recovery was defined as an absolute neutrophil count of $\geq 0.5 \times 10^9$/L for 3 consecutive days. The incidences of grade II–IV or III–IV acute and chronic or extensive chronic GVHD were based on standard criteria [12, 13].

### Statistical analysis

The probability of OS was calculated by the Kaplan–Meier method. The cumulative incidences of engraftment, NRM and GVHD were evaluated using Gray's method. In the competing risk models for engraftment and GVHD, relapse and death before these events were defined as competing risks. In the competing risk models for NRM, relapse was defined as a competing risk. A two-sided $P$ value of $<0.05$ was considered statistically significant. Standard risk was defined as the first complete remission of acute leukemia, the first chronic phase of chronic myeloid leukemia, or non-malignant diseases. High risk was defined as other hematological malignancies. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University), which is a graphical user interface for R (The R Foundation for Statistical Computing, version 2.13.0) [14]. More precisely, it is a modified version of R commander (version 1.6-3) that was designed to add statistical functions that are frequently used in biostatistics.

## Results

### Patients' characteristics

The details of the patients' characteristics are shown in Table 1. The median age was 31 years (range 1–68). The underlying disease was non-malignant and hematologically malignant in 51 and 35 patients, respectively. Among hematological malignancies, 27 patients (84 %) had a high-risk disease. In pediatric patients (age < 18), 31 of 34 patients had non-malignant disease. Among the 85 patients for whom data on HLA typing were available, 41 received bone marrow from a donor with an HLA mismatch (one antigen mismatch $n = 13$, one allele mismatch $n = 24$, 2–3 allele mismatch $n = 4$). As GVHD prophylaxis, tacrolimus was used in 67 patients (78 %). Most patients received a reduced-intensity conditioning regimen (RIC $n = 33$) or a non-myeloablative conditioning regimen (NMA $n = 46$) [15, 16]. NMA was only used in patients with a non-malignant disease.

Regarding the total dose of ATG-F, 10 and 20 mg/kg were used in 35 and 20 patients, respectively. ATG-F was administered on 2 and 4 days in 28 patients and 44 patients, respectively.

**Table 1** Patients' characteristics

| | *N* (%) |
|---|---|
| No. of patients | 86 |
| Age, median (range), year | 31 (1–68) |
| Pediatrics | 34 (40) |
| Adults | 52 (60) |
| Sex (Male/Female) | 54/32 |
| Diagnosis | |
| SAA | 45 (52.5) |
| AML/MDS | 21 (24.5) |
| NHL | 9 (10) |
| MM | 1 (1) |
| CML/MPD | 4 (5) |
| Primary immunodeficiency | 3 (3.5) |
| Inherited metabolic diseases | 3 (3.5) |
| HLA mismatch | |
| None | 44 (51) |
| HLA antigen mismatch | 13 (15) |
| B | 3 |
| DR | 10 |
| HLA allele mismatch | 28 (29)[a] |
| A | 7 |
| B | 7 |
| DRB1 | 15 |
| Unknown | 1 |
| GVHD prophylaxis | |
| CSP ± MTX | 17 (20) |
| TAC ± MTX | 59 (69) |
| TAC + MTX + PSL | 8 (9) |
| Others | 2 (2) |
| Conditioning | |
| Myeloablative | |
| MEL + TBI | 3 (3) |
| BU + CY | 2 (2) |
| CY + TBI | 2 (2) |
| Reduced-intensity | |
| Flu + BU-based | 28 (33) |
| Flu + MEL-based | 5 (6) |
| Non-myeloablative | |
| Flu + CY-based | 25 (29) |
| CY + TBI/TLI | 20 (23) |
| CY alone | 1 (1) |

*SAA* severe aplastic anemia, *AML* acute myeloid leukemia, *MDS* myelodysplastic syndrome, *NHL* non-Hodgkin's lymphoma, *MM* multiple myeloma, *CML* chronic myeloid leukemia, *MPD* myeloproliferative disorder, *CSP* cyclosporin, *MTX* methotrexate, *TAC* tacrolimus, *PSL* prednisolone, *Flu* fludarabine, *BU* busulfan, *CY* cyclophosphamide, *TBI* total body irradiation, *TLI* total lymphoid irradiation, *MEL* melphalan

