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Presumptive treatment strategy for aspergillosis in allogeneic haematopoietic stem cell transplant recipients

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***Background*: The onset of invasive aspergillosis (IA) after allogeneic haematopoietic stem cell transplantation (HSCT) is bimodal. However, IA early after HSCT has become less frequent due to the shortened neutropenic period, and the clinical significance of empirical treatment for aspergillosis based on persistent febrile neutropenia (FN) became less clear. Therefore, we started a presumptive treatment strategy, in which anti-*Aspergillus* agents were started when patients developed positive serum test and/or infiltrates or nodules on X-ray or CT-scan associated with persistent FN, in 2002.**

***Methods*: We retrospectively reviewed the records of 114 adult patients who underwent allogeneic HSCT between September 2002 and December 2005 in high-efficiency particulate air-filtered clean rooms. Fluconazole was given as anti-*Candida* prophylaxis. The primary endpoint was the development of early IA, which was defined as probable or proven IA according to the EORTC/MSG criteria that developed between the day of HSCT and 7 days after engraftment.**

***Results*: Among 73 patients who experienced persistent FN for 7 days or longer, anti-*Aspergillus* agents were empirically started in 13 patients at the discretion of attending physicians, whereas 60 patients actually followed presumptive treatment strategy. Only 4 of 60 patients received anti-*Aspergillus* agents. Two patients in the presumptive group developed early IA, but were successfully treated with anti-*Aspergillus* agents started after the diagnosis of IA.**

***Conclusions*: These findings suggested the feasibility of a presumptive treatment strategy for aspergillosis in HSCT recipients. A randomized controlled trial is warranted to compare empirical and presumptive anti-*Aspergillus* strategy in allogeneic HSCT recipients.**

Keywords: empirical treatment, febrile neutropenia, invasive aspergillosis

Introduction

Invasive fungal infection (IFI) is one of the leading causes of transplant-related mortality and its incidence in allogeneic haematopoietic stem cell transplantation (HSCT) recipients ranges from 8 to 15%.[1–3] Invasive aspergillosis (IA) is the most common IFI after allogeneic HSCT.[1–4] The development of IA after allogeneic HSCT shows bimodal distribution, one in the neutropenic period early after HSCT and the other 2–3 months after HSCT when patients are taking glucocorticosteroid for acute graft-versus-host disease (GVHD).[1,3,5,6] IA early after HSCT, however, has become less frequent because of the shortened neutropenic period due to the use of peripheral blood stem cells (PBSC), granulocyte colony-stimulating factor (G-CSF) and high-efficiency particulate air (HEPA) filtration and/or laminar air flow.[5–12] Therefore, the clinical significance of empirical treatment for aspergillosis based on persistent febrile neutropenia (FN) has become less clear, although it is supported by old evidence and recent guidelines.[11–16]

Our transplantation unit moved to a new building in September 2002. At the same time, we changed the strategy against aspergillosis during the neutropenic period from

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empirical strategy to presumptive strategy, in which anti-*Aspergillus* agents were started based on positive serum test and/or infiltrates or nodules on X-ray or CT-scan associated with persistent FN.[17,18] In this report, we reviewed the outcomes of 114 patients who underwent allogeneic HSCT in the new transplant unit and evaluated the feasibility of the presumptive strategy during the early neutropenic period after allogeneic HSCT.

Materials and methods

Study patients

Medical records of 124 consecutive adult patients who underwent allogeneic HSCT at the University of Tokyo Hospital between September 2002 and December 2005 were reviewed. All patients received prophylactic antifungal agents. Of the 124 patients, 114 who received fluconazole at 200 mg/day as anti-*Candida* prophylaxis were included in this study.[19,20] The remaining 10 patients were excluded from this study, because they had recent IA and prophylactically received anti-*Aspergillus* agents including micafungin and itraconazole. Characteristics of the 114 patients are summarized in Table 1. The median age was 43 years (range, 20–66 years). Patients' underlying diseases included acute myeloblastic leukaemia (AML), acute lymphoblastic leukaemia (ALL), chronic myelogenous leukaemia (CML), myelodysplastic syndrome (MDS), non-Hodgkin lymphoma (NHL), aplastic anaemia (AA) and so on. Standard-risk diseases were defined as AML/ALL in first complete remission (CR1) or CR2, CML in first chronic phase (CP1) or CP2, chemosensitive NHL, MDS in refractory anaemia or refractory anaemia with ringed sideroblasts and non-malignant haematological disorders. All other diseases were classified as high-risk diseases. Eight patients had received previous autologous or allogeneic stem cell transplantation. Two patients had a previous history of probable IA prior to HSCT.

Transplantation procedure

The stem cell source was bone marrow (BM) from a related donor in 8, BM from an unrelated donor in 47 and PBSC from a related donor in 59. Myeloablative conditioning regimens were used in 74 patients, mainly with total body irradiation plus cyclophosphamide or busulfan plus cyclophosphamide. Fludarabine-based reduced-intensity conditioning regimens were conducted in 40 patients. In these regimens, fludarabine was combined with either busulfan at 8–16 mg/kg in total or melphalan at 140 mg/m^2 in total. In some patients, total body irradiation of 4 Gy in total was added. Therefore, the intensities of regimens were close to the myeloablative conventional regimens. Prophylaxis against GVHD was performed with calcineurin inhibitors (cyclosporine or tacrolimus) with or without short-term methotrexate in the majority of patients. *In vivo* T cell depletion using alemtuzumab or anti-thymocyte globulin was performed in 27 patients, concomitant with cyclosporine and short-term methotrexate.

Neutrophil engraftment was defined as an absolute neutrophil count >500 cells/mm^3 for 3 consecutive days. All patients were housed in double-door HEPA-filtered laminar air flow rooms and provided with low microbial diets until neutrophil engraftment. New quinolones were given prophylactically in all patients. Recombinant G-CSF was routinely administered for patients with non-malignant disease and those with lymphoid malignancies after HSCT. Chest X-ray and non-invasive screening serum tests for IA including galactomannan antigen test (Platelia Aspergillus, Bio-Rad Laboratories, Marnes-la-Coquette, France) and β-D-glucan (BDG) test (β-glucan Test Wako, Wako Pure Chemical Industries, Tokyo, Japan) were performed weekly. Initial empirical antibacterial treatment for FN was started with fourth-generation cephalosporins or carbapenems.[11] For patients with persistent or recurrent FN for 7 days or longer, we did not start anti-*Aspergillus* agents as an early presumptive treatment for aspergillosis until patients developed positive serum test and/or infiltrates or nodules on X-ray or CT-scan (presumptive group). Thirteen patients, however, received

Table 1. Characteristics of the 114 patients who were included in this study

Characteristic	
Median recipient age, years (range)	43 (20–66)
Male/female	66/48
Underlying diagnosis, *n* (%)	
AML	30 (26.3)
ALL	20 (17.5)
AUL	1 (0.9)
CML	13 (11.4)
MDS	13 (11.4)
NHL	16 (14.0)
ATL	4 (3.5)
AA	7 (6.1)
Others	10 (8.8)
IA before HSCT, *n* (%)	2 (1.8)
Disease status, *n* (%)	
Standard-risk	65 (57.0)
High-risk	49 (43.0)
Donor, *n* (%)	
Related	67 (58.8)
Unrelated	47 (41.2)
Stem cell source, *n* (%)	
BM	55 (48.2)
PBSC	59 (51.8)
Number of transplantation, *n* (%)	
1	106 (93.0)
2	7 (6.1)
3	1 (0.9)
HLA mismatches at serological level, *n* (%)	30 (26.3)
HLA mismatches at genetic level, *n* (%)	36 (31.6)
Conditioning regimen, *n* (%)	
Myeloablative conditioning	74 (64.9)
Reduced-intensity conditioning	40 (35.1)
GVHD prophylaxis, *n* (%)	
Cyclosporine alone	4 (3.5)
Cyclosporine and short-term MTX	80 (70.2)
Tacrolimus and short-term MTX	3 (2.6)
In vivo T cell depleted	27 (23.7)
Engraftment, *n* (%)	112 (98.1)
Days of engraftment, median (range)	16.5 (9–43)
Antibacterial prophylaxis, *n* (%)	
Tosufloxacin	110 (96.5)
Ciprofloxacin	4 (3.5)
Use of G-CSF, *n* (%)	68 (59.6)

MTX, methotrexate; G-CSF, granulocyte colony-stimulating factor; AUL, acute unclassified leukaemia; ATL, adult T-cell leukaemia/lymphoma.

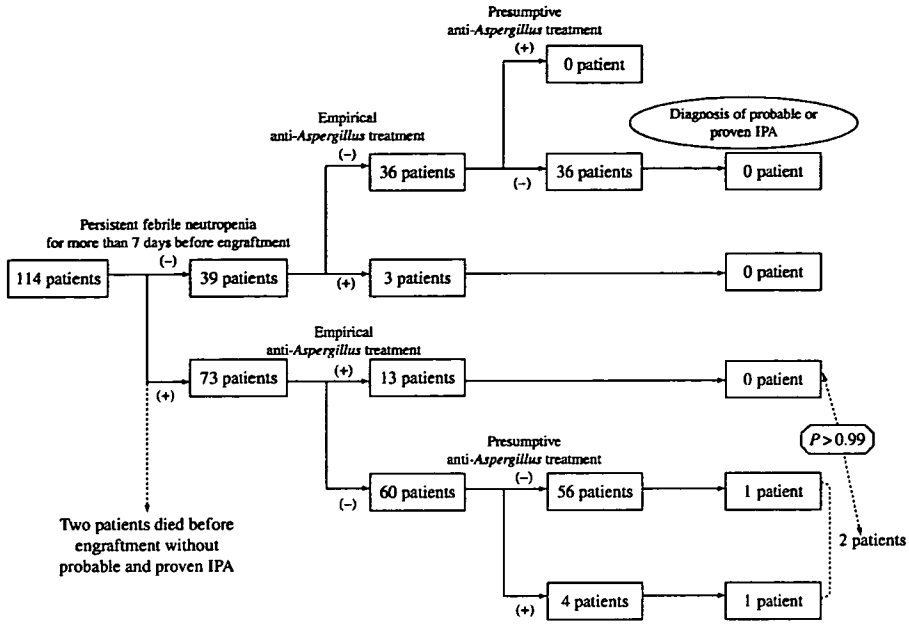

Figure 1. Treatment and outcome of patients included in the study. One hundred and fourteen patients were included in the study. One hundred and nine patients experienced FN. Seventy-three patients experienced persistent or recurrent FN for 7 days or longer. Thirteen patients in the empirical group received anti-*Aspergillus* treatment and the remaining 60 patients were included in the presumptive group. Four patients actually received anti-*Aspergillus* treatment presumptively. In total, early IA was observed in two patients in the presumptive group and none in the empirical group (3.3% versus 0%, $P > 0.99$). IPA, invasive pulmonary aspergillosis.

anti-*Aspergillus* agents empirically at the discretion of attending physicians (empirical group, Figure 1).

