of nosocomial infections due to the renovation of transplant buildings[10,11] and via the water system in transplant wards has been reported.[12] The CDC guidelines recommend the use of air conditioning systems/tools.[3] However, the guidelines may have become outdated due to changing circumstances. First, the timing of the development of aspergillosis has changed. Most *Aspergillus* infections occur late beyond 100 days after transplantation.[13] This trend may be enhanced by the wider application of reduced-intensity stem cell transplantation (RIST) on an outpatient basis.[14] Second, newly developed antifungal agents have been shown to be effective in the prevention of *Aspergillus* infection.[15,16] Moreover, a recent drastic change is that RIST has been extended to unrelated transplantation,[17] umbilical cord blood transplantation[18] and elderly patients.[19] RIST has the advantage of a shorter period of neutropenia, and it is likely that the incidence of *Candida* infection is reduced. On the other hand, the incidence of steroid-therapy requiring GVHD remains the same as in conventional transplantations, and the intensity of immune suppression is similar.[20] It is expected that late-occurring fungal infections by *Aspergillus*, etc may become a significant problem after RIST.[21-23]

Thus, a re-evaluation and update of the guidelines appears to be necessary, particularly in the area of RIST. There have been only a few studies on fungal infections after RIST.[21-23] The objective of this study was to survey antifungal prophylaxis in Japanese transplantation practice, and to compare the findings in RIST, conventional allogeneic transplantation (CST), and autologous transplantation.

Patients and methods

Data collection

Of the 418 Japanese medical institutions in which HSCT is performed, 122 institutions agreed to participate in this survey. Each of these institutions received a questionnaire that included the following items: the number of transplantations performed in 2001, the type of the patient's disease and the type of transplantation, use of a clean room or designated room for transplantation, practice regarding prophylactic use of antifungal agents, and occurrence of systemic fungal infections. For cases that developed deep fungal infections, age, sex, date of onset, diagnostic approaches, infected organs, pathogens, neutrophil count at onset, coexistence of GVHD, use of immunosuppressants, prophylactic use of antifungal agents, and patient outcome were also recorded.

We used the EORTC/NIH-MSG criteria for the diagnosis of deep fungal infections.[24] We defined both proven and probable infectious cases as deep fungal infection.

Antifungal prophylaxis measures

We defined the nine measures listed below as measures for preventing fungal infection. All of the methods were evaluated as A or B based on the evidence level in the

CDC guidelines, as previously reported.[25] The guidelines use a combination of category (A–D) and the recommendation (I–III). We used two recommendation levels, A and B, which indicated strong or moderate evidence supported by well-established clinical trials (I or II) or respected authorities (III).

(a) Yeast infection following allogeneic HSCT

(a1) Medical staff in contact with HSCT recipients should follow appropriate hand-washing practices to safeguard patients from exposure (Evidence level A III).

(a2) Fluconazole (400 mg/day, orally or intravenously) should be administered from the day of transplant until engraftment (Evidence level A II).

(b) Mold infections following allo-SCT

(b3) HSCT recipients who remain immunocompromised should avoid hospital construction or renovation areas (Evidence level A III).

(b4) Use of high efficiency particulate air (HEPA) filtration (Evidence level B III).

(b5) Air should flow from patient rooms to corridors (Evidence level B III).

(b6) Correctly sealed rooms, including correctly sealed windows and electrical outlets (Evidence level B III).

(b7) High rates of room air exchange (ie, >12 air changes/h) (Evidence level B III).

(b8) Barriers between patient care areas and renovation or construction areas to prevent dust from entering patient care areas. The barriers must be impermeable to Aspergillus species.

(c) Autologous HSCT

(c9) Autologous HSCT recipients generally are at lower risk of invasive fungal infection than allogeneic HSCT recipients. Autologous HSCT recipients do not require routine intense anti-yeast prophylaxis. Nevertheless, some researchers recommend the use of an anti-yeast prophylaxis in patients who have underlying hematologic malignancies, and who have or will have prolonged neutropenia and mucosal damage from intense conditioning regimens or graft manipulation, or who have recently received purine analogues (Evidence level B III). Regarding mold infections, no guideline has been reported for autologous HSCT.

Prophylaxis for deep fungal infections in Japan

In Japan, the antifungal agents approved for the treatment of deep fungal infections in 2001 included amphotericin B, fluconazole, itraconazole, miconazole and terbinafine. With the exception of itraconazole, they can be administered either orally or intravenously. The only available formula of itraconazole was a capsule, and in this form, absorption through the gastrointestinal tract is inconsistent.[26] Itraconazole oral solution, voriconazole, liposomal amphotericin B and echinocandin have not yet been approved.

Governmental approval has not yet been granted for the prophylactic use of any antifungal agents, although this is widely applied as a practice in most medical institutions.[27] Fluconazole, up to 400 mg, is approved only for the treatment of fungal infection.

Use of a clean room within a transplant ward is reimbursed by insurance. We considered that prophylactic measures (b3)–(b8) were satisfied if HSCT was performed in a clean room. A clean room was defined as an isolated room equipped with HEPA filtration and air flow toward an exit of the room with sealed windows.

End points and statistical methods

The objective of this study was to conduct a survey of antifungal prophylaxis in HSCT. The levels of compliance with the established prophylactic measures in RIST and CST were compared. The second objective was to compare the CST, RIST and auto-SCT groups with respect to the incidence and characteristics of fungal infections.

We examined (a2) and (b3)–(b8) of the nine items listed above. Item (a1) was excluded from the survey because of inconsistency of proper evaluation in a retrospective survey. We considered that items (b3)–(b8) were satisfied when a clean room was used. We aimed to evaluate differences in fungal prophylaxis between conventional and reduced-intensity transplants. We did not collect detailed information on transplantation procedures such as stem cell sources and drugs used in the preparative regimens. A univariate analysis using Fisher's exact test and the Mann–Whitney U test was performed to compare the differences in prophylactic measures between RIST and CST. Values of $P < 0.05$ were considered significant.

Results

Patients background

We received questionnaires from 63 medical institutions, representing 935 transplantations. In Japan, a total of 1964 transplantations were performed in 2001,[28] and approximately half of the patients were surveyed in this study. The median number of transplantations per institute/year was 10 (range, 1–108). The types of transplantation were autologous HSCT (367, 40%), CST (414, 45%), and RIST (154, 15%). All of the RIST recipients received purine-analog-based preparative regimens with or without low-dose TBI.

Patients' diseases included malignant lymphoma (191 cases, 21%), acute myelocytic leukemia (147, 16%), acute lymphocytic leukemia (107, 11%), multiple myeloma (80, 9%), chronic myelocytic leukemia (74, 8%), myelodysplastic syndrome (51, 5%), solid tumors (50, 5%), aplastic anemia (16, 2%), and others (219, 23%).

All patients were hospitalized during transplantation. The number of autologous HSCT, CST and RIST recipients who were in a clean room at the time of transplantation was 261/367 (71%), 409/414 (99%) and 93/154 (66%), respectively.

Prophylactic use of antifungal agents

All patients including autologous HSCT cases received an antifungal agent as a prophylaxis, and azole antifungal agents were administered in 742 patients (79%). The most commonly used azole antifungal agent was fluconazole (89%). Figure 1 shows the types of agents used in CST, RIST and autologous HSCT.

Fluconazole was administered to 324 (78%) CST and 112 (73%) RIST recipients. The numbers of patients who received fluconazole 400, 200 and 100 mg/day were 135 (42%), 169 (52%) and 20 (6%), respectively. Those patients who were given fluconazole 400, 200 and 100 mg/day were 34 (30%), 70 (63%) and 8 (7%), respectively. Significantly larger doses of fluconazole were used in CST than in RIST ($P = 0.037$).

