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1 **Article**

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3 **Title of the paper**

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

6

7 **Running title**

8 Low-dose ATG in unrelated PBSCT

9

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11 **Conflict of interest**

12 SF reports grants and personal fees from CSL Behring, outside the submitted work. MS

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13

1 **Abstract**

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

17

1 **Introduction**

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

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

1 on transplant outcomes. ALC before the administration of ATG or conditioning was
2 associated with transplant outcomes in several studies,¹⁴⁻¹⁸ while others failed to show any
3 associations.^{19,20}

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

8

9 **Materials and methods**

10 **Patients and study design**

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

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

3

4 **Definitions**

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

1

2 **Statistical analysis**

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

16

17 **Results**

18 **Patients and transplant characteristics**

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

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

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

13

14 **Engraftment and acute GVHD**

15 There were no statistical significances in the incidence of both neutrophil and platelet
16 engraftment between the ATG and non-ATG groups (Supplemental Fig 2a, b). The
17 cumulative incidence of grade II - IV and grade III - IV aGVHD at day 100 were 34.2% (95%
18 CI, 24.7 - 43.8%) and 5.5% (95% CI, 2.0 - 11.5%) in the ATG group and 39.4% (95% CI,
19 32.1 - 46.6%) and 10.1% (95% CI, 6.1 - 15.2%) in the non-ATG group, respectively. There
20 were no statistical significances in the incidence of both grade II - IV and grade III - IV

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

2

3 **Chronic GVHD**

4 The primary endpoint, cumulative incidence of M/S cGVHD at 2 years was significantly
5 less in the ATG group than that in the non-ATG group (22.1% [95% CI, 14.3 - 30.9%] vs
6 36.3% [95% CI, 29.4 - 43.1%], $P = 0.025$, Figure 1a). The cumulative incidences of overall
7 cGVHD at 2-years was also significantly lower in the ATG group than those in the non-ATG
8 group (31.5% [95% CI, 22.4 - 41.0%] vs 47.5% [95% CI, 40.2 - 54.5%], $P = 0.022$, Figure 1b).

9 We evaluated the incidence of cGVHD by organ between 2 groups. In the ATG group, mouth,
10 eye, and multiple (≥ 3) organ involvements were significantly lower compared to the
11 non-ATG group (mouth: 10.6% [95% CI, 5.4 - 17.9%] vs 27.2% [95% CI, 21.0 - 33.7%], $P =$
12 0.003, eye: 10.7% [95% CI, 5.4 - 17.9%] vs 20.2% [95% CI, 14.8 - 26.3%], $P = 0.031$,
13 multiple organs: 12.8% [95% CI 7.0 - 20.4%] vs 24.0% [95% CI, 18.1 - 30.3%], $P = 0.042$,
14 Figure 1c).

15

16 **Relapse, NRM, off-immunosuppressants, causes of death, cytomegalovirus infection,** 17 **PTLD, and survival**

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

1 56.1%] vs. 25.2% [95% CI, 19.2 - 31.8%], $P < 0.001$, Figure 1d). Causes of death were
2 similar between the groups (Supplemental Table 2). The cumulative incidence of
3 cytomegalovirus infection at 2 years were 7.3% in the ATG group and 5.8% in the non ATG
4 group ($P = 0.65$). The cumulative incidence of PTLD at 2 years were 1.0% in ATG group and
5 0.5% in the non ATG group ($P = 0.52$). Two-year OS was 62.5% (95% CI, 51.9 - 71.5%) in
6 the ATG group and 57.2% (95% CI, 49.8 - 63.9%) in the non-ATG group ($P = 0.34$,
7 Supplemental Fig 3c). Two-year PFS was 52.8% (95% CI, 42.3 - 62.3%) in the ATG group
8 and 52.5% (95% CI, 45.1 - 59.3%) in the non-ATG group ($P = 0.97$, Supplemental Fig 3d). In
9 the ATG group, GRFS and M/S CRFS at 2 year were both superior to those in the non-ATG
10 group (GRFS: 40.2% [95% CI, 30.3 - 49.9%] vs 26.9% [95% CI, 20.7 - 33.4%], $P = 0.034$,
11 Figure 1e, M/S CRFS: 36.0% [95% CI, 26.5 - 45.6%] vs 23.3% [95% CI, 17.5 - 29.6%], $P =$
12 0.034, Figure 1f).