[a] Four patients had a 2–3 allele mismatch

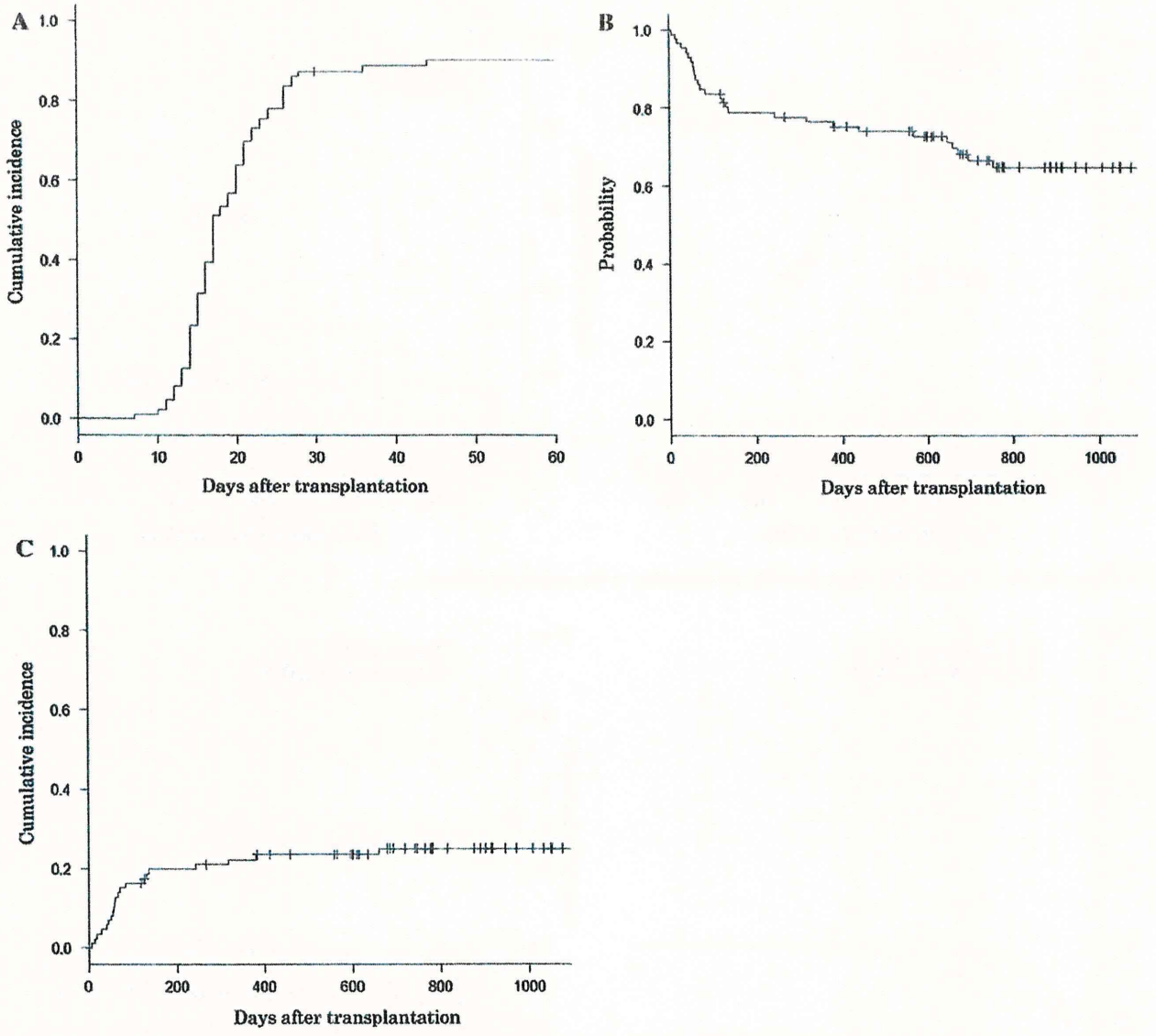


**Fig. 1** Engraftment (**a**), overall survival (**b**) and non-relapse mortality (**c**)

## Clinical outcomes

The median follow-up of surviving patients was 888 days after uBMT (range 118–2122 days). The cumulative incidence of neutrophil engraftment was 90 % (Fig. 1a). The probability of 2-year OS was 67 % (Fig. 1b). The cumulative incidence of 2-year NRM was 25 % (Fig. 1c).

Patients with a non-malignant disease had a significantly better 2-year OS than those with a malignant disease (81 vs. 44 %, $P = 0.0004$; Fig. 2a), but there was no significant difference in the incidence of NRM between the 2 groups (19 vs. 35 %, $P = 0.16$, Fig. 2b). UBMT from an HLA antigen-mismatched donor had a significantly inferior OS compared to uBMT from an HLA-matched donor (37 vs. 69 %, $P = 0.008$), but there was no significant difference between uBMT from an HLA allele-mismatched donor and that from an HLA-matched donor (78 vs. 69 %, $P = 0.50$). Patients who received an uBMT from an HLA antigen-mismatched donor tended to have a higher incidence of NRM than those who received uBMT from an HLA-matched donor (46 vs. 21 %, $P = 0.051$). However, there was no significant difference between uBMT from an HLA allele-mismatched donor and that from an HLA-matched donor (22 vs. 21 %, $P = 0.91$).

## GVHD and post-transplant lymphoproliferative disease (PTLD)

The cumulative incidences of grade II–IV and grade III–IV acute GVHD were 20 and 8 %, respectively (Fig. 3a).

