (95% CI, 6.1 - 15.2%) in the non-ATG group, respectively. There were no statistical significances in the incidence of both grade II - IV and grade III - IV

aGVHD between the groups (Supplemental Fig 2c, d).

**Chronic GVHD**

The primary endpoint, cumulative incidence of M/S cGVHD at 2 years was significantly less in the ATG group than that in the non-ATG group (22.1% [95% CI, 14.3 - 30.9%] vs 36.3% [95% CI, 29.4 - 43.1%], *P* = 0.025, Figure 1a). The cumulative incidences of overall cGVHD at 2-years was also significantly lower in the ATG group than those in the non-ATG group (31.5% [95% CI, 22.4 - 41.0%] vs 47.5% [95% CI, 40.2 - 54.5%], *P* = 0.022, Figure 1b). We evaluated the incidence of cGVHD by organ between 2 groups. In the ATG group, mouth, eye, and multiple (≥ 3) organ involvements were significantly lower compared to the non-ATG group (mouth: 10.6% [95% CI, 5.4 - 17.9%] vs 27.2% [95% CI, 21.0 - 33.7%], *P* = 0.003, eye: 10.7% [95% CI, 5.4 - 17.9%] vs 20.2% [95% CI, 14.8 - 26.3%], *P* = 0.031, multiple organs: 12.8% [95% CI 7.0 - 20.4%] vs 24.0% [95% CI, 18.1 - 30.3%], *P* = 0.042, Figure 1c).

**Relapse, NRM, off-immunosuppressants, causes of death, cytomegalovirus infection, PTLD, and survival**

The cumulative incidences of relapse and NRM at 2 years were equivalent between the groups (Supplemental Fig 3a, b). The ATG group had higher incidence of immunosuppressant discontinuation without relapse at 2 years (46.4% [95% CI, 36.0 -

56.1%] vs. 25.2% [95% CI, 19.2 - 31.8%], *P* < 0.001, Figure 1d). Causes of death were similar between the groups (Supplemental Table 2). The cumulative incidence of cytomegalovirus infection at 2 years were 7.3% in the ATG group and 5.8% in the non ATG group (*P* = 0.65). The cumulative incidence of PTLD at 2 years were 1.0% in ATG group and 0.5% in the non ATG group (*P* = 0.52). Two-year OS was 62.5% (95% CI, 51.9 - 71.5%) in the ATG group and 57.2% (95% CI, 49.8 - 63.9%) in the non-ATG group (*P* = 0.34, Supplemental Fig 3c). Two-year PFS was 52.8% (95% CI, 42.3 - 62.3%) in the ATG group and 52.5% (95% CI, 45.1 - 59.3%) in the non-ATG group (*P* = 0.97, Supplemental Fig 3d). In the ATG group, GRFS and M/S CRFS at 2 year were both superior to those in the non-ATG group (GRFS: 40.2% [95% CI, 30.3 - 49.9%] vs 26.9% [95% CI, 20.7 - 33.4%], *P* = 0.034, Figure 1e, M/S CRFS: 36.0% [95% CI, 26.5 - 45.6%] vs 23.3% [95% CI, 17.5 - 29.6%], *P* = 0.034, Figure 1f).

**Multivariate analysis**

We evaluated the risk factors for transplant outcomes from pre-transplant parameters by univariate analysis (Supplemental Table 3). In multivariate analysis, only ATG administration was identified as a significant favorable risk factor for M/S cGVHD (HR, 0.37; 95% CI, 0.19 - 0.69; *P* = 0.002) and overall cGVHD (HR, 0.64; 95% CI, 0.43 - 0.95; *P* = 0.028). HCT-CI ≥ 2 (HR, 1.49; 95% CI, 1.10 - 2.00; *P* = 0.009) and ATG administration (HR, 0.73; 95% CI, 0.54 - 0.99; *P* = 0.040) for GRFS, and high or very high of DRI (HR, 1.39; 95%

CI, 1.04 - 1.85; *P* = 0.026) and ATG administration (HR, 0.76; 95% CI, 0.56 - 1.00; *P* = 0.0496) for M/S CRFS were identified as significant risk factors (Table 2). High or very high of DRI, HCT-CI ≥ 2 and recipient age ≥ 53 years were identified as risk factors for OS and PFS, and high or very high of DRI and recipient age ≥ 53 years were identified as risk factors for relapse, while HCT-CI ≥ 2 was identified as a risk for NRM (Supplemental Table 4).