13

14 **Multivariate analysis**

15 We evaluated the risk factors for transplant outcomes from pre-transplant parameters
16 by univariate analysis (Supplemental Table 3). In multivariate analysis, only ATG
17 administration was identified as a significant favorable risk factor for M/S cGVHD (HR, 0.37;
18 95% CI, 0.19 - 0.69; $P = 0.002$) and overall cGVHD (HR, 0.64; 95% CI, 0.43 - 0.95; $P =$
19 0.028). HCT-CI ≥ 2 (HR, 1.49; 95% CI, 1.10 - 2.00; $P = 0.009$) and ATG administration (HR,
20 0.73; 95% CI, 0.54 - 0.99; $P = 0.040$) for GRFS, and high or very high of DRI (HR, 1.39; 95%

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

6

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

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

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

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

7 We evaluated the risk factors for M/S cGVHD, relapse, GRFS, and M/S CRFS in the
8 ATG group (Supplemental Table 6). Multivariate analysis showed that high-ALC (HR, 5.50;
9 95% CI, 1.86 – 16.28; $P = 0.002$) for M/S cGVHD, donor age ≥ 43 (HR, 0.44; 95% CI, 0.20 –
10 0.98; $P = 0.044$) and low-ALC (HR, 2.41; 95% CI, 1.20 – 4.86; $P = 0.014$) for relapse,
11 HCT-CI ≥ 2 (HR, 2.01; 95% CI, 1.16 - 3.48; $P = 0.013$) for GRFS, and MAC (HR, 0.56; 95%
12 CI, 0.34 – 0.95; $P = 0.032$) and intermediate-ALC (HR, 0.53; 95% CI, 0.30 – 0.97; $P = 0.038$)
13 for M/S CRFS were identified as significant risk factors, respectively (Table 3). Moreover, in
14 patients transplanted in remission, the intermediate-ALC group had significantly favorable
15 GRFS and M/S CRFS at 2 years compared to the other groups (Supplemental Fig 5).

16

17 **Discussion**

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

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

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

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

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

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

5

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2

3 **Figure legends**

4 **Figure 1. Impacts of ATG on the incidence of cGVHD and off-immunosuppressants,**

5 **and the probability of GRFS and M/S CRFS.** The cumulative incidences of M/S cGVHD (a),

6 overall cGVHD (b) in the ATG (*solid lines*) and non-ATG (*dashed lines*) groups. Incidence of

7 cGVHD at 2 year by organs in the ATG (*white bars*) and non-ATG (*black bars*) groups (c).

8 Error bar indicates 95% confidence interval. * $P < 0.05$. The cumulative incidences of

9 off-immunosuppressants (d), and Kaplan-Meier plots of GRFS (e) and M/S CRFS (f) in the

10 ATG (*solid lines*) and non-ATG (*dashed lines*) groups.

11

12 **Figure 2. Association of ALC before ATG with aGVHD, cGVHD and relapse.** ALC before

13 ATG in patients with or without grade III - IV aGVHD (a), M/S cGVHD (b), and relapse (c).

14 The ends of the center box indicate the upper and lower quartile of the data, the line inside

15 the rectangle indicates the median, the whiskers indicate the maximum and minimum values,

16 and the dots outside the rectangle indicate outliers. The cumulative incidence of grade III - IV

17 aGVHD (d), M/S cGVHD (e), and relapse (f) in patients with the low-ALC (*solid lines*, N = 32),

18 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 (×10⁶/kg) (median, range)	4.45 (1.69 – 10.91)	3.80 (1.00 – 13.50)	0.25
ALC before ATG (×10⁹/L) (median, range)	0.061 (0 – 1.30)		
ALC before conditioning (×10⁹/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 ≥ 4.9×10 ⁶ /kg	< 4.9×10 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 \geq 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 \geq 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 \geq 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.