Definition of IA

The primary endpoint of this study was the development of probable or proven IA, that was diagnosed according to the criteria of the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG).[21] Microbiological criteria included two consecutive positive galactomannan tests using a reduced cutoff of 0.6 optical density index (O.D.I.).[22] IA that occurred between the day of HSCT and 7 days after engraftment was defined as early IA. The cumulative incidences of IA were calculated using Gray's method considering death without IA as a competing risk.[23]

Results

Clinical outcomes after allogeneic HSCT

Engraftment was observed in all patients at a median of 16.5 days (9–43 days) after HSCT, except for two who experienced early death before engraftment. Forty-two patients, 37.5% of those who achieved engraftment, developed grade II–IV acute GVHD at a median of 21 days after HSCT. Fifty-eight patients, 58.6% of those who survived more than 100 days after HSCT, developed chronic GVHD. Thirty-six patients relapsed at a median of 123.5 days after HSCT. Two-year overall survival of all subjects was 52.4% with a median follow-up duration of surviving patients of 822 (range 107–1603) days after HSCT.

Incidence of IA

Sixteen patients developed probable or proven IFI with a cumulative incidence of 15.1%, including 13 IA, 2 mucormycosis and 1 candidiasis (Table 2). The cumulative incidence of IA was 11.6% (Figure 2) and the median onset was 169.5 days (range, 12–531 days) after HSCT. Twelve out of 13 IA patients suffered from invasive pulmonary aspergillosis (IPA), whereas one developed gastrointestinal aspergillosis. No statistically significant risk factor was identified for the incidence of IA except for male sex (17.8% for male patients versus 2.1% for female patients, $P = 0.018$).

FN and early IA

One hundred and nine patients experienced FN with median duration of 12 days (range, 1–39 days). A median of three (range 1–5) antibiotics per patient were used for empirical antibacterial treatment during FN. Seventy-three patients experienced persistent or recurrent FN for 7 days or longer. The median duration of neutropenia was 21 and 20 days in the empirical group and presumptive group, respectively ($P = 0.91$). Thirteen patients in the empirical group received anti-*Aspergillus* treatment at a median of 9 days (range, 3–21 days) after the onset of FN (Figure 1). Amphotericin B was administered empirically in three patients, which was terminated within 2 days because of renal dysfunction. Micafungin was given to the other 10 patients for a median of 16.5 days (range, 3–76 days). Sixty patients followed the presumptive treatment strategy. Of the 60 patients in the presumptive group, 4 patients actually received anti-*Aspergillus* treatment presumptively, triggered by an elevation of BDG in 1 and infiltrates or nodules on chest

Table 2. Incidence of probable or proven IFI after allogeneic HSCT

	No.
Diagnosis of IFI after HSCT	
Proven diagnosis	4
Probable diagnosis	12
Onset of IFI after HSCT	
Early IFI	2
Late IFI	14
Incidence of late IFI	
Patients without FN	6
Empirical group	0
Presumptive group	8
Organisms that caused IFI	
Aspergillus spp.	13
Candida glabrata	1
Mucor spp.	2
Treatment for IFI	
Amphotericin B	5
Itraconazole	2
Micafungin	4
Voriconazole	3
None	2
Outcome	
Improved	8
No change or progression	6

X-ray or CT-scan in 3. One of them was subsequently diagnosed to have probable IA within a week, because galactomannan test became positive. We changed the anti-*Aspergillus* agent from micafungin to voriconazole and IPA was successfully treated (patient no. 4 in Table 3). Another patient in the presumptive group, who did not receive empirical or presumptive anti-*Aspergillus* agents, developed positive galactomannan test and nodules on CT-scan simultaneously, and was diagnosed to have probable IA (patient no. 5 in Table 3). This patient was also successfully treated with micafungin.

In total, early IA was observed in two patients in the presumptive group and none in the empirical group (3.3% versus 0%, $P > 0.99$). There was no significant difference in the duration of FN between the two groups (15.6 days versus 17.7 days, $P = 0.26$). There was no death that was directly associated with early IA in the whole population.

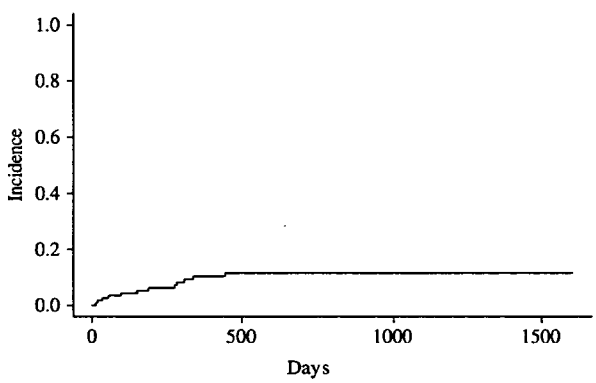

Figure 2. Cumulative incidence of IA after allogeneic HSCT. The cumulative incidence of IA was 11.6% in this study.

Discussion

In this study, the incidences of probable or proven IFI and IA were 15.1% and 11.6%, respectively, which were compatible with other recent studies.[1–3,5,7,8] Only three patients developed IFI other than IA, probably due to the prophylactic use of fluconazole.[5,7,8] Among the 13 patients with IA, only 2 developed IA early after HSCT. Both patients were successfully treated with anti-*Aspergillus* agents and therefore there was no death that was directly related to early IA.

Empirical anti-*Aspergillus* treatment has been recommended for patients with persistent FN.[11,24] However, this strategy is based on two old randomized controlled trials published in the 1980s, before the era of fluconazole prophylaxis.[12,25] Until recently, the standard antifungal agent in this setting has been amphotericin B deoxycholate.[12,25] This approach is limited by the substantial infusion-related toxicity and nephrotoxicity caused by this agent. Recently, lipid formulations of amphotericin B and intravenous itraconazole appeared to have equivalent efficacy compared with conventional amphotericin B with less toxicity.[13,16] Voriconazole and caspofungin were also reported to have similar efficacy.[14,15] However, these alternative agents are very expensive and still more toxic than fluconazole.

A presumptive strategy has been expected to decrease the use of these anti-*Aspergillus* agents by postponing anti-*Aspergillus* treatment until more specific findings are detected in patients with persistent FN. Several findings have been considered specific for IA, such as halo sign on CT-scan in neutropenic patients.[21] In addition, blood tests to detect *Aspergillus* constituents have been investigated, including galactomannan antigen test, BDG test and PCR to detect *Aspergillus* DNA.[22,26,27] Their clinical roles, however, have not been clarified.[26] Previously, we prospectively compared the sensitivity and specificity of these tests and found that the galactomannan test was the most suitable test for the diagnosis of IA with the best cutoff of 0.6 O.D.I.[22] In this study, we included not only blood galactomannan test with this cutoff index and halo sign on CT-scan but also blood BDG test and infiltrates or nodules on X-ray or CT-scan as triggers to start anti-*Aspergillus* treatment to increase sensitivity rather than specificity. By this presumptive strategy, only 2 of the 60 patients with persistent FN developed early IA, both of whom were successfully treated with anti-*Aspergillus* agents after the diagnosis of probable IA. This enabled us to decrease the use of anti-*Aspergillus* agents that are expensive and potentially toxic (4 of 60 in the presumptive group versus 13 of 13 in the empirical group). Considering the low incidence of early IA in the presumptive group, most patients in the empirical group might have been unnecessarily exposed to anti-*Aspergillus* agents. This is a retrospective study and therefore there are several limitations. Especially, we could not exclude the possibility of selection bias that high-risk patients tended to be treated empirically at the discretion of the attending physicians. However, there was no difference in the duration of neutropenia between the two groups. Both patients with a previous history of IA were included in the presumptive group.

Maertens *et al.*[28] recently showed the feasibility of preemptive therapy against IA. They started liposomal amphotericin B

Table 3. Characteristics of patients who received anti-*Aspergillus* agents presumptively (nos. 1–4) and patients who developed early IA (nos. 4 and 5)

No.	Age	Sex	Diagnosis	Prior IA	Triggers to start anti-*Aspergillus* agents	Anti-*Aspergillus* agents	Diagnosis of early IA	Outcome
1	57	Male	AML	—	Elevation of BDG	MCFG	No	Death due to AML progression
2	56	Male	AML	—	XP findings (consolidation)	MCFG	No	Alive
3	35	Male	CAEBV	—	CT findings (small multiple nodules with halo)	MCFG → ITC	No	Alive
4	56	Female	ALL	—	CT findings (nodules with halo) (positive galactomannan test after a week)	MCFG → VRC	Yes	Alive
5	54	Male	MDS	Probable IPA	CT findings (nodules with halo) and positive galactomannan test	MCFG → ITC	Yes	Alive

CAEBV, chronic active Epstein–Barr virus infection; IPA, invasive pulmonary aspergillosis; MCFG, micafungin; ITC, itraconazole; VRC, voriconazole; XP, X-ray photograph.

for patients with two consecutive positive galactomannan tests or with CT findings suggestive of IFI, regardless of the presence or absence of FN. They successfully reduced the use of anti-*Aspergillus* agents and no undetected cases of IA were identified. This approach may be more sensitive than our presumptive strategy to add anti-*Aspergillus* agents only for patients with persistent FN associated with positive serum test and/or radiological evidence. However, frequent galactomannan testing (thrice weekly) is required for this preemptive approach and thus it can be performed in only a limited number of centres.

Recently, prophylactic use of itraconazole, an anti-*Aspergillus* agent, has been evaluated in allogeneic HSCT recipients in two randomized controlled trials.[29,30] The incidence of IA was lower in the itraconazole group than the fluconazole group in both trials. The difference in the incidence of IA appeared 2 or 3 months after HSCT, not in the neutropenic period early after HSCT. Therefore, the prophylactic use of anti-*Aspergillus* agents should be considered for patients at higher-risk for IA, including patients receiving steroid for GVHD or neutropenic patients with a recent history of IA. However, for patients who are receiving anti-*Aspergillus* prophylaxis, another approach other than empirical or presumptive therapy, may be required.

In conclusion, these findings suggested the feasibility of a presumptive strategy for IA in HSCT recipients, provided that they were treated in a HEPA-filtered laminar air flow room. A randomized controlled trial is warranted to compare the efficacy and safety of presumptive and empirical strategy early after HSCT.

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Transparency declarations

None to declare.

References

1. Brown JMY. *Fungal Infection After Hematopietic Stem Cell Transplantation. Third Edition.* Cambridge, UK: Cambridge University Press, 2003.

2. Imataki O, Kami M, Kim SW *et al.* A nationwide survey of deep fungal infections and fungal prophylaxis after hematopoietic stem cell transplantation in Japan. *Bone Marrow Transplant* 2004; **33**: 1173–9.

3. Martino R, Subira M, Rovira M *et al.* Invasive fungal infections after allogeneic peripheral blood stem cell transplantation: incidence and risk factors in 395 patients. *Br J Haematol* 2002; **116**: 475–82.

4. Tollemar J. *Fungal Infections. Third Edition.* Oxford, UK: Blackwell Publishing Ltd, 2004.

5. Grow WB, Moreb JS, Roque D *et al.* Late onset of invasive aspergillus infection in bone marrow transplant patients at a university hospital. *Bone Marrow Transplant* 2002; **29**: 15–9.