The numbers of patients who received fluconazole until engraftment and beyond day 75 in the CST and RIST groups were, respectively, 324 (100%) and 39 (12%), and 109 (97%) and 18 (16%). Duration of fluconazole use was not significantly different between CST and RIST.

Incidence and clinical characteristics of deep fungal infections

Of the 935 transplant cases, 37 (4.0%) were diagnosed with deep fungal infections (13 proven and 24 probable cases); 0.03% (1/367) of autologous HSCT, 6.0% (25/414) of CST, and 7.1% (11/154) of RIST. The causative organisms included Candida ($n = 9$), Aspergillus ($n = 16$), Mucor ($n = 1$), Fusarium ($n = 1$) and unknown ($n = 10$). The median onset date from the time of transplant was 85 days (range, 1–392 days): 92 days in CST and 117 days in RIST, with nine cases developing within 30 days of transplant (one case in autologous HSCT, seven in CST and one in RIST). Three cases of 37 deep fungal infections had a previous history of fungal infection. All of them received fluconazole and AMPH-B intravenously as a prophylaxis.
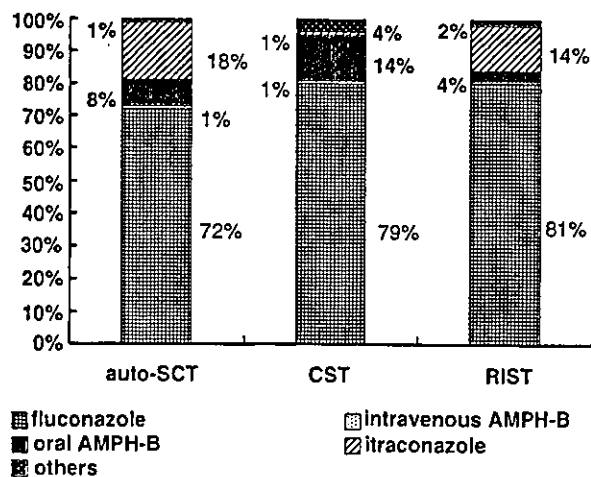

Figure 1 Types of prophylactic antifungal agents used in each type of HSCT. Azole agents were the most-administered drugs for prophylactic use in HSCT patients.

The causative organisms for the nine cases of candidiasis included *C. albicans* (n = 4), *C. glabrata* (n = 2), *C. tropicalis* (n = 1), *C. inconspicua* (n = 1), and a combination of *C. lusitaniae* and *C. guilliermondii* (n = 1). The infected organs included blood (fungemia, six cases), the respiratory system (n = 2) and the liver and spleen (n = 1). Among four patients who were infected with *C. albicans*, two had not received any antifungal agents including fluconazole as a prophylaxis, while the remaining two cases had been on fluconazole prophylaxis and were considered to be prophylaxis failure. Among these four patients, three started or continued to receive fluconazole following infection, and three patients subsequently died.

The causative organisms for the 16 cases of aspergillosis included *Aspergillus* spp. (n = 14), *A. fumigatus* (n = 1) and *A. terreus* (n = 1). Six and 10 of these patients received RIST and CST, respectively. In all, 14 and two patients had received prophylactic fluconazole and itraconazole, respectively. GVHD requiring corticosteroid therapy was documented at the onset of the disease in 13 of the 16 cases. The initially infected organ was the respiratory system in all cases, with a median onset of invasive aspergillosis of 85 days (range, 9–392). Four of the 16 patients were neutropenic at the diagnosis of aspergillosis. Treatment for invasive aspergillosis included intravenous AMPH-B (13 cases), oral itraconazole (two cases), and fluconazole (one case). Eight patients subsequently died without resolution of invasive aspergillosis.

Comparison of CST and RIST recipients

Table 1 shows the characteristics of fungal infections developed in CST and RIST recipients. There was no significant difference between the two groups with respect to the incidence, date of onset or death rate.

Discussion

This study highlights the current problems in antifungal prophylaxis. In Japan, a total of 1964 transplantations were performed in 2001,[28] and about half of these were surveyed in this study. We believe that sufficient data were collected to characterize prophylaxis practice and fungal infection in this country, while it is limited by bias as a retrospective design, and we should be careful in interpreting the results of this study.

With improvements in prophylactic measures, the incidence of early mold infections has decreased,[14,29,30]

and most infections occur late in association with GVHD.[13] The need for a HEPA filter or laminar air flow (LAF) may be questioned in RIST. All transplant wards in Japan, but not all nontransplant wards, are equipped with these systems. Although 99% of CST was performed in transplant wards, this percentage decreased to 71% in autografts and 66% in RIST. The potential economic benefits of RIST may be related to an increase in RIST performed in nontransplant wards without expensive air filtration systems.

In autologous HSCT, the prophylactic use of antifungal agents is recommended only for high-risk patients[3] based on several clinical studies.[4,31] However, this study demonstrates that all of the Japanese autologous HSCT recipients received some prophylactic antifungal agents. This is an overuse of antifungal agents because the overall risk of fungal infection in autologous transplants is lower than allogeneic transplant. There are several possible explanations for this overuse. Since the definition of 'low-risk patients' in the CDC guidelines is unclear, physicians take a more conservative way to reduce the risk of fungal infection. It has been suggested that long-term use of azole antifungal agents may cause resistance.[32] Additionally, azole antifungal agents are expensive, and long-term use is not economical.[33] On the other hand, for the prophylactic use of antifungal agents in allogeneic transplant, the CDC recommends prophylactic fluconazole 400 mg/day during the neutropenic period following transplantation. However, the rationale for the selection of fluconazole from among many other available antifungal agents, its dose setting and prophylaxis period remain unclear. Its usefulness in RIST recipients has not been established.

After the CDC issued its guidelines in 2000, various drugs, including voriconazole, itraconazole (oral solution and intravenous formulation), and echinocandin, have been developed and are now commercially available. Moreover, *Aspergillus* is not susceptible to fluconazole, and recent comparative studies suggest that itraconazole is more effective than fluconazole in the prevention of *Aspergillus* infection.[15,34] Hence, the recommendation of fluconazole over any other agent may no longer be defensible. Nevertheless, this study clearly documented that fluconazole was used in 75% of allogeneic transplant in Japan. This bias is likely due to the CDC recommendation and to the fact that an alternative antifungal agent is not commercially available in Japan. As of June 2003, the only available itraconazole formula in Japan is a capsule, which carries an obvious risk of inadequate

Table 1   Comparison on clinical features of deep fungal infection between CST and RIST

		CST (n = 414)	RIST (n = 154)
Number of deep fungal infection		26 (6.3%)	11 (7.1%)
Median onset (days after transplant) (range)		77 (1–392)	117 (28–182)
Presence of GVHD at the onset of fungal infection	Present/absent	12/14	9/2
Presence of neutropenia at the onset of fugal infection[a]	Present/absent	8/18	1/10
Use of corticosteroid at the onset of fungal infection	Yes/no	15/11	7/4
Causative organisms	Aspergillus/Candida/Others	17/7/2	9/1/1
Mortality		81%	82%

[a]Neutropenia was defined as neutrophils below 0.5×10^9/l.

absorption of the drug. Voriconazole and caspofungin have not yet been approved, and micafungin was approved only recently. Regarding *Aspergillus* infections, it is difficult to conclude that the CDC's recommendation for the prophylactic administration of fluconazole is useful. With the present availability of all of these alternative agents, a comparative study to identify a suitable procedure will be required.