**Association of ALC before ATG with transplant outcomes**

We evaluated the association of ALC before ATG and transplant outcomes in the ATG group. There was no significant association between the ATG dose and aGVHD and cGVHD (data not shown). ALC before ATG was significantly higher in patients with grade III - IV aGVHD (median ALC: 0.407 vs. 0.060 × $10^9$/L, *P* = 0.015, Figure 2a) and M/S cGVHD (median ALC: 0.21 vs. 0.050 × $10^9$/L, *P* = 0.002, Figure 2b) compared to those without it. ALC before ATG was significantly lower in patients with relapse compared to those without it (median ALC: 0.024 vs. 0.080 × $10^9$/L, *P* = 0.020, Figure 2c).

Based on these results, ATG group was stratified into 3 groups according to ALC before ATG; low-ALC (< 0.030 × $10^9$/L), intermediate-ALC (0.030 ≤ ALC < 0.154 × $10^9$/L), and high-ALC (0.154 × $10^9$/L ≤) groups. Patients and transplant characteristics were comparable among the groups, except for lower recipient age and higher proportion of MAC in the high ALC group, and lower donor age in the intermediate ALC group (Supplemental Table 5). The cumulative incidence of grade III - IV aGVHD (0 vs. 0 vs. 14.8%, *P* = 0.014, Figure 2d) and

M/S cGVHD (9.5 vs. 10.4 vs. 48.3%, $P < 0.001$, Figure 2e) were significantly higher in the high-ALC group than those in the others, while relapse rate was significantly higher in the low-ALC group than those in the others (44.7 vs. 31.0 vs. 17.2%, $P = 0.026$, Figure 2f). We used ratios of ALC and a total dose of ATG (ALC / ATG) to evaluate the association of the interaction between the ATG dose and ALC with GVHD and relapse according to a recent study,[27] and we confirmed the same results to those of ALC only (Supplemental Fig 4).

We evaluated the risk factors for M/S cGVHD, relapse, GRFS, and M/S CRFS in the ATG group (Supplemental Table 6). Multivariate analysis showed that high-ALC (HR, 5.50; 95% CI, 1.86 – 16.28; $P = 0.002$) for M/S cGVHD, donor age ≥ 43 (HR, 0.44; 95% CI, 0.20 – 0.98; $P = 0.044$) and low-ALC (HR, 2.41; 95% CI, 1.20 – 4.86; $P = 0.014$) for relapse, HCT-CI ≥ 2 (HR, 2.01; 95% CI, 1.16 - 3.48; $P = 0.013$) for GRFS, and MAC (HR, 0.56; 95% CI, 0.34 – 0.95; $P = 0.032$) and intermediate-ALC (HR, 0.53; 95% CI, 0.30 – 0.97; $P = 0.038$) for M/S CRFS were identified as significant risk factors, respectively (Table 3). Moreover, in patients transplanted in remission, the intermediate-ALC group had significantly favorable GRFS and M/S CRFS at 2 years compared to the other groups (Supplemental Fig 5).