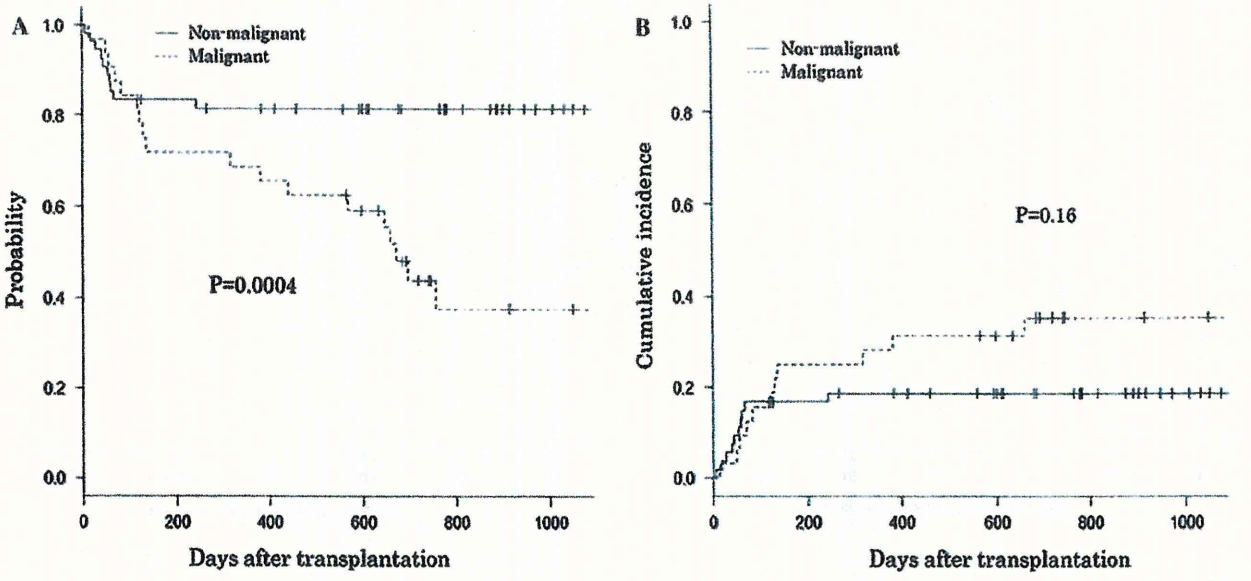


**Fig. 2** Overall survival (**a**) and non-relapse mortality (**b**) according to the underlying disease

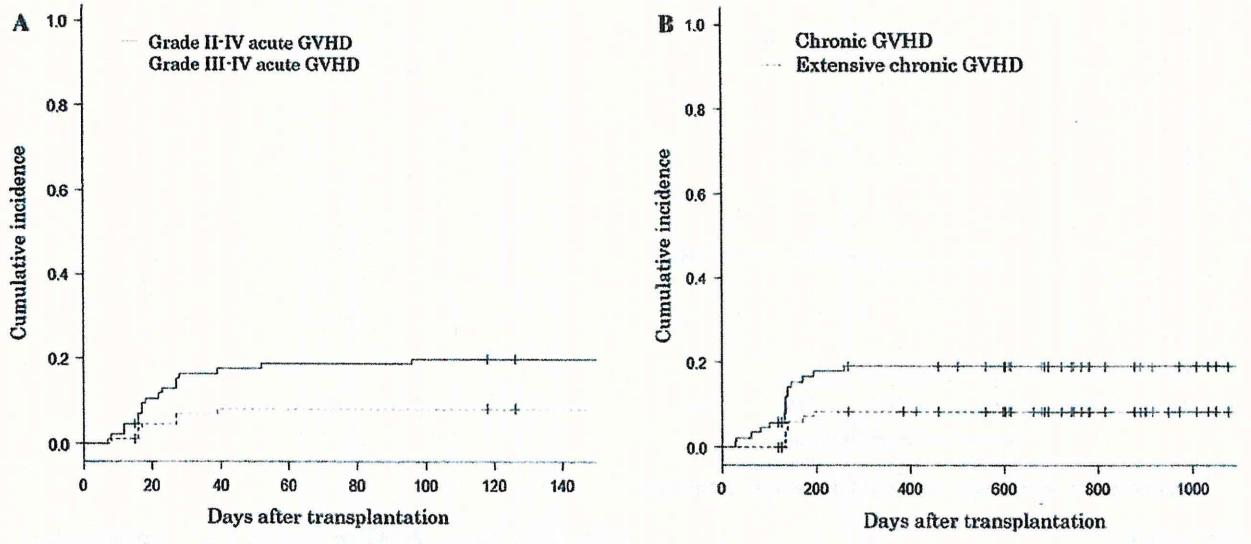


**Fig. 3** Grade II–IV and grade III–IV acute GVHD (**a**) and chronic and extensive chronic GVHD (**b**)

The cumulative incidences of chronic GVHD and extensive chronic GVHD were 19 and 8 %, respectively (Fig. 3b). There was no significant difference in the incidence of acute GVHD between patients with a non-malignant disease and those with a malignant disease (19 vs. 22 %, $P = 0.79$). In terms of the incidences of grade II–IV and grade III–IV acute GVHD, there was no significant difference regardless of the presence of an HLA mismatch (grade II–IV 31, 18, 16 % and grade III–IV 23, 4, 7 % in patients with an HLA antigen-mismatched donor, HLA allele-mismatched donor, and HLA-matched donor, respectively). There were no reported cases of PTLD.