While all of the patients received prophylactic fluconazole at least until engraftment, only 44% received the recommended dose of 400 mg/day. Moreover, only 20% of both the CST and RIST recipients received fluconazole beyond 75 days following transplantation, as recommended in a previous study.[6] It has been reported that *C. albicans* can be controlled at a lower dose of 200 mg/day.[35,36] Many physicians believe that 400 mg of fluconazole is not required for prophylactic use, and optimal duration of fluconazole prophylaxis remains to be established. Since fluconazole is expensive and costs about 100 000 yen ($850) when used at 400 mg/day to cover from the commencement of pre-transplant treatment and engraftment, validation of the adequate dose and the duration for prophylactic use is important.

With an increasing number of patients undergoing transplant, establishment of fungal management is important in RIST. The practice for the prevention and treatment of fungal infection varies among institutions. Mortality of invasive aspergillosis was 50% in this survey, which were far lower than reported previously.[1] The differences might be attributable to diagnostic approaches. In Japan, diagnostic measures using computed tomography and blood tests such as beta-D-glucan assay or an enzyme-linked immunosorbent test detecting galactomannan antigen are widely used.[37-39] These tests might have contributed to make an early diagnosis of aspergillosis, improving its prognosis. These situations are similar to antifungal prophylaxis. The guidelines for antifungal prophylaxis, which were prepared based on previous clinical studies, should be updated, since the circumstances surrounding transplantation have been changing. However, there are little data to make new recommendations for guidelines of antifungal prophylaxis, and more information is needed regarding fungal infections following RIST. Further investigation is needed to determine what measures are effective to accommodate the changes in transplant practices.

Acknowledgements

References

1 Wingard JR. Fungal infections after bone marrow transplant. *Biol Blood Marrow Transplant* 1999; 5: 55–68.

2 Cornely OA, Ullmann AJ, Karthaus M. Evidence-based assessment of primary antifungal prophylaxis in patients with hematologic malignancies. *Blood* 2003; 101: 3365–3372.

3 Guidelines for preventing opportunistic infections among hematopoietic stem cell transplant recipients. *Biol Blood Marrow Transplant* 2000; 6(6a): 659–713; 715; 717–727; quiz 729–733.

4 Goodman JL, Winston DJ, Greenfield RA et al. A controlled trial of fluconazole to prevent fungal infections in patients undergoing bone marrow transplantation. *N Engl J Med* 1992; 326: 845–851.

5 Slavin MA, Osborne B, Adams R et al. Efficacy and safety of fluconazole prophylaxis for fungal infections after marrow transplantation – a prospective, randomized, double-blind study. *J Infect Dis* 1995; 171: 1545–1552.

6 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: 2055–2061.

7 Marr KA, Seidel K, White TC et al. Candidemia in allogeneic blood and marrow transplant recipients: evolution of risk factors after the adoption of prophylactic fluconazole. *J Infect Dis* 2000; 181: 309–316.

8 Wingard JR, Merz WG, Rinaldi MG et al. Increase in *Candida krusei* infection among patients with bone marrow transplantation and neutropenia treated prophylactically with fluconazole. *N Engl J Med* 1991; 325: 1274–1277.

9 Kami M, Machida U, Okuzumi K et al. Effect of fluconazole prophylaxis on fungal blood cultures: an autopsy-based study involving 720 patients with haematological malignancy. *Br J Haematol* 2002; 117: 40–46.

10 Loo VG, Bertrand C, Dixon C et al. Control of construction-associated nosocomial aspergillosis in an antiquated hematology unit. *Infect Control Hosp Epidemiol* 1996; 17: 360–364.

11 Oren I, Haddad N, Finkelstein R et al. Invasive pulmonary aspergillosis in neutropenic patients during hospital construction: before and after chemoprophylaxis and institution of HEPA filters. *Am J Hematol* 2001; 66: 257–262.

12 Anaissie EJ, Stratton SL, Dignani MC et al. Pathogenic molds (including *Aspergillus* species) in hospital water distribution systems: a 3-year prospective study and clinical implications for patients with hematologic malignancies. *Blood* 2003; 101: 2542–2546.

13 Marr KA, Carter RA, Boeckh M et al. Invasive aspergillosis in allogeneic stem cell transplant recipients: changes in epidemiology and risk factors. *Blood* 2002; 100: 4358–4366.

14 McSweeney PA, Niederwieser D, Shizuru JA et al. Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects. *Blood* 2001; 97: 3390–3400.

15 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: 705–713.

16 Herbrecht R, Denning DW, Patterson TF et al. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. *N Engl J Med* 2002; 347: 408–415.

17 Bornhauser M, Thiede C, Platzbecker U et al. Dose-reduced conditioning and allogeneic hematopoietic stem cell transplantation from unrelated donors in 42 patients. *Clin Cancer Res* 2001; 7: 2254–2262.

18 Barker JN, Weisdorf DJ, DeFor TE et al. Rapid and complete donor chimerism in adult recipients of unrelated donor umbilical cord blood transplantation after reduced-intensity conditioning. *Blood* 2003; 102: 1915–1919.

19 Childs R, Chernoff A, Contentin N et al. Regression of metastatic renal-cell carcinoma after nonmyeloablative allogeneic peripheral-blood stem-cell transplantation. *N Engl J Med* 2000; 343: 750–758.

20 Mielcarek M, Martin PJ, Leisenring W et al. Graft-versus-host disease after nonmyeloablative versus conventional hemato-poietic stem cell transplantation. Blood 2003; 102: 756–762.

21 Fukuda T, Boeckh M, Carter RA et al. Invasive fungal infections in recipients of allogeneic hematopoietic stem cell transplantation after nonmyeloablative conditioning: risks and outcomes. Blood 2003; e-pub.

22 Hagen EA, Stern H, Porter D et al. High rate of invasive fungal infections following nonmyeloablative allogeneic trans-plantation. Clin Infect Dis 2003; 36: 9–15.

23 Kojima R, Kusumi E, Nannya Y et al. Invasive pulmonary aspergillosis (IPA) after reduced-intensity hematopoietic stem cell transplantation (RIST). Blood 2002; 100: 438b.

24 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.

25 USPHS/IDSA. 1999 USPHS/IDSA guidelines for the preven-tion of opportunistic infections in persons infected with human immunodeficiency virus. U.S. Public Health Service (USPHS) and Infectious Diseases Society of America (IDSA). MMWR Recomm Rep 1999; 48 (RR-10): 1–59, 61–66.

26 Kanda Y, Kami M, Matsuyama T et al. Plasma concen-tration of itraconazole in patients receiving chemotherapy for hematological malignancies: the effect of famotidine on the absorption of itraconazole. Hematol Oncol 1998; 16: 33–37.

27 Yoshida M, Tsubaki K, Kobayashi T et al. Infectious complications during remission induction therapy in 577 patients with acute myeloid leukemia in the Japan Adult Leukemia Study Group studies between 1987 and 1991. Int J Hematol 1999; 70: 261–267.

28 Kojima R, Kusumi E, Nannya Y et al. Invasive pulmonary aspergillosis (IPA) after reduced-intensity hematopoietic stem cell transplantation (RIST). Blood 2002; 100: 438b.

29 Ruiz-Arguelles GJ, Gomez-Almaguer D, Ruiz-Arguelles A et al. Results of an outpatient-based stem cell allotransplant program using nonmyeloablative conditioning regimens. Am J Hematol 2001; 66: 241–244.

30 Gonzalez-Ryan L, Haut PR, Coyne K et al. Developing a pediatric outpatient transplantation program. The Children's Memorial Hospital experience. Front Biosci 2001; 6: G1–G5.