## Discussion

Our study showed that low-dose ATG with a median of 2.0 mg/kg effectively inhibits cGVHD and improves GRFS and CRFS in unrelated PBSCT. Excessive dose of ATG is a risk for infection and posttransplant lymphoproliferative disorder, while insufficient dose of

ATG may increase a risk of GVHD.[28] The optimal dose of ATG in unrelated PBSCT remains undefined because of a lack of well-addressed clinical studies. Although doses of thymoglobulin vary ranging from 2.5 mg/kg to 15 mg/kg in previous studies,[10,29-46] lower doses (≤ 3.0mg/kg) of thymoglobulin has been used in recent studies.[39,41-45] We have shown that only 2 mg/kg of ATG given on days -2 and -1 was sufficient to reduce naïve T cells, which are responsible for GVHD induction, at day 28 after PBSCT and efficiently prevented severe aGVHD and cGVHD in HLA-matched PBSCT following MAC.[45,46] European Society for Blood and Marrow Transplantation also recommends low doses of ATG (2.5 - 5 mg/kg) in HLA-matched related donor transplant, but relatively high doses of ATG (4.5 - 6 mg/kg) in HLA-matched unrelated donor transplant.[7] The current study including 49.5% of HLA-mismatch PBSCT showed that 1.0 - 3.0 mg/kg of ATG effectively inhibits both aGVHD and cGVHD in unrelated PBSCT.

Recent studies have reported an association between ALC before transplantation and transplant outcomes. An optimum exposure of ATG was associated with favorable survival in unrelated HSCT and ALC before conditioning was the most significant factor in determining the dose of ATG.[14] A previous study showed that 60 mg/kg of ATLG significantly inhibited cGVHD but impaired OS, particularly in patients with low ALC before ATLG using cyclophosphamide + total body irradiation conditioning regimen.[15] In our study, such an association was not observed. The discrepancy between the studies may be due to the difference in ATG dose or small number of patients in our study; ATG dose was significantly

lower in our study compared to this previous study. Recently, we investigated the association of ALC before ATG with aGVHD in patients who underwent MAC-PBSCT with low-dose ATG (2 mg/kg). ALC before ATG was significantly higher in patients with aGVHD requiring SCs compared to patients without it, and high ALC before ATG (≥ 0.15 × $10^9$/L) suggested to be a risk factor for aGVHD requiring SCs.[49] This study further extended these data by showing the association of ALC before ATG with GVHD and relapse. To our knowledge, our study is the first to demonstrate association of ALC with GVHD and relapse in the setting of unrelated PBSCT using low-dose ATG. As ATG binds to various types of lymphocytes, dose of ATG may be insufficient in high recipient ALC at the time of ATG administration, leading to an increased risk of GVHD. In vice versa, ATG dose may be excessive in low ALC at the time of ATG administration, leading to an increased risk of infection or relapse.[28] Remarkably, outcomes of intermediate ALC before ATG group in complete remission at transplantation were significantly improved with 72.2% GRFS and M/S CRFS at 2 year compared with those of high or low ALC before ATG group, which seems to be more favorable compared to results from previous studies.[3-6,9,15] Based on these results, we propose a strategy to individualize GVHD prophylaxis by modulating ATG doses according to ALC before ATG administration.

Our study has several limitations that should be considered when reviewing the results, including a retrospective design with heterogenous patient characteristics and small sample size in several subgroups. As our result was obtained by low-dose ATG with mainly

tacrolimus, it is unclear whether similar results can be obtained with cyclosporine. Nevertheless, our data indicate the efficacy of low-dose ATG in unrelated PBSCT, and highlight the possibility of individualized modification in the ATG administration according to ALC before ATG. Larger prospective studies should be conducted to confirm our findings.

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## Figure legends

**Figure 1. Impacts of ATG on the incidence of cGVHD and off-immunosuppressants, and the probability of GRFS and M/S CRFS.** The cumulative incidences of M/S cGVHD (a), overall cGVHD (b) in the ATG (*solid lines*) and non-ATG (*dashed lines*) groups. Incidence of cGVHD at 2 year by organs in the ATG (*white bars*) and non-ATG (*black* bars) groups (c). Error bar indicates 95% confidence interval. * $P < 0.05$. The cumulative incidences of off-immunosuppressants (d), and Kaplan-Meier plots of GRFS (e) and M/S CRFS (f) in the ATG (*solid lines*) and non-ATG (*dashed lines*) groups.