6. Wald A, Leisenring W, van Burik JA *et al.* Epidemiology of *Aspergillus* infections in a large cohort of patients undergoing bone marrow transplantation. *J Infect Dis* 1997; **175**: 1459–66.

7. Baddley JW, Stroud TP, Salzman D *et al.* Invasive mold infections in allogeneic bone marrow transplant recipients. *Clin Infect Dis* 2001; **32**: 1319–24.

8. Jantunen E, Ruutu P, Niskanen L *et al.* Incidence and risk factors for invasive fungal infections in allogeneic BMT recipients. *Bone Marrow Transplant* 1997; **19**: 801–8.

9. Petersen FB, Buckner CD, Clift RA *et al.* Laminar air flow isolation and decontamination: a prospective randomized study of the effects of prophylactic systemic antibiotics in bone marrow transplant patients. *Infection* 1986; **14**: 115–21.

10. Eckmanns T, Ruden H, Gastmeier P. The influence of high-efficiency particulate air filtration on mortality and fungal infection among highly immunosuppressed patients: a systematic review. *J Infect Dis* 2006; **193**: 1408–18.

11. Hughes WT, Armstrong D, Bodey GP *et al.* 2002 guidelines for the use of antimicrobial agents in neutropenic patients with cancer. *Clin Infect Dis* 2002; **34**: 730–51.

12. EORTC International Antimicrobial Therapy Cooperative Group. Empiric antifungal therapy in febrile granulocytopenic patients. *Am J Med* 1989; **86**: 668–72.

13. Walsh TJ, Finberg RW, Arndt C *et al.* Liposomal amphotericin B for empirical therapy in patients with persistent fever and neutropenia. National Institute of Allergy and Infectious Diseases Mycoses Study Group. *N Engl J Med* 1999; **340**: 764–71.

14. Walsh TJ, Pappas P, Winston DJ *et al.* Voriconazole compared with liposomal amphotericin B for empirical antifungal therapy in patients with neutropenia and persistent fever. *N Engl J Med* 2002; **346**: 225–34.

15. Walsh TJ, Teppler H, Donowitz GR *et al.* Caspofungin versus liposomal amphotericin B for empirical antifungal therapy in patients with persistent fever and neutropenia. *N Engl J Med* 2004; **351**: 1391–402.

16. Boogaerts M, Winston DJ, Bow EJ *et al.* Intravenous and oral itraconazole versus intravenous amphotericin B deoxycholate as empirical antifungal therapy for persistent fever in neutropenic patients with cancer who are receiving broad-spectrum antibacterial therapy. A randomized, controlled trial. *Ann Intern Med* 2001; **135**: 412–22.

17. Martino R, Viscoli C. Empirical antifungal therapy in patients with neutropenia and persistent or recurrent fever of unknown origin. *Br J Haematol* 2006; **132**: 138–54.

18. Bow EJ. Of yeasts and hyphae: a hematologist's approach to antifungal therapy. *Hematology 2006 American Society of Hematology Education Program Book.* Washington, DC, USA: American Society of Hematology, 2006; 361–7.

19. Marr KA, Seidel K, Slavin MA *et al.* Prolonged fluconazole prophylaxis is associated with persistent protection against candidiasis-related death in allogeneic marrow transplant recipients: long-term follow-up of a randomized, placebo-controlled trial. *Blood* 2000; **96**: 2055–61.

20. Kanda Y, Yamamoto R, Chizuka A *et al.* Prophylactic action of oral fluconazole against fungal infection in neutropenic patients. A meta-analysis of 16 randomized, controlled trials. *Cancer* 2000; **89**: 1611–25.

21. Ascioglu S, Rex JH, de Pauw B *et al.* Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. *Clin Infect Dis* 2002; **34**: 7–14.

22. Kawazu M, Kanda Y, Nannya Y *et al.* Prospective comparison of the diagnostic potential of real-time PCR, double-sandwich enzyme-linked immunosorbent assay for galactomannan, and a (1→3)-β-D-glucan test in weekly screening for invasive aspergillosis in patients with hematological disorders. *J Clin Microbiol* 2004; **42**: 2733–41.

23. Gooley TA, Leisenring W, Crowley J *et al.* Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. *Stat Med* 1999; **18**: 695–706.

24. Hughes WT, Armstrong D, Bodey GP *et al.* 1997 guidelines for the use of antimicrobial agents in neutropenic patients with unexplained fever. Infectious Diseases Society of America. *Clin Infect Dis* 1997; **25**: 551–73.

25. Pizzo PA, Robichaud KJ, Gill FA *et al.* Empiric antibiotic and antifungal therapy for cancer patients with prolonged fever and granulocytopenia. *Am J Med* 1982; **72**: 101–11.

26. Kami M, Fukui T, Ogawa S *et al.* Use of real-time PCR on blood samples for diagnosis of invasive aspergillosis. *Clin Infect Dis* 2001; **33**: 1504–12.

27. Chamilos G, Kontoyiannis DP. Defining the diagnosis of invasive aspergillosis. *Med Mycol* 2006; **44** Suppl: 163–72.

28. Maertens J, Theunissen K, Verhoef G *et al.* Galactomannan and computed tomography-based preemptive antifungal therapy in neutropenic patients at high risk for invasive fungal infection: a prospective feasibility study. *Clin Infect Dis* 2005; **41**: 1242–50.

29. Winston DJ, Maziarz RT, Chandrasekar PH *et al.* Intravenous and oral itraconazole versus intravenous and oral fluconazole for long-term antifungal prophylaxis in allogeneic hematopoietic stem-cell transplant recipients. A multicenter, randomized trial. *Ann Intern Med* 2003; **138**: 705–13.

30. Marr KA, Crippa F, Leisenring W *et al.* Itraconazole versus fluconazole for prevention of fungal infections in patients receiving allogeneic stem cell transplants. *Blood* 2004; **103**: 1527–33.

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Allogeneic Bone Marrow Transplantation from Unrelated Human T-Cell Leukemia Virus-I–negative Donors for Adult T-Cell Leukemia/Lymphoma: Retrospective Analysis of Data from the Japan Marrow Donor Program

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ABSTRACT

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) from an HLA-matched related donor has been suggested to improve the poor prognosis of adult T-cell leukemia/lymphoma (ATLL). However, the infusion of HTLV-I–infected cells from HTLV-I–positive related donors could lead to the development of donor-derived ATLL under immunosuppressive conditions. Although most ATLL patients lack a suitable HLA-matched related donor and require an HTLV-I–negative unrelated donor, little information is currently available regarding the outcome of unrelated bone marrow transplantation (UBMT) for ATLL. To evaluate the role of UBMT in treating ATLL, we retrospectively analyzed data from 33 patients with ATLL treated by UBMT through the Japan Marrow Donor Program (JMDP). Overall survival (OS), progression-free survival, and cumulative incidence of disease progression and progression-free mortality at 1 year after UBMT were 49.5%, 49.2%, 18.6%, and 32.3%, respectively. Multivariate analysis identified recipient age as an independent prognostic factor for OS (P = .044). Patients age ≥50 years who showed nonremission at transplantation tended to have higher rates of treatment-related mortality. Our observations suggest that UBMT could represent a feasible treatment option for ATLL patients and warrant further investigation based on these risk factors.

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KEY WORDS

Adult T-cell leukemia/lymphoma • Allogeneic hematopoietic stem cell transplantation • Unrelated donor • Graft-versus-adult T-cell leukemia/lymphoma

INTRODUCTION

Adult T-cell leukemia/lymphoma (ATLL) is a peripheral T-cell neoplasm caused by human T-cell leukemia virus type I (HTLV-I) [1,2]. ATLL is generally classified into 4 clinical subtypes based on clinical and laboratory features: acute, chronic, smoldering, and lymphoma type. Clinically, acute- and lymphoma-type ATLL show an aggressive course, with tumor

burden, severe hypercalcemia, multiorgan failure, and poor performance status. ATLL has an extremely poor prognosis, with a median survival of about 6 months for the acute type and about 10 months for the lymphoma type; these patients are usually highly immunocompromised and develop various opportunistic infections. [3] Furthermore, their tumor cells are usually resistant to conventional chemotherapies, because overexpression of multidrug-resistance genes leads to intrinsic drug resistance. [4,5] Intensified chemotherapy [6,7] and autologous stem cell transplantation [8] likewise have failed to improve the prognosis. Thus, alternative treatment strategies for ATLL are needed.

Some cases of successful treatment with allogeneic stem cell transplantation (allo-HSCT) from an HLA-matched related donor have been reported, and a graft-versus-ATLL (GvATLL) effect has been implicated for improving treatments outcomes in transplant patients undergoing transplantation for ATLL. [9–11] However, more than 2/3 of patients with ATLL lack HLA-matched related donors. Furthermore, approximately 2/3 of the siblings of patients with ATLL are HTLV-I carriers [12], and allo-HSCT from an HTLV-I–positive donor may carry a risk of promoting the development of ATLL through the addition of a new HTLV-I load on the immunocompromised host. [13,14] Although most ATLL patients lack a suitable HLA-matched related donor and require an unrelated donor to benefit from allo-HSCT, few reports are available concerning the results of unrelated donor bone marrow transplantation (UBMT) for ATLL [9,11,15–18], and the number of patients in these few reports has been too small on which to base any solid conclusions. Therefore, to clarify the feasibility and efficacy of UBMT from an HTLV-I-negative donor for ATLL, we retrospectively analyzed registered data and clinical outcomes of UBMT for ATLL through the Japan Marrow Donor Program (JMDP).

PATIENTS AND METHODS

Patients and Transplantation Procedure

The subjects of this retrospective study consisted of 33 patients with ATLL (acute type, n = 20; lymphoma type, n = 7; not described, n = 6) who received UBMT from a donor mediated and recruited through the JMDP between September 1999 and January 2004. The clinical indications for UBMT were determined by each individual institution. The median time from diagnosis of ATLL to UBMT was 8 months (range, 5–28 months). At the time of transplantation, 13 patients were in complete remission (CR), 2 patients were in partial remission (PR), and 14 patients were in nonremission (NR); disease status at the time of transplantation was not described in 4 patients. CR status was reported in detail for 13 patients, with 11 patients in first CR (CR1) and 2 patients in second CR (CR2) (Table 1). All unrelated donors were HTLV-I antibody–negative. Serologic typing for HLA-A, -B, and -DR was performed using a standard 2-stage complement-dependent test of microcytotoxicity. [19] Alleles at the HLA-A, -B, and -DRB1 loci were identified by high-resolution DNA typing as described previously. [20] Serologic typing revealed that 22 patients were matched at the HLA-A, -B, and -DR loci. Four patients were mismatched at 1 HLA-DR locus, and 1 patient was mismatched at 2 loci of HLA-A and -DR. DNA typing revealed that 13 patients were matched at HLA-A, -B and -DRB1 loci. Ten patients were mismatched at 1 locus; 9 patients were mismatched at the HLA-DRB1 locus, and the remaining patient was mismatched at 1 HLA-A locus. Another 4 patients were mismatched at 2 loci. HLA typing data were not described in 6 patients. Patient and donor characteristics are summarized in Table 2.