31 Rotstein C, Bow EJ, Laverdiere M et al. Randomized placebo-controlled trial of fluconazole prophylaxis for neutropenic cancer patients: benefit based on purpose and intensity of cytotoxic therapy. The Canadian Fluco-nazole Prophylaxis Study Group. Clin Infect Dis 1999; 28: 331–340.

32 White A, Goetz MB. Azole-resistant Candida albicans: report of two cases of resistance to fluconazole and review. Clin Infect Dis 1994; 19: 687–692.

33 Dranitsaris G, Phillips P, Rotstein C et al. Economic analysis of fluconazole versus amphotericin B for the treatment of candidemia in non-neutropenic patients. Pharmacoeconomics 1998; 13: 509–518.

34 Marr KA, Hoyle M, Balajee A et al. Itraconazole vs fluconazole for antifungal prophylaxis in allogeneic HSCT recipients: results of a randomized trial. Blood 2002; 100: 215a.

35 Kami M, Sawada Y, Mori S et al. Serum levels of fluconazole in patients after cytotoxic chemotherapy for hematological malignancy. Am J Hematol 2001; 66: 85–91.

36 MacMillan ML, Goodman JL, DeFor TE et al. Fluconazole to prevent yeast infections in bone marrow transplantation patients: a randomized trial of high versus reduced dose, and determination of the value of maintenance therapy. Am J Med 2002; 112: 369–379.

37 Kami M, Tanaka Y, Kanda Y et al. Computed tomographic scan of the chest, latex agglutination test and plasma (1-3)-beta-D-glucan assay in early diagnosis of invasive pulmonary aspergillosis: a prospective study of 215 patients. Haemato-logica 2000; 85: 745–752.

38 Obayashi T, Yoshida M, Mori T et al. Plasma $(1 \rightarrow 3)$-beta-D-glucan measurement in diagnosis of invasive deep mycosis and fungal febrile episodes. Lancet 1995; 345: 17–20.

39 Maertens J, Verhaegen J, Lagrou K et al. Screening for circulating galactomannan as a noninvasive diagnostic tool for invasive aspergillosis in prolonged neutropenic patients and stem cell transplantation recipients: a prospective validation. Blood 2001; 97: 1604–1610.

Appendix

This study was conducted at the following institutions under the auspices of the following investigators in Japan:

Masaya Mukai (Sapporo City General Hospital, Hok-kaido), Aoyagi Yumei (Dokkyo University School of Medicine, Tochigi), Kazuaki Yakushiji (Kurume Univer-sity School of Medicine, Fukuoka), Hisashi Tsurumi (Gifu University School of Medicine, Gifu), Yukiyoshi Moriuchi (Sasebo City General Hospital, Nagasaki), Shin Imamura (Fukui Medical University, Fukui), Hiroatu Ago (Shimane Prefectural Central Hospital, Shimane), Sachiko Suzuki (Hakodate Central General Hospital, Hokkaido), Akira Yokota (Chiba Aoba Municipal Hospital, Chiba), Hideki Mitsui (Osaka Medical Center for Cancer and Cardiovas-cular Diseases, Osaka), Yuichi Ishikawa (Chukyo Hospital, Aichi), Yasushi Takamatsu (Fukuoka University School of Medicine, Fukuoka), Kazutaka Sunami (National Okaya-ma Medical Center, Okayama), Nobuo Masauzi (Hako-date Manucipal Hospital, Hokkaido), Tsunehiko Komatsu (Tsukuba Memorial Hospital, Ibaraki), Tadao Ishida (Sapporo Medical University School of Medicine, Hokkai-do), Hideo Hyodo (Hiroshima University Research In-stitute for Radiation Biology and Medicine, Hiroshima), Hiroto Kaneko (Aiseikai-Yamashina Hospital, Kyoto), Hidetaka Takimoto (Kochi Municipal Central Hospital, Kochi), Tadasu Tobita (Yakizu City Hospital, Shiga), Tsutomu Kato (Toyama Medical and Pharmaceutical University, Toyama), Eiichi Ohtsuka (Oita Medical Uni-versity, Oita), Hideo Kimura (Kita-Fukushima Medical Center, Fukushima), Kimiharu Uozumi (Kagoshima Uni-versity, Faculty of Medicine, Kagoshima), Toshiro Ito (Shinshu University, The Department of Medicine, Nagano), Masami Inoue (Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka), Yoshinao Yamamoto (Kishiwada City Hospital, Osaka), Shoichi Doi (Kyoto-Katsura Hospital, Kyoto), Yasuhiko Miyazaki (Kansai Medical University, Osaka), Jyunichi Yamagami (Saitama Prefectural Children Medical Center, Saitama), Toshiharu Tamaki (Rinku General Medical Center, Osaka), Yujiro Yamano (Kyushu Kosei-Nenkin Hospital, Fukuoka), Makoto Hirokawa (Akita University School of Medicine, Akita), Shuji Ozaki (Tokushima University Hospital, Tokushima), Koichiro Muta (Kyushu University Graduate School of Medical Science, Fukuoka), Shuichi Hanada (National Kyushu Cardiovascular Center, Fukuoka), Nobuhiko

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Comparative analysis of clinical outcomes after allogeneic bone marrow transplantation *versus* peripheral blood stem cell transplantation from a related donor in Japanese patients

Tetsuya E. Tanimoto,[1] Takuhiro
Yamaguchi,[2] Yuji Tanaka,[3] Akiko Saito,[2]
Kinuko Tajima,[4] Takahiro Karasuno,[5]
Masanobu Kasai,[6] Kenji Kishi,[7] Takehiko
Mori,[8] Nobuo Maseki,[9] Satoko
Morishima,[10] Shigesaburo Miyakoshi,[11]
Masaharu Kasai,[12] Yuju Ohno,[13]
Sung-Won Kim,[4] Akihiko Numata,[1]
Masahiro Kami,[4] Yoichi Takaue,[4] Shin-
ichiro Mori[3] and Mine Harada[1]

[1]*First Department of Internal Medicine (Medicine and Biosystemic Science), Kyushu University Graduate School of Medical Sciences, Fukuoka,* [2]*Department of Biostatistics, School of Health Sciences and Nursing, University of Tokyo, Tokyo,* [3]*Haematology Division, Tokyo Metropolitan Komagome Hospital, Tokyo,* [4]*Stem Cell Transplant Unit, National Cancer Centre Hospital, Tokyo,* [5]*Fifth Department of Internal Medicine, Osaka Medical Centre for Cancer and Cardiovascular Diseases, Osaka,* [6]*Department of Internal Medicine, Japanese Red Cross Nagoya First Hospital, Aichi,* [7]*Division of Haematology/Oncology, Tokai University School of Medicine, Kanagawa,* [8]*Division of Haematology, Department of Medicine, Keio University School of Medicine, Tokyo,* [9]*Haematology Division, Saitama Cancer Centre Hospital, Saitama,* [10]*Haematology Division, Meitetsu Hospital, Aichi,* [11]*Haematology Division, Toranomon Hospital, Tokyo,* [12]*Research Institute for Artificial Organs, Transplantation and Gene Therapy, Sapporo Hokuyu Hospital, Hokkaido, and* [13]*Department of Internal Medicine, Kitakyushu Municipal Medical Centre, Fukuoka, Japan*

Correspondence: Tetsuya E. Tanimoto MD, First Department of Internal Medicine (Medicine and Biosystemic Science), Kyushu University Graduate School of Medical Sciences, Maidashi 3-1-1, Higashi-Ku, Fukuoka 812-8582, Japan. E-mail: tetanimot@yahoo.co.jp

Summary

A reduced incidence of graft *versus* host disease (GvHD) has been documented among Japanese allogeneic bone marrow transplantation (BMT) patients, as the Japanese are genetically more homogeneous than western populations. To clarify whether this ethnic difference affects the results of allogeneic peripheral blood stem cell transplantation (PBSCT), we conducted a nationwide survey to compare clinical outcomes of allogeneic PBSCT ($n = 214$) and BMT ($n = 295$) from a human leucocyte antigen-identical-related donor in Japanese patients. The cumulative incidence of grades II–IV acute GvHD was 37·4% for PBSCT and 32·0% for BMT. The cumulative incidence of extensive chronic GvHD at 1 year was significantly higher after PBSCT than BMT (42% vs. 27%; $P < 0·01$). The organ involvement patterns of GvHD were different between the two groups. By multivariate analyses, the incidence of chronic GvHD was significantly increased in PBSCT, whereas the stem cell source did not affect the incidence of acute GvHD, transplant-related mortality, relapse or survival. We concluded that Japanese PBSCT patients have an increased risk of chronic GvHD compared with BMT patients, but the incidence of acute GvHD was still lower than in western populations. Thus, the choice of haematopoietic stem cell source should be considered based on data for individual ethnic populations.