**Figure 2. Association of ALC before ATG with aGVHD, cGVHD and relapse.** ALC before ATG in patients with or without grade III - IV aGVHD (a), M/S cGVHD (b), and relapse (c). 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. The cumulative incidence of grade III - IV aGVHD (d), M/S cGVHD (e), and relapse (f) in patients with the low-ALC (*solid lines,* N = 32), intermediate-ALC (*dashed lines,* N = 30), and high-ALC (*dotted lines,* N = 28) groups.

**Table 1 Patient and transplant characteristics**

| | ATG group (N = 97) | Non-ATG group (N = 190) | *P*-value |
|---|---|---|---|
| **Age (median, range)** | 56 (17 - 70) | 52 (17 - 71) | 0.12 |
| **Sex (Male / Female)** | 61 / 36 | 126 / 64 | 0.60 |
| **Disease** | | | 0.44 |
| **AML** | 41 | 86 | |
| **ALL** | 16 | 26 | |
| **MDS** | 19 | 42 | |
| **MPN** | 1 | 9 | |
| **CML** | 3 | 4 | |
| **ML** | 17 | 23 | |
| **Disease risk index** | | | 0.19 |
| **Low** | 8 | 11 | |
| **Intermediate** | 67 | 113 | |
| **High** | 17 | 53 | |
| **Very high** | 5 | 13 | |
| **HCT-CI** | | | 0.57 |
| **0** | 51 | 104 | |
| **1** | 19 | 32 | |
| **2** | 10 | 27 | |
| **3 ≤** | 17 | 27 | |
| **Recipient CMV serostatus** | | | 0.080 |
| **CMV-IgG (+)** | 77 | 165 | |
| **CMV-IgG (-)** | 20 | 22 | |
| **Unknown** | 0 | 3 | |
| **Donor age (median, range)** | 40 (20 - 51) | 39 (19 - 55) | 0.72 |
| **Donor–recipient sex combination** | | | 0.39 |
| **Female to Male** | 12 | 32 | |
| **Others** | 84 | 158 | |
| **HLA (graft-versus-host direction)** | | | < 0.001 |
| **Match** | 49 | 160 | |
| **1-locus mismatch** | 43 | 26 | |
| **2-locus mismatch** | 5 | 4 | |
| **Conditioning** | | | 0.53 |
| **MAC** | 46 | 98 | < 0.001 |
| **CY + TBI based** | 15 | 44 | |
| **Flu + TBI based** | 0 | 2 | |
| **BU + CY** | 12 | 43 | |
| **FLU + BU based** | 18 | 9 | |
| **FLU + MEL + TBI** | 1 | 0 | |
| **RIC** | 51 | 92 | 0.88 |
| **FLU + BU based** | 33 | 53 | |
| **FLU + MEL based** | 15 | 31 | |
| **FLU + BU + MEL** | 2 | 6 | |
| **Others** | 1 | 2 | |
| **GVHD prophylaxis** | | | 0.053 |
| **TAC + MTX** | 95 | 175 | |
| **TAC** | 1 | 2 | |
| **TAC + MMF** | 0 | 1 | |
| **CSP + MTX** | 0 | 10 | |
| **Unknown** | 1 | 2 | |
| **$CD34^+$ cells ($\times 10^6$/kg) (median, range)** | 4.45 (1.69 – 10.91) | 3.80 (1.00 – 13.50) | 0.25 |
| **ALC before ATG ($\times 10^9$/L) (median, range)** | 0.061 (0 – 1.30) | | |
| **ALC before conditioning ($\times 10^9$/L) (median, range)** | 0.69 (0.090 – 2.06) | | |
| **Median follow-up days (median, range)** | 801 (25 – 2263) | 804 (9 – 2681) | 0.34 |

**Abbreviations:** CY, cyclophosphamide; TBI, total body irradiation; BU, busulfan, FLU, fludarabine; MEL, melphalan; RIC, reduced-intensity conditioning; TAC, tacrolimus; MTX, methotrexate; MMF, mycophenolate mofetil; CSP, cyclosporine.