Table 1. *Patient characteristics*

Characteristic	Value
Median age at transplantation, years (range)	49 (range, 24–59)
Sex, n	
Male	18
Female	15
Performance status, n	
0–1	21
2–4	4
ND	8
Subtypes of ATLL, n	
Acute	20
Lymphoma	7
ND	6
Disease status at transplantation, n	
CR or PR	15
NR	14
ND	4
Duration from diagnosis to UBMT, n	
Within 1 year	21
Beyond 1 year	11
ND	1
Conditioning, n	(TBI-containing, 22; non-TBI-containing, 11)
CST	27
RIST	6
Cell dose, n	
$< 3.0 \times 10^8$/kg	16
$\geq 3.0 \times 10^8$/kg	14
ND	3
GVHD prophylaxis, n	
CsA + MTX	13
TCR + MTX	20

ND indicates not described; CR, complete remission; PR, partial remission; NR, nonremission; UBMT, unrelated bone marrow transplantation; TBI, total body irradiation; CST, conventional stem cell transplantation; RIST, reduced-intensity stem cell transplantation; GVHD, graft-versus-host disease; CsA, cyclosporine; MTX, methotrexate; TCR, tacrolimus.

Table 2. *Patient and donor characteristics*

Characteristic	Value
HLA-A, -B, and -DRB1 allele mismatches, n	
0	13
1	10
2	4
ND	6
Sex of donor/patient, n	
Male/male	13
Female/female	8
Female/male	5
Male/female	7
Extent of ABO match, n	
Match	19
Minor mismatch	4
Major mismatch	7
Major/minor	2
ND	1

ND indicates not described.

Transplantation was performed according to the protocol of each institution; therefore, conditioning regimens and prophylaxis against graft-versus-host disease (GVHD) differed among patients. Conditioning regimens were myeloablative in 27 patients; total body irradiation (TBI) was incorporated in 22 patients. Reduced-intensity conditioning regimens were used in 6 patients. GVHD prophylaxis included cyclosporine (n = 13) and tacrolimus (n = 20) combined with methotrexate. All recipients received bone marrow transplantation, which was not manipulated.

Assessment of Engraftment, GVHD, Survival, and Progression-Free Mortality

The day of sustained engraftment was defined as the first of 3 consecutive days with an absolute neutrophil count exceeding 0.5×10^9/L. Acute GVHD was diagnosed and graded according to the standard criteria described previously. [21,22] Chronic GVHD was evaluated according to standard criteria [23] in patients who survived more than 100 days after transplantation. Overall survival (OS) was defined as the duration (in days) from transplantation to death from any cause. Progression-free survival (PFS) was defined as days from transplantation to disease progression or death from any cause. Progression-free mortality was defined as death without disease progression.

Data Management and Statistical Considerations

Data were collected by the JMDP using a standardized report form. Follow-up reports were submitted at 100 days, 1 year, and every subsequent year after transplantation. The cumulative incidence of disease progression and progression-free mortality were evaluated using Gray's method, [24] considering each other risk as a competing risk. OS and PFS were estimated using the Kaplan-Meier method. Potential confounding factors considered in the analysis were age, sex, disease status, duration from diagnosis to transplantation, Eastern Cooperative Oncology Group (ECOG) performance status, [25] conditioning regimen, number of bone marrow cells transplanted, and presence of grade II–IV acute GVHD. Proportional hazard modeling was used to evaluate any influence of these factors on OS, treating development of acute GVHD as a time-dependent covariate. Factors associated with at least borderline significance ($P < .05$) in univariate analyses were subjected to multivariate analyses using backward-stepwise proportional hazards modeling. P values $P < .10$ were considered statistically significant.

RESULTS

Engraftment and GVHD

Transplantation outcomes are summarized in Table 3. The median number of cells transplanted was 2.44×10^8 nucleated cells/kg of recipient body weight (range, $0.58–3.58 \times 10^8$ nucleated cells/kg of recipient body weight). Five patients (15%) died within 20 days. Neutrophil engraftment was achieved in 28 patients. Late graft failure occurred in 1 of these 28 patients, although the patient showed engraftment on

Table 3. *Transplantation outcome*

	Value
Alive/dead, n	19/14
Median follow-up for survivors, days (range)	139 (87–600)
Cause of death	
Progression, n	2
Death without progression, n	9
Median days after transplantation (range)	32 (10–71)
Late graft failure, n	1
GVHD, n	1
Infection, n	3
TMA, n	2
VOD, n	1
Arrhythmia, n	1
Not described, n	3
Disease progression, n	5
Median days after transplantation (range)	122 (61–223)
Engraftment, n	
Engraftment	28
Death within 20 days	5
Late graft failure	1
Acute GVHD, n	
None	3
Grade I	8
Grade II	12
Grade III	3
Grade IV	2
Chronic GVHD, n	
None	14
Limited	1
Extensive	3

GVHD indicates graft-versus-host disease; TMA, thrombotic microangiopathy; VOD, venooculusive disease.

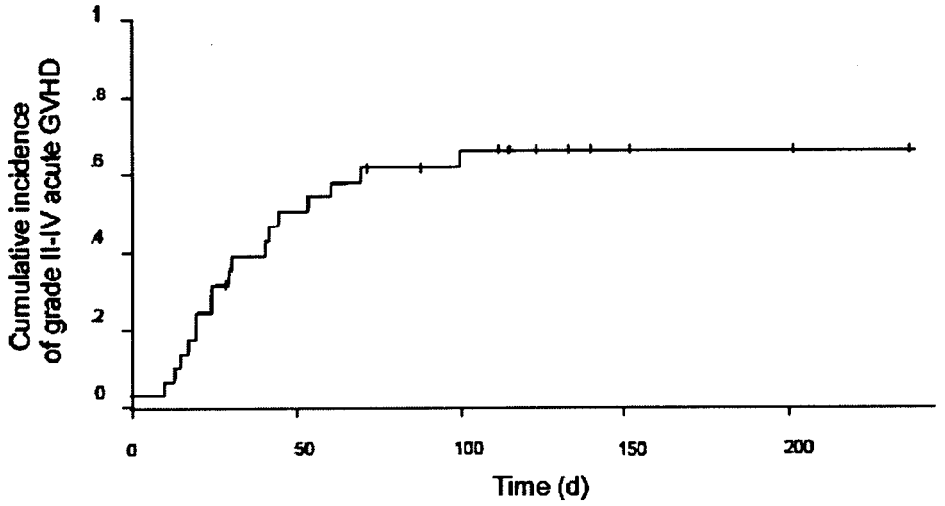

Figure 1. Cumulative incidence of grade II–IV acute GVHD in patients who achieved neutrophil engraftment.

day 14. Acute GVHD developed in 25 of the 28 patients who achieved engraftment (89%): grade I GVHD in 8 patients, grade II in 12 patients, grade III in 3 patients, and grade IV in 2 patients. The cumulative incidence of grade II–IV acute GVHD was 61% (Figure 1). Chronic GVHD developed in 4 of 18 patients, with limited disease in 1 patient and extensive disease in the other 3 patients.

Survival and disease progression

The 1-year OS and PFS were 49.5% (95% confidence interval [CI], 31.2%–78.5%) and 49.2% (95% CI, 33.6%–72.1%), respectively (Figure 2). Disease progression was observed in 5 patients, and the median number of days from transplantation to disease progression was 122 (range, 61–223 days). As of the last follow-up, 14 deaths had been reported. Primary cause of death was disease progression in 2 patients and was not described in 3 patients, but the other 9 deaths were not due to disease progression (see Table 3). Primary causes of transplantation-related death within 100 days after transplantation were late graft failure in 1 patient, GVHD in 1 patient, infection in 3 patients (with methicillin-resistant *Staphylococcus aureus*–positive sepsis in 1 patient and pulmonary infection in 2 patients), thrombotic microangiopathy (TMA) in 2 patients, veno-occlusive disease (VOD) in 1 patient, and arrhythmia in 1 patient.

Univariate and Multivariate Analyses for OS

Pretransplantation and posttransplant factors were calculated for OS (Table 4). In univariate analyses, OS was not significantly associated with sex, duration from diagnosis to transplantation, ECOG performance status, conditioning regimen, number of bone marrow cells transplanted, or presence of grade II–IV acute GVHD. On the other hand, patient age and disease status at transplantation were identified as significant independent risk factors. In multivariate analyses, only patient age at transplantation was identified as exerting a significant independent risk impact on OS (≥50 years vs <50 years; relative risk, 3.47; 95% CI, 1.03–11.6; $P = .044$). Disease status at transplantation exerted a marginally significant impact on OS (NR vs CR or PR; relative risk, 3.17; 95% CI, 0.96–10.5; $P = .059$) (Figure 3).

Influence of Pretransplantation Factors on Disease Progression and Progression-Free Mortality

The cumulative incidence of disease progression and progression-free mortality at 1 year were 18.6% and 32.3%, respectively (Figure 4). To clarify how age and disease status at transplantation affected OS, we evaluated the relationship between these factors and the incidence of progression-free mortality. The cumulative incidence of progression-free mortality was significantly higher in patients age ≥50 years at transplantation (50% vs 18%; $P = .048$; Figure 5A). NR at transplantation exerted a marginally significant effect on increased progression-free mortality (54% vs 20%; $P = .070$; Figure 5B).

DISCUSSION

This study analyzed the data and evaluated treatment outcomes for 33 patients with ATLL who received UBMT. Two important findings were identified regarding UBMT for ATLL. First, UBMT from HTLV-I–negative donors for ATLL represents a feasible treatment. Second, recipient age (≥50 years) and NR disease status at transplantation were independent risk factors for OS, and patients with ATLL displaying these risk factors tended to exhibit higher frequencies of treatment-related mortality.