## Dose of ATG-F

For a comparison of the effect of the ATG-F dose, we included only adult patients (≥18 years old). The median dose of ATG-F was 10 mg/kg. We divided patients into 2 groups: those who received more than 10 mg/kg of ATG-F ($n = 21$, high ATG group) and those who received 10 mg/kg or less of ATG-F ($n = 31$, low ATG group). The characteristics of the 2 groups are shown in Supplementary Table 1. The high-ATG group included significantly more patients with a non-malignant disease (76 vs. 23 %, $P < 0.001$) and more younger patients compared to the

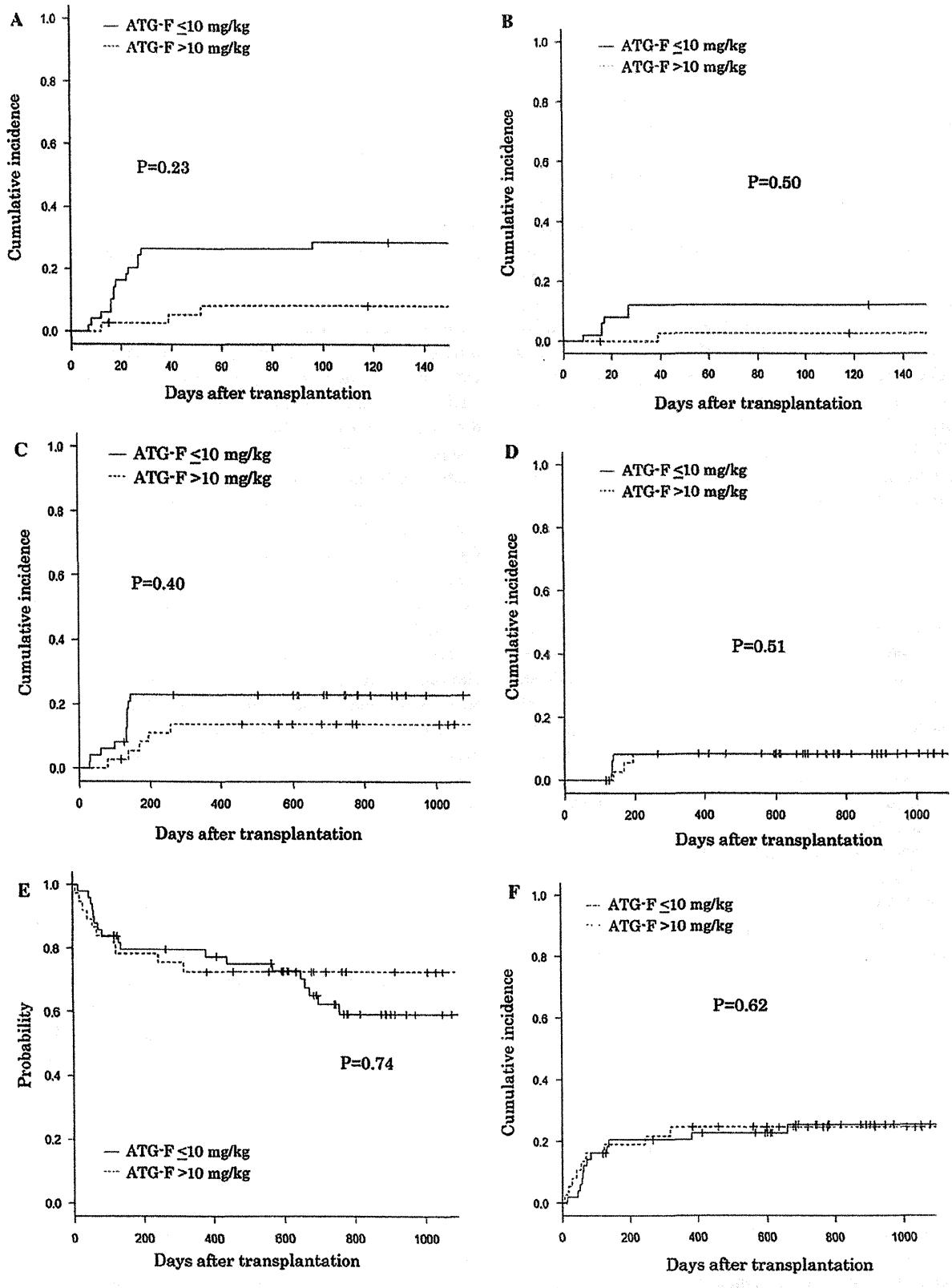


**Fig. 4** Grade II–IV acute GVHD (**a**), grade III–IV acute GVHD (**b**), chronic GVHD (**c**), extensive chronic GVHD (**d**), overall survival (**e**) and non-relapse mortality (**f**) according to the dose of ATG-F in adult patients