Keywords: Japanese, marrow transplantation, stem cell transplantation, graft *versus* host disease.

During the past decade, peripheral blood stem cell transplantation (PBSCT) has been explored in the autologous as well as the allogeneic haematopoietic stem cell transplantation (HSCT) setting as an alternative to bone marrow transplantation (BMT). Although there were some inconsistencies in the early reports, it appears that haematological recovery is faster, but the incidence of acute graft versus host disease (GvHD) is similar, and chronic GvHD is more frequent in allogeneic PBSCT patients than in BMT patients (Schmitz et al, 1998, 2002; Blaise et al, 2000; Champlin et al, 2000; Heldal et al, 2000; Powles et al, 2000; Bensinger et al, 2001; Cutler et al, 2001; Couban et al, 2002; Ringden et al, 2002). Additionally, some investigators have reported improved survival after PBSCT compared with BMT (Powles et al, 2000; Bensinger et al, 2001; Couban et al, 2002).

Although a number of small prospective randomized-controlled trials (RCTs) have been published, cautious interpretation is required because the primary end points of these studies were safety (Schmitz et al, 1998), engraftment (Blaise et al, 2000; Heldal et al, 2000; Powles et al, 2000) and equivalency of acute GvHD (Bensinger et al, 2001). Because of the small sample size in these studies, the statistical power was not enough to detect differences in important, clinically relevant outcomes between PBSCT and BMT, such as chronic GvHD, relapse rate, transplant-related mortality (TRM) and survival. In an attempt to clarify this, several large RCTs and meta-analyses have recently been published (Cutler et al, 2001; Couban et al, 2002; Schmitz et al, 2002; Horan et al, 2003).

However, findings in western populations cannot be directly transferred to other ethnic populations, where the incidence of GvHD differs. Most previous studies that compared BMT and PBSCT were from western countries. While detailed information on the ethnics of the study population was not provided, most patients would have been Caucasian. In Japanese BMT patients, the incidence of acute GvHD is considered to be lower than in western countries because of the relative genetic homogeneity of the population (Morishima et al, 1989; Oh et al, 2002; Lin et al, 2003). Whether this ethnic difference also affects the results of PBSCT, as reflected in differences in the incidence of GvHD, relapse and survival, has not been established. Apart from the intense eradication of malignant cells by the conditioning regimen, the main therapeutic benefit of allogeneic HSCT relies on the induction of immune-mediated graft versus leukaemia (GVL) effect (Horowitz et al, 1990). This GVL effect may also have a different impact in different ethnic groups. Therefore, to survey outcomes after allogeneic HSCT in Japan, we conducted a retrospective, multi-centre study comparing allogeneic PBSCT with BMT from a human leucocyte antigen (HLA)-identical related donor in 509 patients with leukaemia or myelodysplastic syndrome (MDS). We also aimed to determine the impact of GvHD on relapse and survival after transplantation.

Patients and methods

Methods

Transplantation centres across Japan were contacted and asked to provide data on all consecutive allogeneic HSCT from a family donor using report forms with specific addenda. Recipients of T-cell-depleted blood stem cell transplants, those receiving reduced-intensity stem cell transplantation, and those who had received bone marrow together with PBSC were not reported. Between January 1999 and October 2001, a total of 629 adult patients with leukaemia or MDS received a myeloablative preparative regimen and allogeneic BMT or PBSCT from an HLA-identical-related donor (matched at HLA-A, -B, -DR by serological or molecular testing) in 82 participating centres (Appendix A). Patients who did not receive GvHD prophylaxis using ciclosporin A (CsA) and methotrexate (MTX) ($n = 41$), those who did not receive granulocyte colony-stimulating factor (G-CSF) postallograft ($n = 75$), those who had undergone autografting previously ($n = 3$) and those who had double cancer ($n = 3$) were excluded. Finally, a total of 509 patients were included in this analysis. The stem cell source was decided according to the protocol of each transplantation centre. The medical records were reviewed retrospectively for patients' demographic data, date of engraftment, onset of acute and chronic GvHD, grading and organ involvement from the date of transplantation to the date of death or last contact. Computerized error checks and physician review of submitted data were performed to ensure data quality.

End point definitions

End points were assessed on the date of last patient contact and were analysed as of 31 May 2002. The study focused on haematopoietic recovery, acute and chronic GvHD, target organs of GvHD, TRM, progression-free survival (PFS) and overall survival (OS) after PBSCT compared with BMT. The day of neutrophil engraftment was defined as the first of three consecutive days on which the patient's absolute neutrophil count was above $0.5 \times 10^9/l$. The day of platelet engraftment was defined as the first of seven consecutive days on which the platelet count was above $20 \times 10^9/l$ without platelet transfusion. Engraftment failure was diagnosed as when engraftment was not achieved at any time after transplantation. The diagnosis of GvHD was based on clinical evidence with histological confirmation whenever possible. Acute GvHD within the first 100 d after transplantation was graded according to standard criteria by attending physicians of each hospital (Przepiorka et al, 1995). Patients who survived at least 100 d without relapse or disease progression, with sustained donor engraftment, were evaluated for chronic GvHD. Chronic GvHD was graded as limited (localized skin or single organ involvement) or clinically extensive (Shulman et al, 1980).

Patients without GvHD were censored at the time of relapse, disease progression, death or last follow-up. GvHD after donor leucocyte infusion was not included in this analysis.

Standard risk diseases were defined as acute myeloid leukaemia (AML) or acute lymphoblastic leukaemia (ALL) in first remission; chronic myeloid leukaemia (CML) in chronic phase; and refractory anaemia without excess of blasts (Bensinger *et al*, 2001). All other stages of these diseases and all other types of leukaemia were considered as high risk. The Eastern Cooperative Oncology Group (ECOG) scale was used to evaluate performance status (PS) at the time of transplantation. PFS was measured as the time from the day of transplantation until disease relapse or progression, death from any cause or second transplantation for graft failure or rejection. Both relapse and progression were defined as disease progression with TRM being censored. TRM included all causes of death other than disease progression or relapse occurring at any time after transplantation. Reported causes of death were reviewed and categorized. Patients who died as a result of relapse or disease progression after transplantation were considered to have died of their original disease. Similarly, patients who died of active GvHD were considered to have died of this complication even if other complications (e.g. infection) were recorded as the proximate cause. All deaths were considered for estimating the OS.