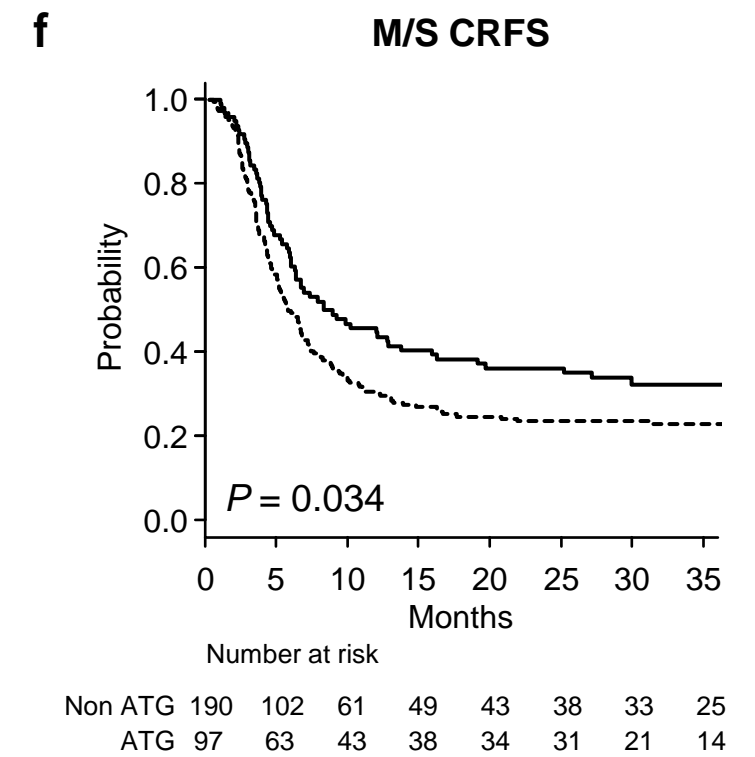
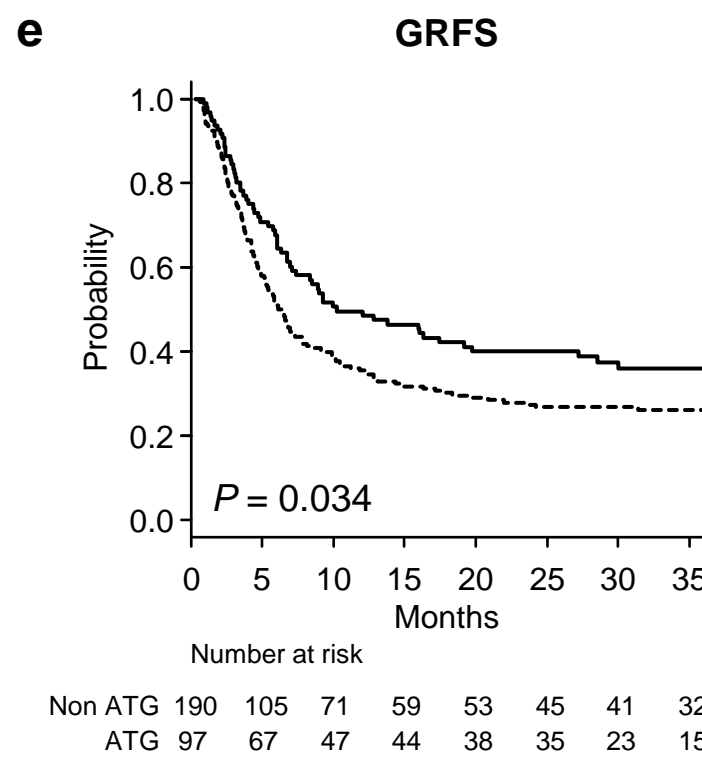