low-ATG group. The high-ATG group included more patients with an HLA mismatch, but this difference was not statistically significant. The cumulative incidences of grade II–IV acute GVHD in the low- and high-ATG groups were 23 and 10 %, respectively ($P = 0.23$; Fig. 4a). The incidence of grade II–IV acute GVHD in the low-ATG group tended to be higher than that in the high-ATG group, but this difference was not statistically significant. The cumulative incidences of grade III–IV acute GVHD in the low- and high-ATG groups were 10 and 5 %, respectively ($P = 0.50$; Fig. 4b). The cumulative incidences of chronic GVHD in the low- and high-ATG groups were 19 and 10 %, respectively ($P = 0.40$; Fig. 4c). The cumulative incidences of extensive chronic GVHD in the low- and high-ATG groups were 10 and 5 %, respectively ($P = 0.51$; Fig. 4d). The probabilities of 2-year OS in the low- and high-ATG groups were 39 and 57 %, respectively ($P = 0.74$; Fig. 4e). The cumulative incidences of 2-year NRM in the low- and high-ATG groups were 34 and 38 %, respectively ($P = 0.62$; Fig. 4f).

## Discussion

We determined the clinical outcomes of Japanese patients who received ATG-F as GVHD prophylaxis for an uBMT. We found low incidences of both acute and chronic GVHD with the use of low-dose ATG-F, considering that all patients received BMT from an unrelated donor and about half received BMT from a donor with an HLA mismatch.

A previous large Japanese retrospective study reported that the incidences of grade II–IV and grade III–IV acute GVHD in patients who received uBMT from an HLA-matched donor were 34.5 and 11.8 %, respectively [17]. In addition, that study reported that the incidence of grade III–IV acute GVHD in patients who received an unrelated BMT from an HLA one allele-mismatched donor was 16.1–27.8 %, depending on the locus of mismatch [17]. In that study, only 176 of 1282 patients (14 %) received ATG. Therefore, it seems that the incidence of acute GVHD in patients who received low-dose ATG-F (grade II–IV 20 %, grade III–IV 8 %) was lower than that for all of the registered patients in Japan.

Our study also showed low incidences of chronic GVHD and extensive chronic GVHD (19 and 8 %, respectively). A previous report that focused on chronic GVHD showed that the incidences of chronic GVHD and extensive chronic GVHD after uBMT in Japan were 45.8 and 28.2 %, respectively [18]. In that study, only 203 of 2937 patients (7 %) received ATG [18]. In patients with an HLA-mismatched donor, the incidence of chronic GVHD was significantly higher than that in patients with an HLA-matched donor in previous studies [12, 13]. Compared to a previous report from Japan, the incidence of chronic GVHD in patients with ATG-F in our study seems to be promising, and the reduction of chronic GVHD was consistent with previous reports [4, 18].

In Western countries, the total dose of ATG-F for GVHD prophylaxis is usually 30–60 mg/kg [3, 4]. In Asian countries, as shown in the current study, a smaller dose of ATG is commonly used, since the incidence of GVHD itself is lower in Asian patients than in Caucasian patients [6, 7]. Kim et al. [8] reported that the use of low-dose ATG (Thymoglobulin 2.5 mg/kg) was associated with a low incidence of acute GVHD in patients who received an HLA-mismatched unrelated HSCT. In our study with a low dose of ATG-F (median 10 mg/kg), the incidences of both acute and chronic GVHD were relatively low. In a subset analysis, the use of a lower dose of ATG-F (≤10 mg/kg) did not significantly increase the incidence of GVHD compared to ATG-F at a higher dose, albeit the size of the study was limited. A reduction of chronic GVHD should lead to not only a reduction of morbidity and mortality associated with chronic GVHD but also an improvement of QOL which is an important clinical outcome in long-term survivors [4, 19]. Such low dosages of ATG-F should be good candidates for testing in a prospective clinical trial.

Another concern with the use of ATG is a possible increase in infectious diseases [20]. The CIBMTR study showed that the incidence of EBV-related PTLD in patients with ATG was significantly higher than that in those without T cell depletion (2 vs. 0.1 %, $P = 0.005$) [20]. Although the number of patients was limited, there were no cases of PTLD in this study, which suggested that the immunosuppression with low-dose ATG-F was not very intense. However, the incidence of PTLD should be confirmed in a larger study.

This study has several limitations. Even though we included all patients in the registry who received ATG-F as GVHD prophylaxis for uBMT, the number of patients was still quite small. This must be because of the use of ATG-F as GVHD prophylaxis was not covered by insurance in Japan in the era of this study. Furthermore, the patients had heterogeneous characteristics. Especially, the underlying disease and the conditioning regimen varied significantly. As recently reported by Soiffer et al. [20], the benefit of ATG could differ depending on the intensity of the conditioning regimen. Therefore, based on the results of the current study, the impact of low-dose ATG-F with a uniform conditioning regimen and GVHD prophylaxis should be assessed.

In conclusion, the use of low-dose ATG-F in Japanese patients who underwent an uBMT was associated with promisingly low incidences of both acute and chronic GVHD, with a low incidence of late NRM. The role of low-dose ATG-F as prophylaxis for GVHD should be further assessed in a prospective clinical trial.