Statistical analysis

The primary end point of the comparison was the cumulative incidence of acute and chronic GvHD. The secondary end points included the incidence of relapse, TRM, PFS and OS. The following patient or transplant characteristics were analysed for their prognostic value on each of the outcomes: patient and donor age (less than or more than 40 years), sex, sex matching, ECOG PS, disease risk, cytomegalovirus serology, stem cell source, conditioning regimen and doses of MTX. To compare the two groups of patients receiving PBSC or BM, we used the chi-square test for categorical variables and the non-parametric Mann–Whitney *U*-test for ordered categorical and continuous variables. The unadjusted probabilities of PFS and OS were estimated from the time of transplantation using the Kaplan–Meier product limit method, according to the risk group, and 95% confidence intervals (CIs) were calculated using the Greenwood formula (Kaplan & Meier, 1958). To compare these two outcomes between the graft types, the log-rank test was used. In calculating the time-to-event for analysis of neutrophil/platelet engraftment, acute/chronic GvHD, TRM or relapse where competing risks alter the assessment of frequency, cumulative incidences were estimated (Gooley *et al*, 1999).

Association of graft type and each of the outcomes were mainly evaluated with multivariate Cox proportional hazards models (Cox, 1972). The occurrence of acute and/or chronic GvHD was included as a time-dependent covariate. The proportional hazards assumption of the Cox model was

assessed mainly by a graphical approach. To confirm the results concerning the effects of graft type obtained from Cox analyses, we also presented results that adjusted the baseline confounding by the inverse probability-of-treatment weighted (IPTW) method (Robins *et al*, 2000). This method is less restrictive than the Cox model because we did not need to correctly specify any assumption between time to each event and baseline factors. We modelled the probability that a patient received PBSCT using the logistic regression with all the baseline factors described above as explanatory variables. From this logistic regression model, estimates of the patient specific weight, i.e. the inverse of the conditional probability of receiving his/her own graft type, were obtained. The subject-specific weight was used to estimate the effect of graft type. This weight is the probability that a subject would have his/her own observed transplantation. For IPTW estimates, the conservative robust variance estimates were used to construct confidence intervals (Lin & Wei, 1989). For end points other than relapse, cumulative incidence functions were predicted from the proportional (subdistribution) hazards model (Fine & Gray, 1999) and adjusted for effects of significant covariates in the multivariate Cox models explained above. The weights were the sample population value for each prognostic factor. SAS version 8.2 (SAS Institute Inc., Cary, NC, USA) and S Plus 2000 (Mathsoft, Seattle, WA, USA) were used for all statistical analyses.

Results

Patient and transplantation characteristics

Patient and transplantation characteristics are summarized in Table I; 295 patients received BMT and 214 received PBSCT. Regarding the diagnosis of their disease, 188 (36·9%) had AML, 144 (28·3%) had CML, 108 (21·2%) had ALL, 50 (9·8%) had MDS, and 19 (3·7%) had other types of leukaemia. The standard risk disease cohort consisted of 307 patients (60·3%), and the remaining 202 (39·7%) were of high-risk disease status. Conditioning before transplantation was a total body irradiation (TBI)-based regimen (74·9% in BMT, 64·5% in PBSCT), most often TBI plus cyclophosphamide, or a chemotherapy-based regimen (25·1% in BMT, 35·5% in PBSCT), most often busulphan plus cyclophosphamide. The median dose of nucleated cells given in the BMT group was $3·0 \times 10^8$/kg recipient body weight (range $0·3–18·4 \times 10^8$/kg). The median number of CD34$^+$ cells infused was $5·0 \times 10^6$/kg recipient body weight ($1·0–19·7 \times 10^6$/kg) in the PBSCT group. Prophylaxis for GvHD mainly consisted of a combination of CsA and three doses of short-term MTX (90·2% in BMT, 87·4% in PBSCT). The remaining patients received the four doses (day +1, +3, +6, +11) of MTX (6·8% in BMT, 8·9% in PBSCT) or less than two doses (3·1% in BMT, 3·7% in PBSCT). There were significant differences in the following variables: both patients and donors were older, and chemotherapy-based conditioning regimen was more frequent

Table I. Patient, donor and graft characteristics.

	BM		PBSC		
	n	%	n	%	P-value
No. of patients	295		214		
Median patient age, years (range)	38 (16–58)		41 (15–67)		0·028
Patient sex (male/female)	179/116		113/101		0·076
Female donor	137		114		0·137
Female to male	78		58		0·886
Median donor age, years (range)	37 (12–80)		41 (11–71)		0·045
ECOG PS					0·060
0–1	287	97·3	201	93·9	
2–4	8	2·7	13	6·1	
Risk group					0·352
Standard risk	183	62·0	124	57·9	
High risk	112	38·0	90	42·1	
Diagnosis					
Standard risk					0·485
AML	49 ·	26·8	36	29·0	
CML	74	40·4	47	37·9	
ALL	42	23·0	34	27·4	
MDS	18	9·8	7	5·6	
High-risk					0·920
AML	57	50·9	46	51·1	
CML	14	12·5	9	10·0	
ALL	16	14·3	16	17·8	
MDS	15	13·4	10	11·1	
Others	10	8·9	9	10·0	
Conditioning regimen					0·011
TBI-based	221	74·9	138	64·5	
Chemotherapy-based	74	25·1	76	35·5	
Schedule of MTX					0·528
Abbreviated (one or two doses)	9	3·1	8	3·7	
Three doses	266	90·2	187	87·4	
Four doses	20	6·8	19	8·9	
Patient and donor CMV seronegative	23	7·8	6	2·8	0·014

BM, bone marrow; PBSC, peripheral blood stem cell; ECOG PS, Eastern Cooperative Oncology Group performance status; HLA, human leucocyte antigen; AML, acute myeloid leukaemia; ALL, acute lymphoid leukaemia; CML, chronic myeloid leukaemia; TBI, total body irradiation; MDS, myelodysplastic syndrome; GvHD, graft *versus* host disease; MTX, methotrexate; CMV, cytomegalovirus.

Standard risk disease included AML or ALL in first remission, CML in chronic phase and refractory anaemia. High-risk diseases included all other disease and stages.

in the PBSCT group. However, the two groups did not differ significantly for other patient, disease and transplant-related characteristics. Median follow-up period for the surviving patients at the time of analysis was 15 months in the PBSCT group (3–40 months) and 23 months in the BMT group (1–40 months).

Haematopoietic recovery

Among the patients surviving more than 28 d (BMT, n = 287; PBSCT, n = 208), engraftment occurred in 286 (99·7%) of the BMT patients and in 206 (99·0%) of the PBSCT patients. Patients who received PBSCT had significantly faster neutrophil and platelet recovery. The median time to a neutrophil count of at least $0·5 \times 10^9$/l was 16 d (interquartile range 14–19 d) for the BMT group and 14 d (interquartile range 12–16 d) for the PBSCT group. The median time to a platelet count of at least 20×10^9/l was 22 d (interquartile range 18–28 d) for the BMT group and 18 d (interquartile range 13–25 d) for the PBSCT group. In multivariate Cox analyses, PBSCT was significantly associated with faster neutrophil recovery to at least $0·5 \times 10^9$/l compared with BMT [hazard ratio (HR) = 1·84, 95% CI 1·53–2·22, P < 0·001; Table II]. On the contrary, the high-risk disease (HR = 0·73, 95% CI 0·61–0·89, P = 0·001) was associated with slower neutrophol recovery. Likewise, the significant factor associated