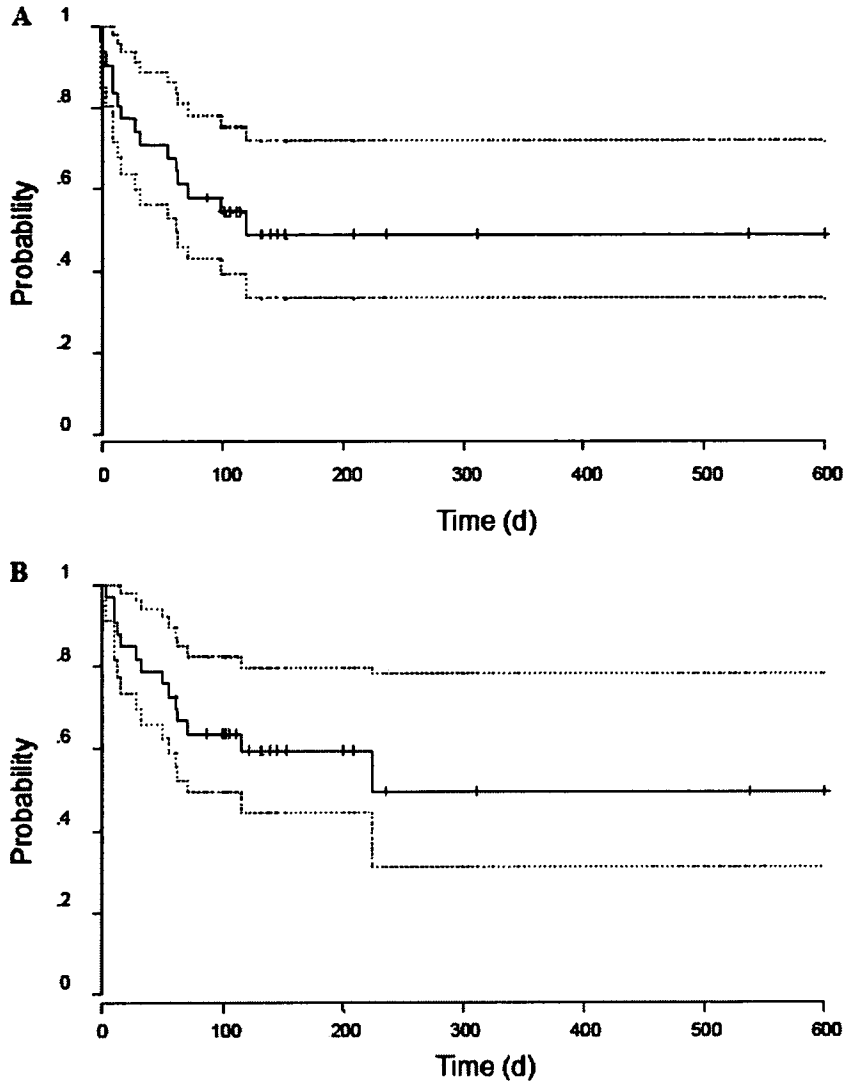

Figure 2. Probability of progression-free survival (A) and overall survival (B) after unrelated bone marrow transplantation for adult T-cell leukemia/lymphoma. Dashed lines represent 95% confidence intervals.

Table 4. *Prognosis factors in univariate and multivariate analyses*

	Univariate		Multivariate	
	Relative risk (95% CI)	P	Relative risk (95% CI)	P
Age ≥50 versus <50 years	4.03 (1.23–13.3)	.022	4.03 (1.23–13.3)	.022
Male versus female	0.97 (0.34–2.80)	.95		
PS 0–1 versus 2–4	0.44 (0.11–1.70)	.23		
NR versus CR or PR	3.37 (1.03–11.0)	.044		.059
UBMT within 1 year versus beyond 1 year	0.54 (0.15–2.00)	.35		
RIST versus CST	0.71 (0.19–2.59)	.60		
TBI versus non-TBI	1.35 (0.45–4.04)	.59		
Cell dose < 3.0 × 10^8/kg versus ≥ 3.0 × 10^8/kg	0.98 (0.31–3.05)	.97		
GVHD II–IV present versus absent	1.91 (0.50–7.26)	.34		

CI indicates confidence interval; PS, performance status; NR, nonremission; CR, complete remission; PR, partial remission; UBMT, unrelated bone marrow transplantation; RIST, reduced-intensity stem cell transplantation; CST, conventional stem cell transplantation; TBI, total body irradiation; GVHD, graft-versus-host disease.

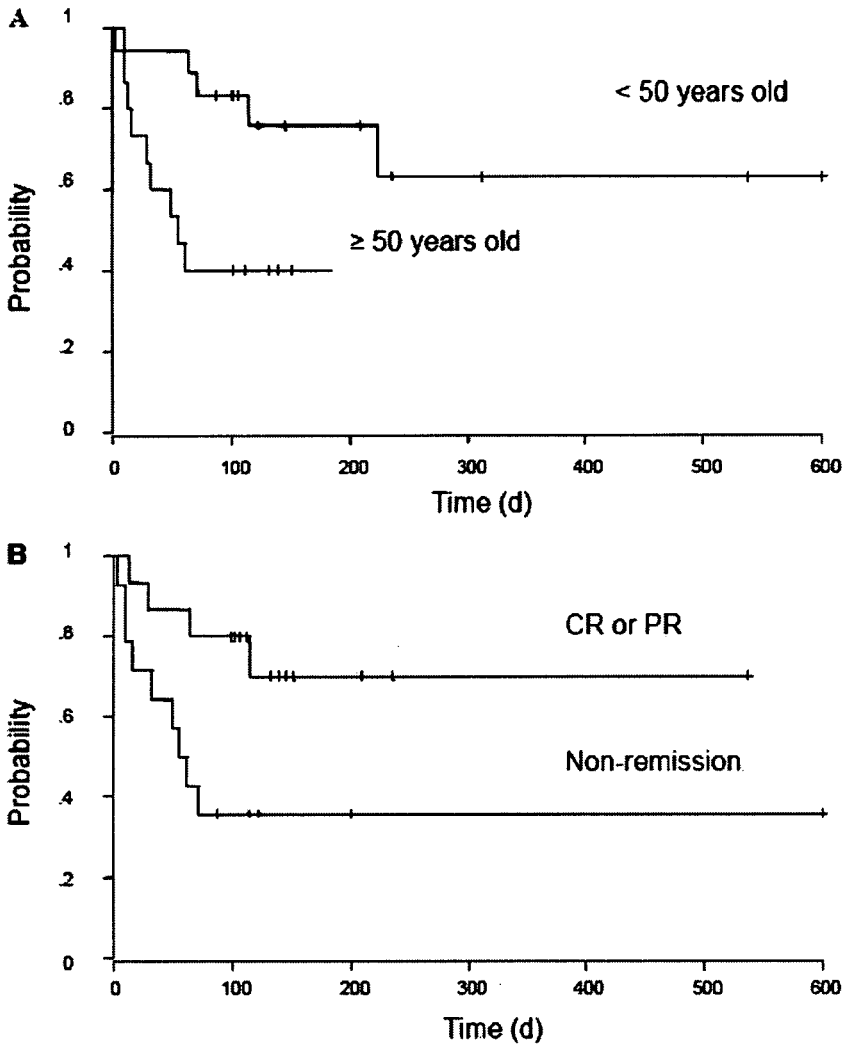

Figure 3. Overall survival according to pretransplantation factors, age (A) and disease status at transplantation (B).

ATLL has an extremely poor prognosis, with projected 2- and 4-year survival rates of 16.7% and 5.0% for the acute type and 21.3% and 5.7% for the lymphoma type, respectively. [3] Neither intensified chemotherapy nor autologous stem cell transplantation have improved the prognosis. Encouraging results for allo-HSCT for ATLL from HLA-matched related donors have been reported by several groups; thus, allo-HSCT may improve the poor prognosis of ATLL. However, the number of patients in most reports has been too small to allow evaluation of the efficacy of allo-HSCT for ATLL. The present results were derived from a large number of patients who underwent transplantation (33 patients) performed through the JMDP. Longer follow-up is, of course, needed to confirm the curative potential of allo-HSCT for ATLL. However, the good survival rates noted here suggest that allo-HSCT is an effective treatment for ATLL, and that patients with ATLL will benefit from allo-HSCT through HTLV-I–negative unrelated donors, because the OS and PFS rates at 1 year after UBMT were 49.5% and 49.2%, respectively. Compared with the results for patients with non-Hodgkin's lymphoma in the National Marrow Donor Program, the incidence of grade III–IV acute GVHD in the present study was low (18% vs 30%). [26] The outcome in the present study appears to be favorable, possible due to the lower incidence of grade III–IV acute GVHD. This observation is compatible with previous studies showing a lower incidence of acute GVHD in Japanese patients compared with Western patients, which might reflect the less diverse genetic background of in the Japanese population. [27,28]

Frequency of relapse after transplantation differs between autologous and allo-HSCT for ATLL. The use of high-dose chemotherapy with autologous HSCT has been reported in only 9 patients, all of whom relapsed or died from transplantation-related mortality. [8] In contrast, the cumulative incidence of

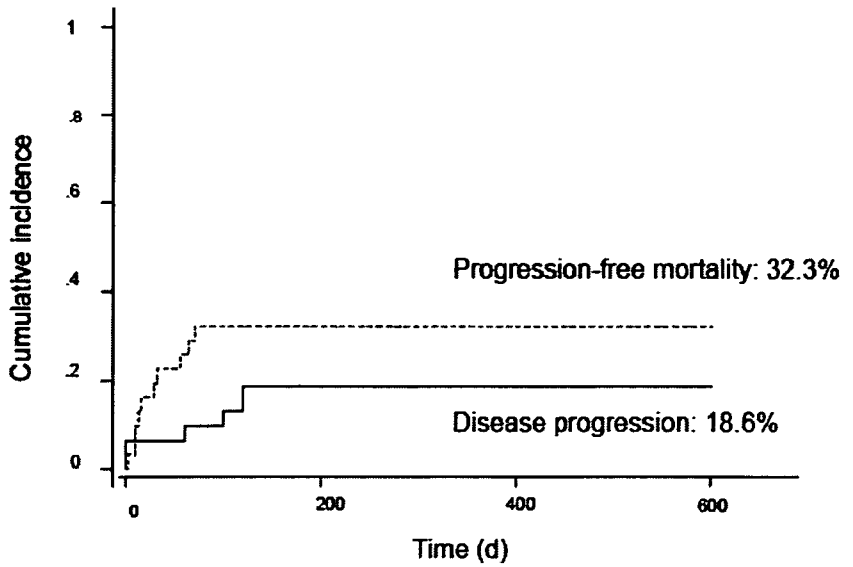

Figure 4. Cumulative incidence of disease progression (—) and progression-free mortality (---) after transplantation.

disease progression was lower after UBMT in this study. Interestingly, patients with ATLL displaying acute or chronic GVHD reportedly did not relapse. [9] In another report, patients with ATLL who relapsed after allo-HSCT reachieved CR after tapering or discontinuation of immunosuppressive agents and donor lymphocyte infusions. [10,11] Reactivation in tax-specific CD8-positive cytotoxic T lymphocytes (CTLs), which has been recently shown in patients with ATLL after allo-HSCT, may indicate a potential contribution of CTLs to anti-ATLL immunity and induction of a GvATLL effect. [29] These results strongly suggest that a GvATLL effect could work on some patients with ATLL to prevent relapse after allo-HSCT. In the present study, neither univariate nor multivariate analysis showed a survival benefit for acute GVHD. We were unable to analyze the relationship between chronic GVHD and relapse, because of the low number of patients with chronic GVHD. In fact, the number of patients may have been insufficient to confirm GvATLL in this study. On the other hand, the absence of benefit from GVHD in preventing relapse suggests that a GvATLL effect could occur in patients with ATLL after allo-HSCT without clinically obvious GVHD. [11]