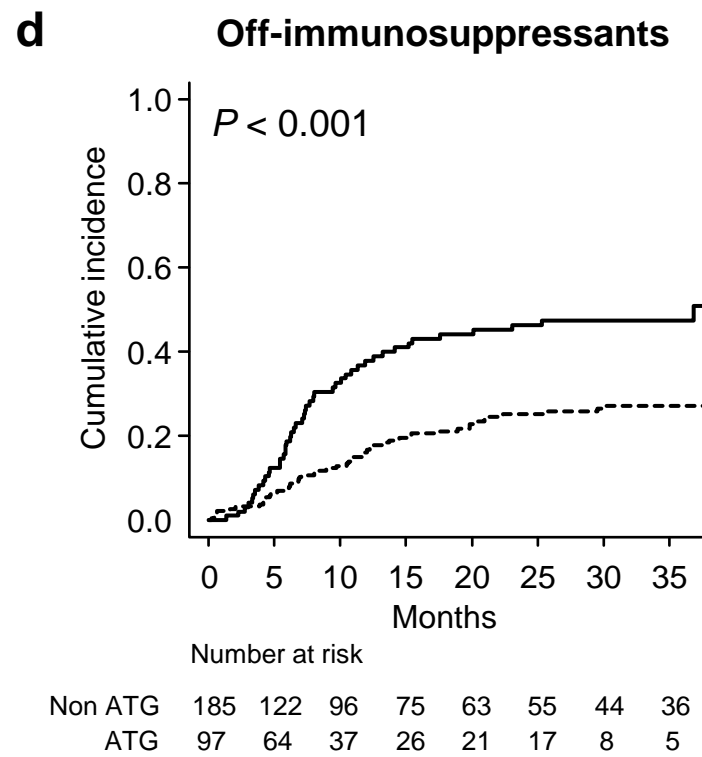
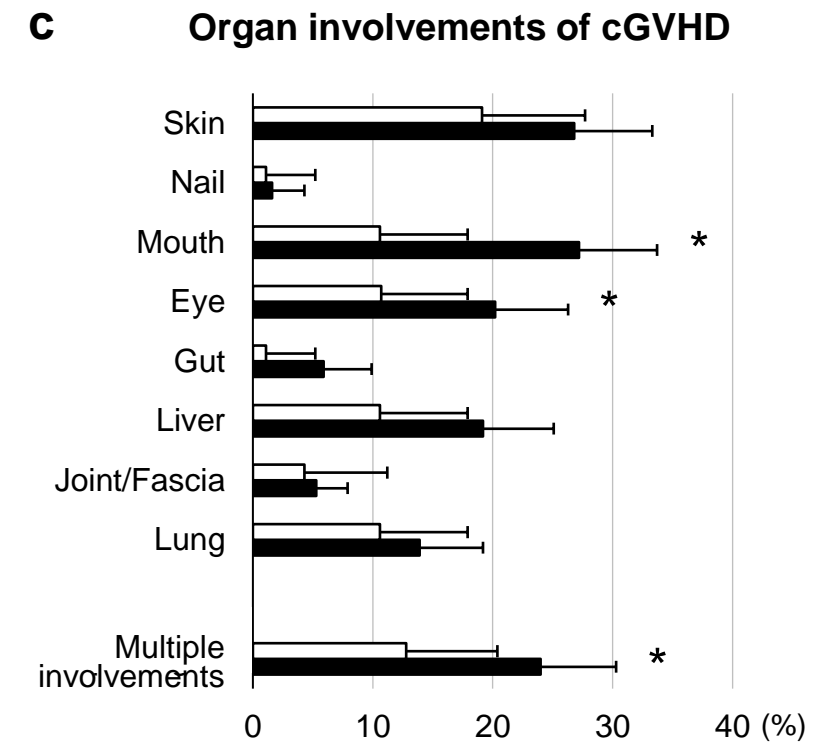