Outcomes	Analysis	Variables	HR (95% CI)	*P*-value
Neutrophils >0·5 × 10⁹/l	Cox	Stem cell source: PBSCT	1·84 (1·53–2·22)	<0·001
		Disease risk: high	0·73 (0·61–0·89)	0·001
	IPTW	Stem cell source: PBSCT	1·77 (1·57–2·00)	<0·001
Platelets >20 × 10⁹/l	Cox	Stem cell source: PBSCT	1·52 (1·25–1·84)	<0·001
		Donor age: ≥40 years	0·75 (0·57–0·98)	0·033
	IPTW	Stem cell source: PBSCT	1·46 (1·29–1·66)	<0·001
Grades II–IV acute GvHD	Cox	Stem cell source: PBSCT	1·13 (0·83–1·53)	0·454
	IPTW	Stem cell source: PBSCT	1·14 (0·93–1·41)	0·217
Any grade chronic GvHD	Cox	Stem cell source: PBSCT	1·41 (1·06–1·87)	0·017
		Donor age: ≥40 years	1·56 (1·06–2·29)	0·026
		Disease risk: high	1·40 (1·06–1·87)	0·020
		Prior acute GvHD: grades II–IV	1·66 (1·26–2·20)	<0·001
	IPTW	Stem cell source: PBSCT	1·56 (1·30–1·88)	<0·001
Extensive chronic GvHD	Cox	Stem cell source: PBSCT	1·65 (1·15–2·36)	0·007
		Donor age: ≥40 years	1·65 (1·01–2·70)	0·046
		Disease risk: high	1·45 (1·01–2·07)	0·043
		Prior acute GvHD: grades II–IV	2·36 (1·68–3·33)	<0·001
	IPTW	Stem cell source: PBSCT	1·88 (1·49–2·39)	<0·001

Table II. Multivariate Cox regression analysis and inverse probability-of-treatment weighted (IPTW) method analysis comparing haematopoietic reconstitution and graft *versus* host disease (GvHD) after bone marrow transplantation (BMT) and peripheral blood stem cell transplantation (PBSCT).

The following covariates were included in the Cox models as explanatory variables; patient and donor age (less than or more than 40 years), sex, sex matching, ECOG PS, disease risk, cytomegalovirus (CMV) serology, stem cell source, conditioning regimen, and doses of MTX. The values of stem cell source and significant covariates are shown.

with faster recovery to a platelet count of at least 20 × 10⁹/l was PBSCT (HR = 1·52, 95% CI 1·25–1·84, *P* < 0·001; Table II). Significant factors for slower platelet recovery were donor age less than 40 years (HR = 0·75, 95% CI 0·57–0·98, *P* = 0·033) and high-risk disease (HR = 0·77, 95% CI 0·64–0·94, *P* = 0·008). Using the IPTW method, we confirmed that PBSCT was significantly associated with faster neutophil and platelet recovery (Table II).

Acute GvHD

Table III summarizes clinical characteristics of patients with acute GvHD and the adjusted cumulative incidence of grades II–IV acute GvHD in the two treatment groups is shown in Fig 1. The cumulative incidence of grades II–IV acute GvHD was 37·4% (95% CI 30·9–43·9) in the PBSCT group and 32·0% (95% CI 26·8–37·2) in the BMT group. By multivariate Cox analysis, haematopoietic stem cell source was not a significant factor for the incidence of grades II–IV acute GvHD (BMT vs. PBSCT: HR = 1·13, 95% CI 0·83–1·53, *P* = 0·454; Table II). We found no significant factor for the incidence of grades II–IV acute GvHD in our model. This result was the same when we used the IPTW method (Table II). The prevalence of organ involvement was different depending on the stem cell source (Table III). Liver and gastrointestinal involvement was more frequent in PBSCT patients than BMT (liver: 14·1% vs. 7·6%, *P* < 0·019; gut: 27·3% vs. 19·0%, *P* < 0·014; Table III), whereas skin involvement was similar between the two groups (46·8% vs. 52·6%, *P* = 0·207).

Table III. Clinical characteristics of patients with acute GvHD.

	BMT (*n* = 289)	PBSCT (*n* = 205)	*P*-value
Acute GvHD			0·213
Grade 0	125 (43·3)	88 (42·9)	
Grade I	70 (24·2)	37 (18·0)	
Grade II	69 (23·9)	44 (21·5)	
Grade III	22 (7·6)	24 (11·7)	
Grade IV	3 (1·0)	12 (5·9)	
Onset after transplantation among patients with grades II–IV acute GvHD			
Median	21	22	
Interquartile range	13·5–28·5	13–31	
Organ involvement			
Skin	152 (52·6)	96 (46·8)	0·207
Liver	22 (7·6)	29 (14·1)	0·019
Gut	52 (17·9)	56 (27·3)	0·014

GvHD, graft *versus* host disease; BMT, bone marrow transplantation; PBSCT, peripheral blood stem cell transplantation.
Values are given as *n* (%).

Chronic GvHD

The adjusted cumulative incidence of any grade chronic GvHD is shown in Fig 2 and the data on the incidence, severity and organ involvement of chronic GvHD are summarized in Table IV. The risk of any grade chronic GvHD in the first year after transplantation was higher in PBSCT than BMT
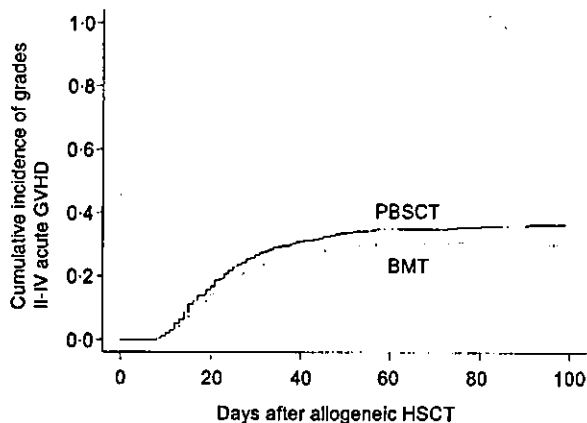
Fig 1. Cumulative incidences of grades II–IV acute graft versus host disease (GvHD) after allogeneic peripheral blood stem cell transplantation (PBSCT) compared with bone marrow transplantation (BMT). Cumulative incidence functions were predicted from the proportional subdistribution hazards model and adjusted for effects of significant covariates.
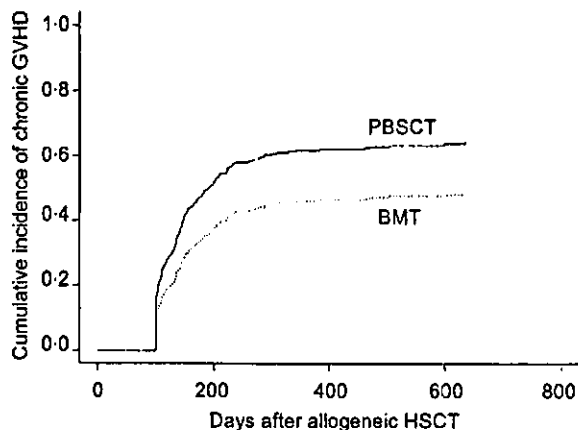

Fig 2. Cumulative incidences of any grade chronic graft versus host disease (GvHD) after allogeneic peripheral blood stem cell transplantation (PBSCT) compared with bone marrow transplantation (BMT). Cumulative incidence functions were predicted from the proportional subdistribution hazards model and adjusted for effects of significant covariates, except occurrence of prior grades II–IV acute GvHD.