Transplantation-related mortality was a significant problem in this study. Five patients (15%) died within 20 days, from infection in 3 patients and TMA in 2 patients. Nine patients (27%) died within 100 days, due to infection in 3 patients, TMA in 2 patients, and VOD in 1 patient. Patients with ATLL might have an increased risk of frequent opportunistic infection, because they have an associated T-cell immunodeficiency. Furthermore, ATLL is usually systemic in distribution, and the accumulated organ damages as a result of repeated cytotoxic chemotherapy seen in patients before transplantation may have contributed to the onset of TMA. In univariate and multivariate analysis, recipient age (≥50 years) and NR disease status at transplantation represented significant risk factors for OS. The multivariate analyses were limited by the small number of patients in each subgroup; however, patients displaying these risk factors tended to have a higher rate of treatment-related mortality than patients without these factors, and it can be assumed that these risk factors have a significant relationship with outcome clinically. In this study, mostly myeloablative conditioning regimens were used before transplantation. Given that conventional allo-HSCT is designed to eradicate tumor cells with myeloablative intensity using maximally tolerated doses of high-dose chemotherapy and radiotherapy, the desirable effects often may be offset by overwhelming toxicity in patients age ≥50 years. Moreover, the number of patients with ATLL who are eligible for allo-HSCT with myeloablative conditioning is limited, because the typical patient with ATLL has a relatively advanced age at presentation (about 60 years). To reduce treatment-related mortality, allo-HSCT with reduced-intensity conditioning offers a new treatment option for patients with ATLL who are ineligible for allo-HSCT with myeloablative conditioning due to advanced age or medical infirmity. [30,31] Okamura et al [32] reported on 16 patients age > 50 years with ATLL who underwent allo-HSCT with reduced-intensity conditioning from HLA-matched related donors and found that treatment-related mortality was acceptable and that allo-HSCT with reduced-intensity conditioning was a feasible treatment for ATLL. Given these findings, UBMT

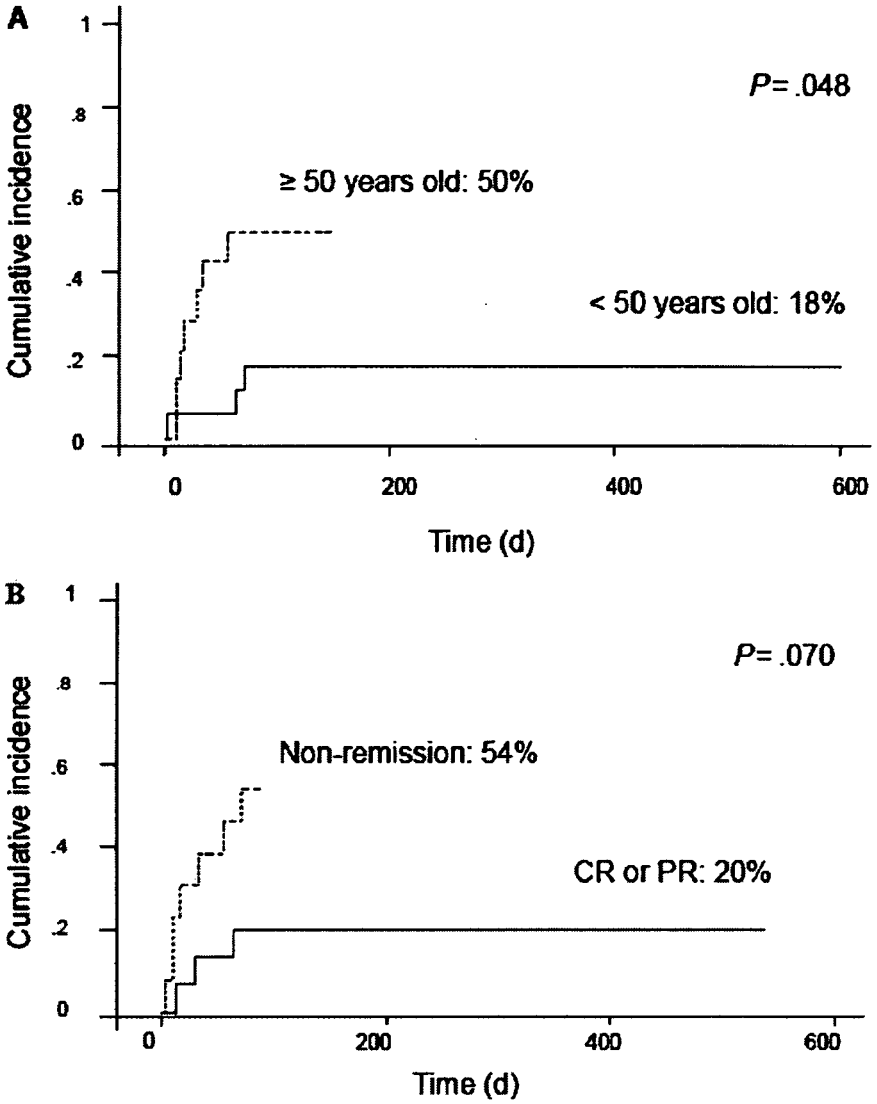

Figure 5. Cumulative incidence of progression-free mortality grouped according to pretransplantation factors, age (A) and disease status at transplantation (B).

with reduced-intensity conditioning should be considered for elderly patients with ATLL.

Another concern related to allo-HSCT for ATLL involves the use of HTLV-1–positive carrier donors. About 2/3 of siblings of patients with ATLL are HTLV-I carriers. From the perspective of HTLV-I–positive donor risk, granulocyte colony–stimulating factor (G-CSF) can reportedly stimulate the proliferation of ATLL cells [33], and HTLV-I–positive donors may be at increased risk of developing ATLL due to the administration of G-CSF in the setting of allogeneic peripheral blood stem cell transplantation. From the perspective of patients with ATLL, allo-HSCT from an HTLV-I–positive donor may carry a risk of HTLV-I–associated disease after allo-HSCT [34] or a risk of promoting the future development of ATLL due to the new HTLV-I load on immunocompromised recipients [13,14]. On the other hand, to date there is no evidence in the JMDP or the literature that ATLL can develop from infected HTLV-I–negative donor cells due to the HTLV-I load of the recipient. The HTLV-I proviral load dramatically decreased to an undetectable level after transplantation, especially after transplantation from HTLV-I–negative donors. [18, 32] This decreased HTLV-I proviral load was observed after both myeloablative and reduced-intensity conditioning. Transplantation from an HTLV-I–positive donor is reportedly associated with a higher frequency of relapse compared with transplantation from an HTLV-I–negative donor. [11] Therefore, the uninfected normal donor T cells might overwhelm infected HTLV-I recipient T cells due to a GvATLL response and might act as an antiviral therapy. However, an HTLV-I–positive do-

nor might avoid clonal expansion of HTLV-I–infected T lymphocytes after allo-HSCT through the provision of cytotoxic T cells. Thus, it is currently difficult to determine whether an HTLV-I–positive or–negative donor should be selected. Longer follow-up is needed to resolve this issue. In the meantime, a prudent clinical attitude toward both HTLV-I–positive donors and recipients with ATLL is warranted.

In conclusion, allo-HSCT from an HTLV-I–negative unrelated donor appears to be an feasible alternative treatment for patients with ATLL for whom an HLA-matched related donor is unavailable. Further prospective controlled studies are needed to assess the efficacy of allo-HSCT for ATLL and to define the clinical indications of allo-HSCT for ATLL, taking into account donor selection, the conditioning regimen, and the prognostic factors identified in this study.

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APPENDIX: PARTICIPATING INSTITUTIONS

The following centers in Japan participated in this study: Hokkaido University Hospital, Sapporo University Hospital, Sapporo Hokuyu Hospital, Japanese Red Cross Asahikawa Hospital, Asahikawa Medical College Hospital, Hirosaki University Hospital, Tohoku University Hospital, Yamagata University Hospital, Akita University Hospital, Fukushima Medical College, National Cancer Center Central Hospital, Institute of Medical Science at the University of Tokyo, Toho University Hospital, Omori Hospital, Tokyo Metropolitan Komagome Hospital, Nihon University Hospital, Itabashi Hospital, Jikei University Hospital, Keio University Hospital, Tokyo Medical College Hospital, Tokyo Medical and Dental University Hospital, Tokyo University Hospital, Yokohama City University Hospital, Kanagawa Children's Medical Center, Kanagawa Cancer Center, Tokai University Hospital, St Marianna University Hospital, Chiba University Hospital, Chiba Children's Hospital, Matsudo Municipal Hospital, Kameda General Hospital, Saitama Children's Medical Center, Saitama Cancer Center Hospital, Saitama Medical School Hospital, Ibaraki Children's Hospital, Jichi Medical School Hospital, Dokkyo University Hospital, Fukaya Red Cross Hospital, Saiseikai Maebashi Hospital, Gunma University Hospital, Niigata University Hospital, Niigata Cancer Center Hospital, Shinshu University Hospital, Saku Central Hospital, Hamamatsu University Hospital, Hamamatsu Medical Center, Shizuoka General Hospital, Shizuoka Children's Hospital, Japanese Red Cross Nagoya First Hospital, Nagoya Daini Red Cross Hospital, Meitetsu Hospital, Nagoya University Hospital, Nagoya Ekisaikai Hospital, National Nagoya Hospital, Aichi Medical School Hospital, Nagoya City University Hospital, Showa Hospital, Anjo Kousei Hospital, Fujita Health University Hospital, Mie University Hospital, Kanazawa University Hospital, Kanazawa Medical University Hospital, Toyama Prefectural Central Hospital, Fukui Medical School Hospital, Shiga University of Medical Science, Center for Adult Disease in Osaka, Kinki University Hospital, Osaka University Hospital, Osaka Medical Center and Research Institute for Maternal and Child Health, Matsushita Memorial Hospital, Hyogo College of Medicine Hospital, Hyogo Medical Center for Adults, Kobe City General Hospital, Kobe University Hospital, Kyoto University Hospital, Kyoto Prefectural University of Medicine Hospital, Social Insurance Kyoto Hospital, Tottori Prefectural Central Hospital, Tottori University Hospital, Hiroshima Red Cross Hospital and Atomic-Bomb Survivors Hospital, Yamaguchi University Hospital, Ehime Prefectural Central Hospital, Okayama National Hospital, Kurashiki Central Hospital, Kyushu University Hospital, Harasanshin General Hospital, Hamanomachi General Hospital, National Kyushu Cancer Center, St Mary's Hospital, Kokura Memorial Hospital, Saga Prefectural Hospital, Nagasaki University Hospital, Miyazaki Prefectural Hospital, Kumamoto National Hospital, Kumamoto University Hospital, Oita Medical University Hospital, Kagoshima University Hospital, and Imamura Bun-in Hospital.

REFERENCES

1. Uchiyama T, Yodoi J, Sagawa K, et al. Adult T-cell leukemia: clinical and hematologic features of 16 cases. *Blood.* 1977;50: 481-492.
2. Yoshida M, Miyoshi I, Hinuma Y. Isolation and characterization of retrovirus from cell lines of human adult T-cell leukemia and its implication in the disease. *Proc Natl Acad Sci USA.* 1982;79:2031-2035.
3. Shimoyama M. Diagnostic criteria and classification of clinical subtypes of adult T- cell leukaemia-lymphoma. A report from the Lymphoma Study Group (1984-87). *Br J Haematol.* 1991; 79:428-437.
4. Kuwazuru Y, Hanada S, Furukawa T, et al. Expression of P-glycoprotein in adult T-cell leukemia cells. *Blood.* 1990;76: 2065-2071.