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**Conflict of interest** None.

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ORIGINAL ARTICLE

# Allogeneic hematopoietic stem cell transplantation for acute myeloid leukemia with t(6;9)(p23;q34) dramatically improves the patient prognosis: a matched-pair analysis

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**Acute myeloid leukemia (AML) with t(6;9)(p23;q34) is well known to have a poor prognosis treated with chemotherapy and autotransplantation. The presence of this karyotype is an indicator for allogeneic hematopoietic stem cell transplantation (HSCT); however, the impact of t(6;9)(p23;q34) on the HSCT outcome remains unclear. We conducted a matched-pair analysis of *de novo* AML patients with and without t(6;9)(p23;q34) using data obtained from the Japanese HSCT data registry. A total of 57 patients with t(6;9)(p23;q34) received transplants between 1996 and 2007, and 171 of 2056 normal karyotype patients matched for age, disease status at HSCT and graft source were selected. The overall survival, disease-free survival, cumulative incidence of relapse and the non-relapse mortality in t(6;9)(p23;q34) patients were comparable to those for normal karyotype patients. A univariate analysis showed that t(6;9)(p23;q34) had no significant impact on the overall survival. These findings suggest that allogeneic HSCT may overcome the unfavorable impact of t(6;9)(p23;q34) as an independent prognostic factor.**
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**Keywords:** allogeneic hematopoietic stem cell transplantation; acute myeloid leukemia; unfavorable cytogenetic risk; t(6;9)(p23;q34)

## Introduction

Acute myeloid leukemia (AML) is a hematological malignancy resulting from the proliferation of leukemic stem cells. Because of the resistance of leukemic stem cells to chemotherapy,[1] long-term survival is generally seen in only 50% of patients treated with chemotherapy alone. Therefore, allogeneic stem cell transplantation (HSCT) is often considered as a curative treatment option.[2] AML is the most common indication for HSCT in North America and in Japan, but fatal transplant-related adverse events are difficult to avoid, despite the improvements in supportive treatment in recent years. Therefore, treatment of AML is hard to standardize, and the attending physician must make a decision on a case-by-case basis, weighing the advantages and disadvantages of HSCT.

The results of previous large clinical trials have indicated that abnormalities of the chromosomal karyotype are considered to be one of the most powerful factors to predict the patient prognosis.[3,4] AML with the unfavorable cytogenetic risk group, such as a partial deletion of the long arm of chromosome 7 (del (7q)), monosomy of chromosome 7 (−7) or with a complex karyotype is considered to be a good indication for HSCT, even during the first remission, because of the high cytogenetic risk associated with chemotherapy and the beneficial outcome that can be achieved by HSCT.[5–8]

The translocation of chromosome (6;9)(p23;q34) forming the *DEK/NUP214* fusion mRNA is observed in ~1% of AML cases.[9] The characteristics of AML with t(6;9)(p23;q34) are known to include development at a younger age,[10] resistance to chemotherapy and a very poor prognosis.[9] Therefore, the presence of this karyotype in AML patients is an indication for HSCT; however, the impact of t(6;9)(p23;q34) on the outcome of HSCT remains unclear because of the rarity of this entity. We conducted a retrospective study to examine the outcomes of HSCT in AML patients with t(6;9)(p23;q34) using the data from the Japan Society for Hematopoietic Cell Transplantation Data Registry.

## Materials and methods

### *Study population*

Clinical data were collected from the databases of the Japan Society for Hematopoietic Cell Transplantation and the Japan Cord Blood Bank Network using a standardized report form. Follow-up reports were submitted at 100 days, 1 year and annually after HSCT. Patients with *de novo* AML aged 15 years or older at the time of first HSCT and who received the transplant between January 1996 and December 2007 were extracted from the databases. We compared the clinical features and the outcomes among the patients with t(6;9)(p23;q34) and the patients with a normal karyotype in G-band staining. Cytogenetic data were analyzed according to the Southwestern Oncology Group criteria in each institution[7] instead of by central review. We selected patient pairs with t(6;9)(p23;q34)

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and the normal karyotype using an optimal matching method with the following three matching factors: recipient age, disease status at HSCT and graft source. This study was approved by the Committee for Nationwide Survey Data Management of the Japan Society for Hematopoietic Cell Transplantation. Written informed consent was obtained in accordance with the Declaration of Helsinki.

### *Statistical analysis*

The overall survival (OS) was defined as the number of days from HSCT until death from any cause. Disease relapse was defined as the number of days from HSCT to relapse of the underlying disease. Non-relapse mortality was defined as death without relapse. Any patient who was alive at the last-follow-up date was censored. All statistical analyses were performed using the R version 2.13.0 software program (R Foundation for Statistical Computing; http://www.r-project.org). Probabilities and times-to-events were compared between the two groups using the Mantel–Haenszel method and stratified Cox's proportional hazard modeling, respectively. The cumulative incidences of non-relapse mortality and relapse were calculated considering each other event as a competing risk, and were compared using the stratified Grey test.[11] *P* values were two sided, and outcomes were considered to be significant when $P \leqslant 0.05$.