**Table 2 Multivariate analysis in all cohort**

| Variable | Reference | HR (95%CI) | *P*-value |
|---|---|---|---|
| **M/S cGVHD** | | | |
| Recipient age ≥ 53 years | < 53 years | 0.76 (0.41 - 1.38) | 0.36 |
| Recipient sex Male | Female | 1.63 (0.93 - 2.87) | 0.089 |
| Conditioning MAC | RIC | 1.42 (0.76 - 2.66) | 0.28 |
| $CD34^+$ cells ≥ $4.9\times10^6$/kg | < $4.9\times10^6$/kg | 1.56 (0.94 - 2.61) | 0.087 |
| ATG administration | No ATG | 0.37 (0.19 - 0.69) | 0.002 |
| **Overall cGVHD** | | | |
| Recipient age ≥ 53 years | < 53 years | 0.72 (0.51 - 1.02) | 0.068 |
| Donor age ≥ 43 years | < 43 years | 1.41 (0.99 - 2.01) | 0.055 |
| ATG administration | No ATG | 0.64 (0.43 - 0.95) | 0.028 |
| **GRFS** | | | |
| HCT-CI ≥ 2 | 0, 1 | 1.49 (1.10 - 2.00) | 0.009 |
| Recipient CMV-IgG positive | Negative | 1.39 (0.92 - 2.11) | 0.12 |
| ATG administration | No ATG | 0.73 (0.54 - 0.99) | 0.040 |
| **M/S CRFS** | | | |
| DRI High, Very high | Low, Intermediate | 1.39 (1.04 - 1.85) | 0.026 |
| ATG administration | No ATG | 0.76 (0.56 - 1.00) | 0.0496 |

**Abbreviations:** RIC, reduced-intensity conditioning.

**Table 3 Multivariate analysis in the ATG group**

| Variable | Reference | HR (95%CI) | *P*-value |
|---|---|---|---|
| **M/S cGVHD** | | | |
| Donor age ≥ 43 years | < 43 years | 1.87 (0.67 – 5.23) | 0.23 |
| High-ALC group | The others | 5.50 (1.86 – 16.28) | 0.002 |
| **Relapse** | | | |
| Donor age ≥ 43 years | < 43 years | 0.44 (0.20 – 0.98) | 0.044 |
| Low-ALC group | The others | 2.41 (1.20 – 4.86) | 0.014 |
| **GRFS** | | | |
| HCT-CI ≥ 2 | 0, 1 | 2.01 (1.16 – 3.48) | 0.013 |
| Intermediate-ALC group | The others | 0.60 (0.33 – 1.10) | 0.097 |
| **M/S CRFS** | | | |
| Conditioning MAC | RIC | 0.56 (0.34 – 0.95) | 0.032 |
| Intermediate-ALC group | The others | 0.53 (0.30 – 0.97) | 0.038 |