5. Ikeda K, Oka M, Yamada Y, et al. Adult T-cell leukemia cells over-express the multidrug-resistance-protein (MRP) and lung-resistance-protein (LRP) genes. *Int J Cancer.* 1999;82:599-604.
6. Taguchi H, Kinoshita KI, Takatsuki K, et al. An intensive chemotherapy of adult T-cell leukemia/lymphoma: CHOP followed by etoposide, vindesine, ranimustine, and mitoxantrone with granulocyte colony-stimulating factor support. *J Acquir Immune Defic Syndr Hum Retrovirol.* 1996;12:182-186.
7. Yamada Y, Tomonaga M, Fukuda H, et al. A new G-CSF-supported combination chemotherapy, LSG15, for adult T-cell leukaemia-lymphoma: Japan Clinical Oncology Group Study 9303. *Br J Haematol.* 2001;113:375-382.
8. Tsukasaki K, Maeda T, Arimura K, et al. Poor outcome of autologous stem cell transplantation for adult T cell leukemia/lymphoma: a case report and review of the literature. *Bone Marrow Transplant.* 1999;23:87-89.
9. Utsunomiya A, Miyazaki Y, Takatsuka Y, et al. Improved outcome of adult T-cell leukemia/lymphoma with allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplant.* 2001;27:15-20.
10. Kami M, Hamaki T, Miyakoshi S, et al. Allogeneic haematopoietic stem cell transplantation for the treatment of adult T-cell leukaemia/lymphoma. *Br J Haematol.* 2003;120:304-309.
11. Fukushima T, Miyazaki Y, Honda S, et al. Allogeneic hematopoietic stem cell transplantation provides sustained long-term survival for patients with adult T-cell leukemia/lymphoma. *Leukemia.* 2005;19:829-834.
12. Momita S, Ikeda S, Amagasaki T, et al. Survey of anti-human T-cell leukemia virus type I antibody in family members of patients with adult T-cell leukemia. *Jpn J Cancer Res.* 1990;81:884-889.
13. Chen YC, Wang CH, Su IJ, et al. Infection of human T-cell leukemia virus type I and development of human T-cell leukemia lymphoma in patients with hematologic neoplasms: a possible linkage to blood transfusion. *Blood.* 1989;74:388-394.
14. Tamaki H, Matsuoka M. Donor-derived T-cell leukemia after bone marrow transplantation. *N Engl J Med.* 2006;354:1758-1759.
15. Ogata M, Ogata Y, Imamura T, et al. Successful bone marrow transplantation from an unrelated donor in a patient with adult T cell leukemia. *Bone Marrow Transplant.* 2002;30:699-701.
16. Ishikawa T. Current status of therapeutic approaches to adult T-cell leukemia. *Int J Hematol.* 2003;78:304-311.
17. Izutsu K, Kanda Y, Ohno H, et al. Unrelated bone marrow transplantation for non-Hodgkin lymphoma: a study from the Japan Marrow Donor Program. *Blood.* 2004;103:1955-1960.
18. Nakase K, Hara M, Kozuka T, et al. Bone marrow transplantation from unrelated donors for patients with adult T-cell leukaemia/lymphoma. *Bone Marrow Transplant.* 2006;37:41-44.
19. Terasaki PI, McClelland JD. Microdroplet assay of human serum cytotoxins. *Nature.* 1964;204:998-1000.
20. Sasazuki T, Juji T, Morishima Y, et al. Effect of matching of class I HLA alleles on clinical outcome after transplantation of hematopoietic stem cells from an unrelated donor. *N Engl J Med.* 1998;339:1177-1185.
21. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestation of graft-versus-host disease in human recipients of marrow from HLA-matched sibling donors. *Transplantation.* 1974;18:295-304.
22. Kernan NA, Bartsch G, Ash RC, et al. Analysis of 462 transplantations from unrelated donors facilitated by the National Marrow Donor Program. *N Engl J Med.* 1993;328:593-602.
23. Shulman HM, Sullivan KM, Weiden PL, et al. Chronic cutaneous graft-versus-host syndrome on man: a long-term clinicopathologic study of 20 Seattle patients. *Am J Med.* 1980;69:204-217.
24. Gooley TA, Leisenring W, Crowley J, et al. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. *Stat Med.* 1999;18:695-706.
25. Oken MM, Creech RH, Tormey DC, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. *Am J Clin Oncol.* 1982;5:649-655.
26. Bierman PJ, Molina L, Nelson G, et al. Matched unrelated donor (MUD) allogeneic bone marrow transplantation for Non-Hodgkin's lymphoma (NHL): results from the National Marrow Donor Program (NMDP) [abstract]. *Proc Am Soc Clin Oncol.* 1999;18:3a.
27. Morishima Y, Kodera Y, Hirabayashi N, et al. Low incidence of acute GVHD in patients transplanted with marrow from HLA-A, B, DR-compatible unrelated donors among Japanese. *Bone Marrow Transplant.* 1995;15:235-239.
28. Kodera Y, Morishima Y, Kato S, et al. Analysis of 500 bone marrow transplants from unrelated donors (UR-BMT) facilitated by the Japan Marrow Donor Program: confirmation of UR-BMT as a standard therapy for patients with leukemia and aplastic anemia. *Bone Marrow Transplant.* 1999;24:995-1003.
29. Harashima N, Kurihara K, Utsunomiya A, et al. Graft-versus-tax response in adult T-cell leukemia patients after hematopoietic stem cell transplantation. *Cancer Res.* 2004;64:391-399.
30. Giralt S, Estey E, Albitar M, et al. Engraftment of allogeneic hematopoietic progenitor ccells with purine analog-containing chemotherapy: harnessing graft-versus-leukemia without myeloablative therapy. *Blood.* 1997;89:4531-4536.
31. Slavin S, Nagler A, Naparstek E, et al. Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. *Blood.* 1998;91:756-763.
32. Okamura J, Utsunomiya A, Tanosaki R, et al. Allogeneic stem-cell transplantation with reduced conditioning intensity as a novel immunotherapy and antiviral therapy for adult T-cell leukemia/lymphoma. *Blood.* 2005;105:4143-4245.
33. Matsushita K, Arima N, Ohtsubo H, et al. Granulocyte-colony stimulating factor-induced proliferation of primary adult T-cell leukaemia cells. *Br J Haematol.* 1997;96:715-723.
34. Imirizaldu JJ, Esteban JC, Axpe IR, et al. Post-transplantation HTLV-1 myelopathy in three recipients from a single donor. *J Neurol Neurosurg Psychiatry.* 2003;74:1080-1084.

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CASE REPORT

Graft rejection and hyperacute graft-versus-host disease in stem cell transplantation from non-inherited maternal antigen complementary HLA-mismatched siblings

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Abstract

Human leukocyte antigen (HLA)-mismatched stem cell transplantation from non-inherited maternal antigen (NIMA)-complementary donors is known to produce stable engraftment without inducing severe graft-versus-host disease (GVHD). We treated two patients with acute myeloid leukemia (AML) and one patient with severe aplastic anemia (SAA) with HLA-mismatched stem cell transplantation (SCT) from NIMA-complementary donors (NIMA-mismatched SCT). The presence of donor and recipient-derived blood cells in the peripheral blood of recipient (donor microchimerism) and donor was documented respectively by amplifying NIMA-derived DNA in two of the three patients. Graft rejection occurred in the SAA patient who was conditioned with a fludarabine-based regimen. Grade III and grade IV acute GVHD developed in patients with AML on day 8 and day 11 respectively, and became a direct cause of death in one patient. The findings suggest that intensive conditioning and immunosuppression after stem cell transplantation are needed in NIMA-mismatched SCT even if donor and recipient microchimerisms is detectable in the donor and recipient before SCT.

Key words graft-versus-host disease; rejection; graft failure; non-inherited maternal antigen; fetomaternal microchimerism

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In allogeneic stem cell transplantation (SCT) from human leukocyte antigen (HLA)-mismatched donors, severe acute graft-versus-host disease (GVHD) and graft rejections occur at a higher rate than SCT from HLA-matched donors (1, 2). Recently, allogeneic SCT from non-inherited maternal antigen (NIMA)-complementary donors has received attentions as one of the methods that potentially overcome the barrier of HLA incompatibility. It is well known that a small number of maternal blood cells exist in the newborn's blood, and in turn, blood cells derived from children can be detected in the mother's blood long after labor. This phenomenon is referred to as fetomaternal microchimerism. The existence of fetomaternal microchimerism suggests that immunological tolerance of hematopoietic cells takes place both in mother and child. Van Rood *et al.* (3) reported that the incidence of chronic GVHD was significantly lower in mother-to-child SCTs than in father-to-child SCTs. Their findings also suggest that HLA-haploidentical NIMA complementary siblings can be alternate donor candidates when there is no HLA-matched donor. Shimazaki *et al.* (4) reported five patients treated with allogeneic SCT from two or three loci-mismatched familiy donors who had a small number of recipient-derived cells in their blood before SCT. Engraftment occurred in all patients, and although acute GVHD developed in all five, their

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severity was grade I or II except for one patient who developed grade III acute GVHD. Ichinohe *et al.* (5) reported that in HLA-haploidentical SCTs, NIMA mismatches in the graft-versus-host (GVH) direction, was associated with a lower risk of severe acute GVHD compared with IPA mismatches.

Based on these backgrounds, we treated two acute myeloid leukemia (AML) patients and one severe aplastic anemia (SAA) patient with SCT from NIMA-mismatched sibling donors. Graft rejection and severe acute GVHD occurred despite the fact that donor and recipient-derived microchimerisms were shown in donor and recipient.

Case report

Case 1

A 27-yr-old man was diagnosed as having chronic myloid leukemia (CML) in myeloid crisis. He underwent an HLA-matched unrelated bone marrow transplantation in September 1999. However, he relapsed with CML in blastic crisis in October 2000. He received allogeneic peripheral blood hematopoietic stem cell transplantation from a NIMA-complementary dizygotic sibling in November 2002. A fever of 38°C occurred on day 3 after transplantation and erythema developed in upper and lower extremities on day 8. A diagnosis of acute GVHD, which met the criteria of hyperacute GVHD (6, 7) was made through skin biopsy findings. The patient's GVHD responded to the treatment and both erythema and icterus disappeared on day 26. The complete donor chimerism was confirmed on day 17 by microsatellite marker analysis. Imatinib mesylate was administered on day 21 and he was in molecular remission on day 58. However, CML recurred as subcutaneous nodules on day 153 and the patient died of CML on day 203.