## Results

### *Patients' characteristics*

A total of 2577 AML cases met the inclusion criteria. The number of cases with t(6;9)(p23;q34) and a normal karyotype was 57 and 2056, respectively; and 171 patients with the normal karyotype were selected for matched-pair analysis by a 1:3 matching ratio. The characteristics of the patients are shown in Table 1; there were no statistically significant differences between the t(6;9)(p23;q34) patients and the normal karyotype patients except the use of total body irradiation as a preconditioning regimen.

### *Survival, relapse and non-relapse mortality*

The probability of OS in the patients with t(6;9)(p23;q34) was as good as that for patients with a normal karyotype (the probability of 5-year OS in t(6;9)(p23;q34) and normal karyotype patients was 45 and 40%, respectively; Figure 1a). When the t(6;9)(p23;q34) patients and the normal karyotype patients were further categorized according to the disease status at HSCT, the OS of the t(6;9)(p23;q34) patients and the normal karyotype patients were comparable in both the complete remission (CR) at HSCT patients and the non-CR at HSCT patients (Figure 1b). The probability of disease-free survival in these patients was also not significantly different (the probability of 5-year disease-free survival in patients with t(6;9)(p23;q34) and the normal karyotype was 42 and 33%, respectively; Figure 1c). The cumulative incidence of relapse (Figure 2a) and the non-relapse mortality (Figure 2b) in t(6;9)(p23;q34) patients were also comparable to those for normal karyotype patients (the 5-year cumulative incidence was 42% in t(6;9)(p23;q34) patients and 45% in normal karyotype patients for relapse ($P=0.34$) and 16 and 22% ($P=0.85$) for non-relapse mortality). The prognostic factors affecting OS revealed that there were no significant differences related to karyotype, gender, gender mismatch between donor and recipient, human leukocyte antigen disparity, recipient cytomegalovirus serostatus and use of total body irradiation for the preconditioning regimen by the univariate analyses (Table 2).

**Table 1** Patient characteristics

| | *t(6;9)(p23;q34)* | *Normal karyotype* | P-*value* |
|---|---|---|---|
| *Age* | | | |
| 15–24 | 14 | 42 | 0.999 |
| 25–34 | 14 | 45 | |
| 35–44 | 20 | 58 | |
| 45–54 | 7 | 20 | |
| 55–64 | 2 | 6 | |
| *Gender* | | | |
| Male | 34 | 97 | 0.758 |
| Female | 23 | 74 | |
| *Disease status at HSCT* | | | |
| CR1 or CR2 | 29 | 87 | 1.0 |
| Not in remission | 28 | 84 | |
| *Preconditioning regimen, TBI* | | | |
| No | 21 | 33 | 0.0102 |
| Yes | 33 | 131 | |
| *Donor* | | | |
| Related | 26 | 78 | 1.0 |
| Unrelated bone marrow | 18 | 54 | |
| Unrelated cord blood | 13 | 39 | |
| *Number of HLA mismatch* | | | |
| 0 | 24 | 47 | 0.379 |
| 1 | 5 | 23 | |
| 2 | 10 | 27 | |
| 3 | 0 | 2 | |

Abbreviations: CR, complete remission; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; TBI, total body irradiation.

## Discussion

Previous reports have confirmed the negative impact of t(6;9)(p23;q34) on the outcome after standard-dose chemotherapy and high-dose therapy with autologous stem cell transplantation in patients with AML.[9,10] The current matched-pair analysis of the nationwide survey demonstrated that the OS and the non-relapse mortality, as well as the relapse rate, were independent of the presence of t(6;9)(p23;q34) in allogeneic HSCT recipients, thus suggesting that allogeneic HSCT may be able to overcome the unfavorable effect of t(6;9)(p23;q34) in AML patients.

However, it is difficult to draw any firm conclusions regarding the results of the present analysis owing to the small number of patients in the matched-pairs subsets. These findings require confirmation in larger studies specifically in examining the impact of t(6;9)(p23;q34) status. Nevertheless, the suggestion that allogeneic HSCT appears to overcome the adverse survival impact of t(6;9)(p23;q34) is supported by other studies.[12,13] In a EBMT study of AML patients with t(6;9)(p23;q34), allogeneic HSCT produced responses that were independent of t(6;9)(p23;q34), and the 3-year OS of patients with t(6;9)(p23;q34) was as high as 51 ± 7%, comparable to AML patients with the normal karyotype.[13] Also, the incidence of relapse following allogeneic HSCT appeared to be similar in patients with t(6;9)(p23;q34) compared with those without