**Abbreviations:** RIC, reduced-intensity conditioning.

**Figure 1.**

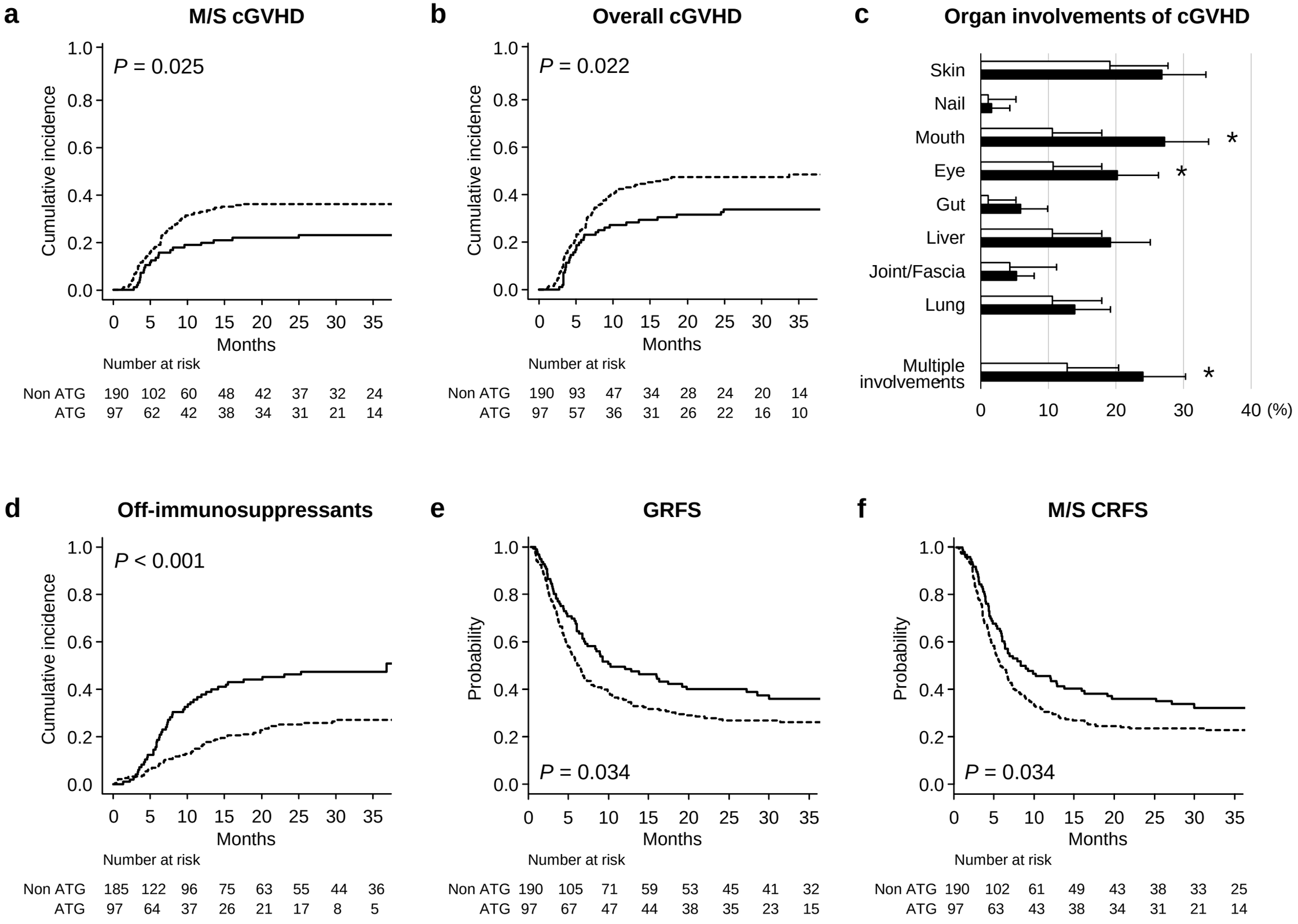
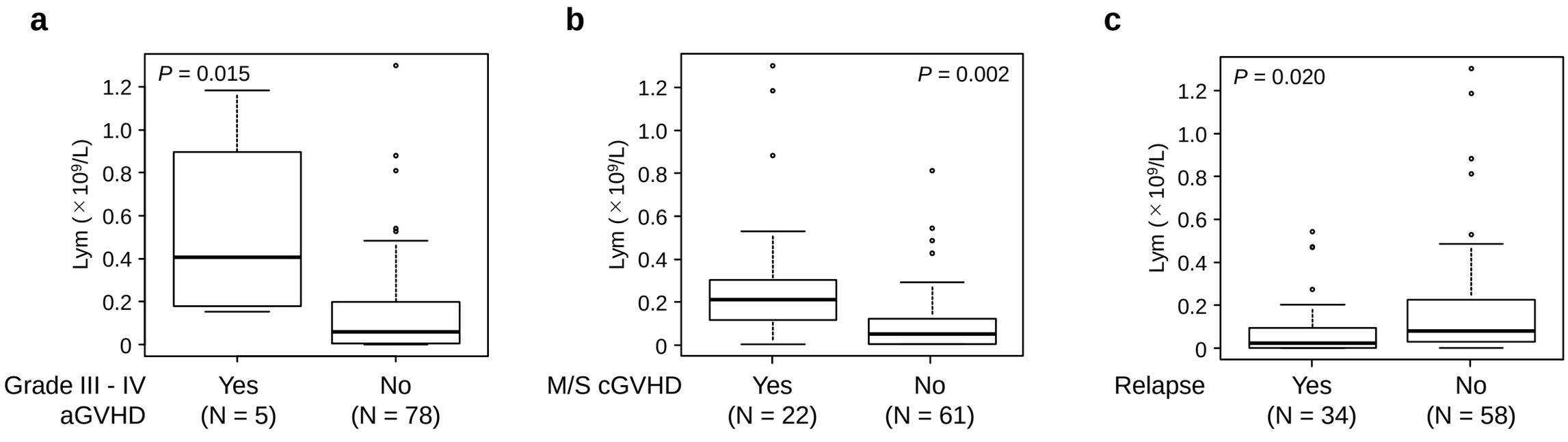