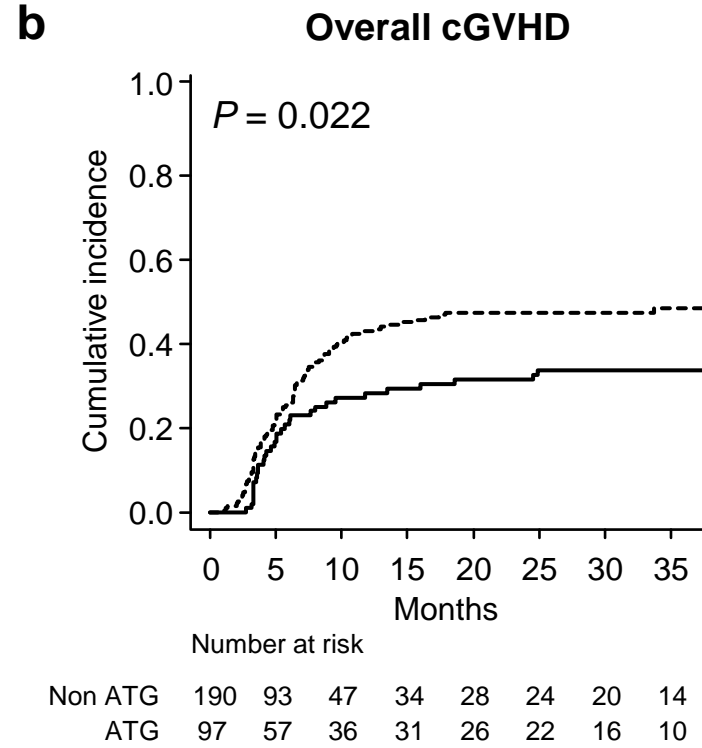
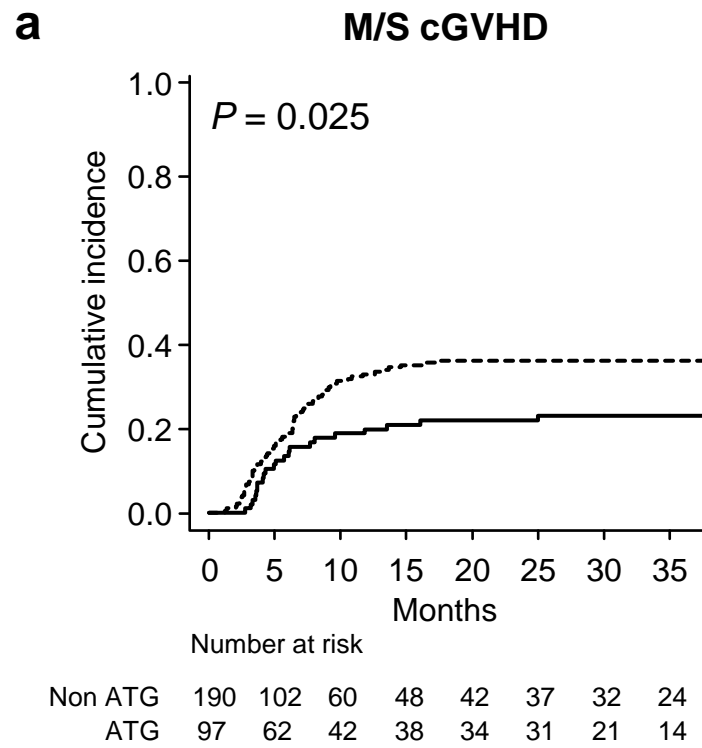


Figure 2.