Case 2

A 15-yr-old woman was diagnosed as having SAA in 2000. She did not respond to all kinds of therapy including ATG and anabolic steroids, and required frequent transfusions. An HLA-matched donor was absent either in relatives or in the bone marrow banks. Allogeneic bone marrow transplantation from the NIMA-complementary sister was performed in September 2003. Microchimerism was revealed in both the patient and donor (8). Her neutrophil count rose to 750/μL on day 25, but it became 0/μL following high fever associated with hyperferritinemia (24 490 ng/dL). Virus-associated hemophagocytic syndrome was suspected and foscarnet was administered without any effect. A chimerism analysis performed on day 34 revealed the absence of donor-derived cells in both the peripheral blood and bone marrow, thus leading to the diagnosis of secondary graft failure. She received an infusion of 1.65×10^6/kg of peripheral blood $CD34^+$ cells collected from the marrow donor without conditioning due to the deteriorating clinical condition, but no hematological recovery occurred. She underwent a cord blood cell transplantation (CBT) following conditioning with fludarabine 125 mg/m^2; melphalan, 160 mg/m^2 and total body irradiation at 4 Gy on day 89 after the first transplantation. She achieved a complete reconstitution of hematopoiesis after CBT and remains well 33 months after CBT.

Case 3

In January 2002, a 32-yr-old man was diagnosed to have AML with a normal karyotype. He achieved a complete remission following standard chemotherapy. A year later, he relapsed with acute lymphocytic leukemia with the Philadelphia chromosome (Ph^+ALL). He was treated with chemotherapy consisting of daunorubicin, vincristine, L-asparaginase, and prednisolone, followed by the administration of imatinib mesylate, but did not achieve a complete remission. There was no HLA-matched family member. The microchimerism by NIMAs possessed by the patient was documented in the blood of one brother. He received allogeneic SCT from this NIMA-complementary brother. He became febrile from day 2 and erythema appeared diffusely on the generalized skin. He was diagnosed to have hyperacute GVHD. His skin GVHD deteriorated thus leading to a diagnosis of grade III acute GVHD. Bohrus methylprednisolone therapy could not improve the symptoms of acute GVHD. As a result, the patient died of thrombotic microangiopathy associated with acute GVHD on day 47.

Results and discussion

This study is observational. The incidence of grade II to IV acute GVHD and graft failure in patients who were transplanted from HLA-haploidentical NIMA-complementary siblings has been reported to be 40–50% and 0–18% respectively (3, 5). Based on the results of these studies, HLA-haploidentical siblings whose NIMA is complementary to that of a patient are thought to be a possible donor candidate when HLA-matched donors are unavailable. However, our experience of hyperacute GVHD and graft rejection in the present report raises a concern about the efficacy of HLA-mismatched SCT from NIMA-complementary siblings.

Tables 1 and 2 summarize the patient characteristics and outcome of SCT for the three patients. Although the HLA disparity was one locus in the GVH direction in case 1, acute GVHD appeared on day 8 before

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Table 1 Patient Characteristics

Case	Age	Sex	Diagnosis	Status at SCT	Preconditioning regimen	GVHD prophylaxis	CD34+ cells ($\times 10^6$/kg)	HLA (A, B, DR)		Microchimerism	
								Recipient	Donor	patient	Donor
1	27	M	CML	BC, relapse after UR-BMT	Flu 150 mg/m^2 + BU 8 mg/kg + ATG 40 mg/kg	CSA	12.5	2/-, 51/38, 4/8	2/33, 51/44, 4/8	ND	ND
2	15	F	AA	Refractory to immunosuppressive therapy	Flu 150 mg/m^2 + CY 120 mg/kg + ATG 25 mg/kg + TBI 2Gy	CSA + sMTX	1.4	11/-, 55/67, 4/-	11/24, 52/67, 4/15	+	+
3	32	M	Ph + ALL	Resistant	TBI 12 Gy + CY 120 mg/kg	FK506 + sMTX	3.1	2/-, 52/51, 9/8	2/11, 51/60, 8/14	-	+

CML = chronic myeloid leukemia; AA = aplastic anemia; Ph + ALL = Philadelphia chromosome positive acute lymphoblastic leukemia; BC = blastic crisis; UR-BMT = unrelated bone marrow transplantation; Flu = fludarabine; BU = busulfan; ATG = antithymocyte globulin; TBI = total body irradiation; CY = cyclophosphamide; CSA = cyclosporine; FK506 = tacrolimus; sMTX = short term methotrexate; NIMA = non-inherited maternal antigens; ND = not done.

Table 2 Clinical outcome

Case	Engraftment		aGVHD		GVHD stage	Treatment of GVHD	Complications	Outcome	Survival after SCT (d)
	Neu (d)	Plt (d)	Onset (d)	Grade					
1	9	9	8	3	Skin 2, Liver 2	2 mg/kg of mPSL started on day 9, 15 mg/m^2 of MTX on day 11 and 1000 mg/d of MMF started on day15	-	Relapse on day 131	203
2	22	NR	-	-	-	-	HPS on day 23, graft rejection on day 27	CBT on day 89	993+
3	13	15	11	4	Skin 4, Liver 4, Gut 3	1 g/d of mPSL for 3 d	TMA, convulsion	Death by GVHD	47

Neu = neutrophil; Plt = platelet; G = granulocyte; T = T lymphocyte; aGVHD = acute graft-versus-host disease; HPS = homophagocytic syndrome; SCT = stem cell transplantation; mPSL = methylprednisolone; MTX = methotrexate; MMF = mycophenolate mofetil; TMA = thrombotic microangiopathy; CMV = cytomegalovirus.

neutrophil engraftment and rapidly progressed to grade III. Case 3 also developed hyperacute GVHD despite the fact that microchimerism was documented in the donor's blood. Acute GVHD is known to occur frequently before engraftment of neutrophil in recipient of HLA-mismatched SCT (6, 7). In the analysis of SCTs between NIMA-complementary family members described by Ichinohe *et al.* (5), the presence of acute GVHD was observed from day 10. The presence of the recipient-specific microchimerism did not necessary predict low incidence of acute GVHD in this study, in line with our experience. Our findings suggest the necessity of intensive immunosuppressive therapy to prevent acute GVHD such as ATG (9, 10) or alemtuzumab (11) even when a donor shows recipient-specific microchimerism. As case 1 had received HLA-matched unrelated bone marrow transplantation before undergoing the second SCT from a NIMA complementary sibling, recipient dendritic cells were probably replaced by the cells of the unrelated donor. The dendritic cells of a recipient play an important role in the development of acute GVHD (12, 13). The absence of the patient-derived dendritic cells, which were educated to tolerate donor T cells, may be responsible for hyperacute GVHD of the patients.

Case 2 was conditioned with fludarabine-based regimen which was known to ensure engraftment of bone marrow from HLA-matched unrelated donors in AA patients (14). Microchimerism by donor cells was documented in the patient. Nevertheless, SCT from the donor ended up with secondary graft rejection. Although the number of of $CD34^+$ cells infused (1.4×10^6/kg) was relatively low, the minimal number of $CD34^+$ cells in the successful SCT was 1.26×10^6/kg in the report by Shimazaki *et al.* (4) and 1.3×10^6/kg in the report by Ichinohe *et al.* (5). It is therefore necessary to intensify the conditioning regimen to prevent graft rejection when HLA-mismatched SCTs from NIMA-complementary siblings are administered to patients with AA even if microchimerism by donor cells is documented in the recipient.

References

1. Anasetti C, Amos D, Beatty PG, Appelbaum FR, Bensinger W, Buckner CD, Clift R, Doney K, Martin PJ, Mickelson E, *et al.* Effect of HLA compatibility on engraftment of bone marrow transplants in patients with leukemia or lymphoma. *N Engl J Med* 1989;**320**:197–204.
2. Beatty PG, Clift RA, Mickelson EM, Nisperos BB, Flournoy N, Martin PJ, Sanders JE, Stewart P, Buckner CD, Storb R, *et al.* Marrow transplantation from related donors other than HLA-identical siblings. *N Engl J Med* 1985;**313**:765–71.
3. van Rood JJ, Loberiza FR Jr, Zhang MJ, *et al.* Effect of tolerance to noninherited maternal antigens on the occurrence of graft-versus-host disease after bone marrow transplantation from a parent or an HLA-haploidentical sibling. *Blood* 2002;**99**:1572–7.
4. Shimazaki C, Ochiai N, Uchida R, Okano A, Fuchida S, Ashihara E, Inaba T, Fujita N, Maruya E, Nakagawa M. Non-T-cell-depleted HLA haploidentical stem cell transplantation in advanced hematologic malignancies based on the feto-maternal microchimerism. *Blood* 2003;**101**:3334–6.
5. Ichinohe T, Uchiyama T, Shimazaki C, *et al.* Feasibility of HLA-haploidentical hematopoietic stem cell transplantation between noninherited maternal antigen (NIMA)-mismatched family members linked with long-term fetomaternal microchimerism. *Blood* 2004;**104**:3821–8.
6. Sullivan KM, Deeg HJ, Sanders J, Klosterman A, Amos D, Shulman H, Sale G, Martin P, Witherspoon R, Appelbaum F, *et al.* Hyperacute graft-v-host disease in patients not given immunosuppression after allogeneic marrow transplantation. *Blood* 1986;**67**:1172–5.
7. Kim DH, Sohn SK, Kim JG, Suh JS, Lee KS, Lee KB. Clinical impact of hyperacute graft-versus-host disease on results of allogeneic stem cell transplantation. *Bone Marrow Transplant* 2004;**33**:1025–30.
8. Tokita K, Terasaki P, Maruya E, Saji H. Tumour regression following stem cell infusion from daughter to microchimeric mother. *Lancet* 2001;**358**:2047–8.
9. Lu DP, Dong L, Wu T, *et al.* Conditioning including antithymocyte globulin followed by unmanipulated HLA-mismatched/haploidentical blood and marrow transplantation can achieve comparable outcomes with HLA-identical sibling transplantation. *Blood* 2006;**107**:3065–73.
10. Aversa F, Terenzi A, Tabilio A, *et al.* Full haplotype-mismatched hematopoietic stem-cell transplantation: a phase II study in patients with acute leukemia at high risk of relapse. *J Clin Oncol* 2005;**23**:3447–54.
11. Kanda Y, Oshima K, Asano-Mori Y, *et al.* In vivo alemtuzumab enables haploidentical human leukocyte antigen-mismatched hematopoietic stem-cell transplantation without ex vivo graft manipulation. *Transplantation* 2005;**79**:1351–7.
12. Wright-Browne V, McClain KL, Talpaz M, Ordonez N, Estrov Z. Physiology and pathophysiology of dendritic cells. *Hum Pathol* 1997;**28**:563–79.
13. Shlomchik WD, Couzens MS, Tang CB, McNiff J, Robert ME, Liu J, Shlomchik MJ, Emerson SG. Prevention of graft versus host disease by inactivation of host antigen-presenting cells. *Science* 1999;**285**:412–5.
14. Bacigalupo A, Locatelli F, Lanino E, Marsh J, Socie G, Maury S, Prete A, Locasciulli A, Cesaro S, Passweg J. Fludarabine, cyclophosphamide and anti-thymocyte globulin for alternative donor transplants in acquired severe aplastic anemia: a report from the EBMT-SAA Working Party. *Bone Marrow Transplant* 2005;**36**:947–50.

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