a
M/S cGVHD
P = 0.025
Cumulative incidence
1.0
0.8
0.6
0.4
0.2
0.0
0 5 10 15 20 25 30 35
Months
Number at risk
Non ATG 190 102 60 48 42 37 32 24
ATG 97 62 42 38 34 31 21 14
b
Overall cGVHD
P = 0.022
Cumulative incidence
Months
Number at risk
Non ATG 190 93 47 34 28 24 20 14
ATG 97 57 36 31 26 22 16 10
c
Organ involvements of cGVHD
Skin
Nail
Mouth
Eye
Gut
Liver
Joint/Fascia
Lung
Multiple involvements
*
0 10 20 30 40 (%)
d
Off-immunosuppressants
P < 0.001
Cumulative incidence
Months
Number at risk
Non ATG 185 122 96 75 63 55 44 36
ATG 97 64 37 26 21 17 8 5
e
GRFS
Probability
P = 0.034
Months
Number at risk
Non ATG 190 105 71 59 53 45 41 32
ATG 97 67 47 44 38 35 23 15
f
M/S CRFS
Probability
P = 0.034
Months
Number at risk
Non ATG 190 102 61 49 43 38 33 25
ATG 97 63 43 38 34 31 21 14

**Figure 2.**


a
P = 0.015
Lym (×10⁹/L)
1.2
1.0
0.8
0.6
0.4
0.2
0
Grade III - IV aGVHD
Yes (N = 5)
No (N = 78)
b
P = 0.002
Lym (×10⁹/L)
M/S cGVHD
Yes (N = 22)
No (N = 61)
c
P = 0.020
Lym (×10⁹/L)
Relapse
Yes (N = 34)
No (N = 58)
d
Grade III - IV aGVHD
P = 0.014
Cumulative incidence
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
Days
Number at risk
Low ALC 32 32 31 30 30 30
Int ALC 30 30 30 29 29 28
High ALC 28 28 28 26 24 23
e
M/S cGVHD
P < 0.001
Cumulative incidence
0
5
10
15
20
25
30
35
Months
Number at risk
Low ALC 32 19 12 11 10 10 4 4
Int ALC 30 22 15 15 14 13 11 5
High ALC 28 17 12 9 8 7 6 5
f
Relapse
P = 0.026
Cumulative incidence
Months
Number at risk
Low ALC 32 20 14 13 12 10 4 4
Int ALC 30 23 19 18 17 16 12 6
High ALC 28 23 21 19 18 17 11 9