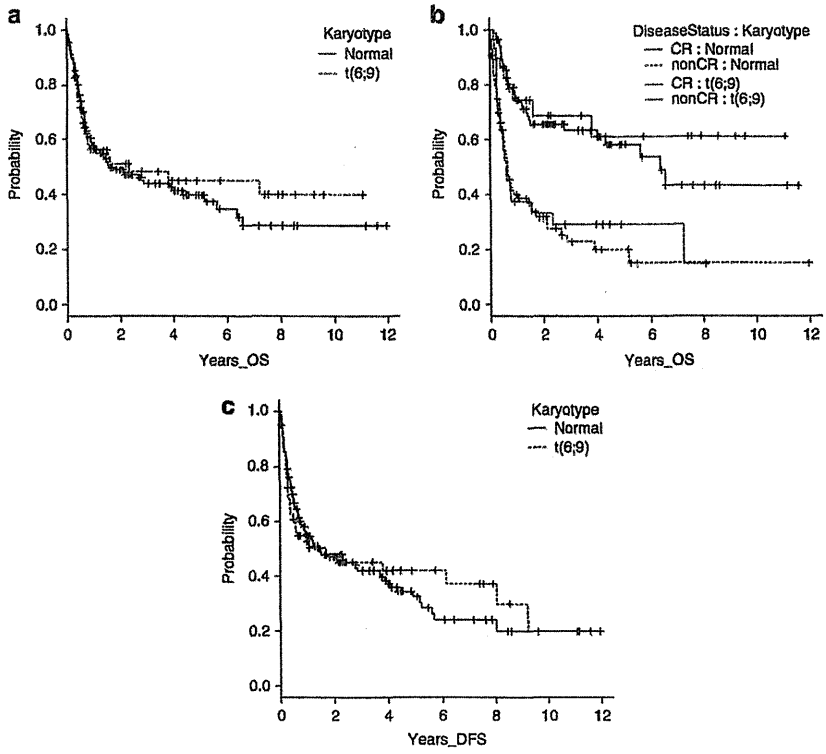


**Figure 1** Survival of the patients. (**a**) The OS of the patients stratified by cytogenetics. Solid line, normal karyotype patients; dotted line, t(6;9) patients. (**b**) The OS of the patients grouped according to their disease status at transplantation. Solid line, normal karyotype patients in CR at HSCT; dashed line, normal karyotype patients in non-CR at HSCT; dotted line, t(6;9) patients in CR at HSCT; chain line, t(6;9) patients in non-CR at HSCT. (**c**) The disease-free survival of the patients. Solid line, normal karyotype patients; dotted line, t(6;9) patients.

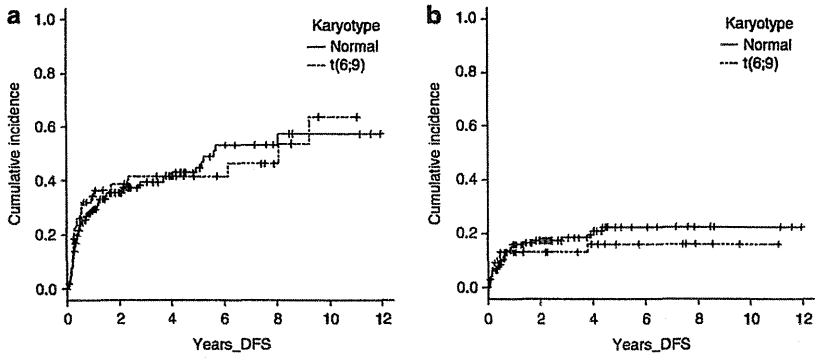


**Figure 2** Cumulative incidence of events after transplantation stratified by cytogenetics. (**a**) The cumulative incidence of relapse of the patients. (**b**) The cumulative incidence of non-relapse mortality of the patients. Solid line, normal karyotype patients; dotted line, t(6;9) patients.

**Table 2** Prognostic factors affecting overall survival

| | *Risk factor* | *Hazard ratio* | *95% CI* | *P-value* |
|---|---|---|---|---|
| Karyotype | t(6;9) | 1.07 | 0.66–1.74 | 0.79 |
| Gender | Male | 1.06 | 0.64–1.73 | 0.83 |
| Gender mismatch | Female to male | 1.41 | 0.74–2.68 | 0.29 |
| HLA compatibility | Mismatch | 0.98 | 0.57–1.75 | 0.94 |
| Recipient CMV | Positive | 0.27 | 0.028–2.70 | 0.27 |
| Donor CMV | Positive | 1.51 | 0.61–3.78 | 0.37 |
| TBI | Yes | 1.47 | 0.75–2.90 | 0.26 |

Abbreviations: CMV, cytomegalovirus; HLA, human leukocyte antigen; TBI, total body irradiation.

t(6;9)(p23;q34). However, the EBMT study made it somewhat difficult to determine whether HSCT would lead to a good outcome, because 87% of the patients were transplanted while in CR, whereas only 29 of 57 (51%) patients in our study received HSCT in CR, which is a more clinically relevant expectation, as a CR is difficult to achieve in these patients.

In conclusion, the current study showed that AML patients with t(6;9)(p23;q34) can be expected to have a post-transplant survival comparable to patients with a normal karyotype, thereby supporting the opinion that they are good candidates for HSCT.

## Conflict of interest

The authors declare no conflict of interest.

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