(cumulative incidence at 1 year: 46·2%, 95% CI 40·4–52·4 with BMT vs. 62·1%, 95% CI 54·8–69·4 with PBSCT). The cumulative incidence of limited chronic GvHD was similar in the two groups (19·2%, 95% CI 14·4–24·0 with BMT and 20·2%, 95% CI 14·3–26·0 with PBSCT). However, the extensive form of chronic GvHD was more prevalent in PBSCT than BMT (27·1%, 95% CI 21·5–32·6 with BMT and 41·9%, 95% CI 34·6–49·3 with PBSCT). Progressive and de novo forms of chronic GvHD were more frequent in PBSCT. In the multivariate Cox analysis, PBSCT, donor age 40 years or older, high-risk disease and prior grades II–IV acute GvHD were significantly associated with increased risk for any grade

chronic GvHD (BMT vs. PBSCT: HR = 1·41, 95% CI 1·06–1·87, P = 0·017; donor age <40 years vs. ≥40 years: HR = 1·56, 95% CI 1·06–2·29, P = 0·026; standard-risk vs. high-risk disease, HR = 1·40, 95% CI 1·06–1·87, P = 0·02; prior grades 0–I acute GvHD vs. grades II–IV acute GvHD: HR = 1·66, 95% CI 1·26–2·19, P < 0·001; Table II). The extensive form of chronic GvHD was associated with the same risk factors (BMT vs. PBSCT: HR = 1·65, 95% CI 1·15–2·36, P = 0·007; donor age <40 years vs. ≥40 years: HR = 1·65, 95% CI 1·01–2·70, P = 0·046; standard-risk vs. high-risk disease: HR = 1·45, 95% CI 1·01–2·07, P = 0·043; prior grades 0–I acute GvHD vs. grades II–IV acute GvHD: HR = 2·36, 95% CI 1·68–3·33, P < 0·001; Table II). Using the IPTW method, we confirmed a significantly increased incidence of any grade and extensive chronic GvHD in PBSCT group. There were differences in the distribution of organ involvement in chronic GvHD during the course of the disease. Rash/scleroderma (38·9% vs. 25·2%, P = 0·006), oral mucositis (45·0% vs. 22·3%, P < 0·001), ocular sicca (28·9% vs. 15·0%, P = 0·002), and liver abnormality (47·0% vs. 30·6%, P = 0·002) were more frequent in PBSCT patients than in BMT patients. The prevalence of organ involvement was otherwise similar in the two groups (Table IV).

Transplantation-related mortality

The cumulative incidence of TRM at 100 d was 9·7% (95% CI 7·0–12·5) with BMT and 15·0% (95% CI 11·6–18·4) with PBSCT, and at 1 year 16·2% (95% CI 12·3–20·1) with BMT and 19·3% (95% CI 14·1–24·4) respectively (Fig 3; Table V). The stem cell source did not affect TRM in the multivariate Cox, or the IPTW method, analysis. The significant adverse risk factor was grades II–IV acute GvHD (HR = 4·92, 95% CI 2·57–9·42, P < 0·001) at 100 d. At 1 year, donor age 40 years or older (HR = 1·98, 95% CI 1·03–3·80, P = 0·040) and grades II–IV acute GvHD (HR = 2·58, 95% CI 1·65–4·05, P < 0·001) increased the risk of TRM. There were 104 deaths in the BMT group and 75 deaths in the PBSCT group (Table VI). The number of TRM was 51 following BMT (49·0%) and 44 following PBSCT (58·7%), and there was a higher incidence of GvHD-related death in the PBSCT group than in the BMT group (17·3% vs. 3·8%). On the contrary, the number of deaths from relapse was lower in PBSCT (n = 31, 41·3%) than in BMT (n = 53, 51·0%). Time to non-relapse death was similar in the two groups.

Relapse

For the standard-risk group, the cumulative incidence of relapse at 1 year was similar (8·1%, 95% CI 4·2–12·0 with BMT vs. 7·5%, 95% CI 3·1–11·9 with PBSCT; Fig 4A). For the high-risk group, this was 37·1% (95% CI 28·0–46·4) with BMT and 33·3% (95% CI 23·3–43·4) with PBSCT respectively (Fig 4B). In multivariate Cox analysis, there was no statistical difference in the risk of relapse after PBSCT and BMT (HR = 0·95, 95%

Table IV. Clinical characteristics of patients with chronic GvHD.

	BMT (*n* = 206)	PBSCT (*n* = 149)	*P*-value
The incidence of chronic GvHD			0·001
All grade	113 (54·9)	107 (71·8)	
Limited	47 (22·8)	33 (22·1)	
Extensive	66 (32·0)	74 (49·7)	
Onset after transplantation among patients with chronic GvHD (days)			
Median	131	127	
Range	100–634	100–598	
Type			0·003
Progressive	12 (5·8)	15 (10·1)	
Quiescent	59 (28·6)	43 (28·9)	
De novo	42 (20·4)	49 (32·9)	
Organ involvement			
Rash/scleroderma	52 (25·2)	58 (38·9)	0·006
Oral mucositis	46 (22·3)	67 (45·0)	<0·001
Ocular sicca	31 (15·0)	43 (28·9)	0·002
Pulmonary disease	14 (6·8)	19 (12·8)	0·057
Liver abnormalities	63 (30·6)	70 (47·0)	0·002
Nausea/vomiting	6 (2·9)	10 (6·7)	0·089
Diarrhoea	7 (3·4)	7 (4·7)	0·534
Esophagitis	2 (1·0)	3 (2·0)	0·411
Arthralgias/arthritis	5 (2·4)	6 (4·0)	0·112
Effusions	1 (0·5)	1 (0·7)	0·818
Auto-antibody	2 (1·0)	2 (1·3)	0·744
Thrombocytopenia (<100 × 10⁹/l)	38 (19·3)	38 (26·6)	0·112

GvHD, graft *versus* host disease; BMT, bone marrow transplantation; PBSCT, peripheral blood stem cell transplantation.
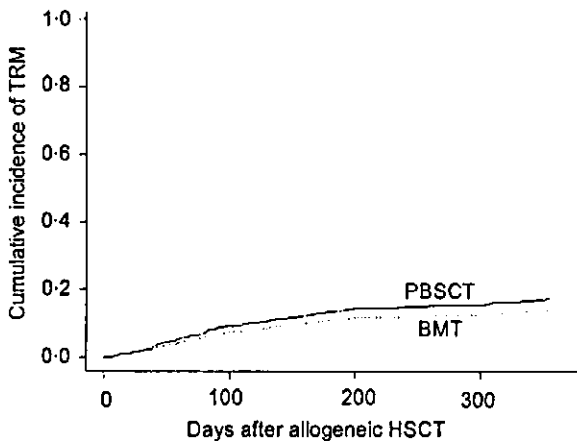Values are given as *n* (%).

Fig 3. Cumulative incidences of treatment-related mortality after allogeneic peripheral blood stem cell transplantation (PBSCT) compared with bone marrow transplantation (BMT). Cumulative incidence functions were predicted from the proportional subdistribution hazards model and adjusted for effects of significant covariates.

CI 0·64–1·41, *P* = 0·806; Table V). We found that the high-risk disease (HR = 3·97, 95% CI 2·66–5·94, *P* < 0·001) and

ECOG PS 2–4 (HR = 3·42, 95% CI 1·73–6·77, *P* < 0·001) had a significantly increased risk of relapse. We did not observe any difference of relapse between the PBSCT and BMT groups using the IPTW method.

Progression-free and overall survival

In standard risk patients, the 2-year PFS and OS in PBSCT and BMT were, respectively, 68·2% (95% CI 58·8–77·5) and 64·7% (95% CI 57·0–72·5) (*P* = 0·993), and 74·1% (95% CI 65·2–83·1) and 73·8% (95% CI 66·9–80·6) (*P* = 0·991). In high-risk patients, PFS and OS in PBSCT and BMT were, respectively, 34·9% (95% CI 23·7–46·0) and 37·7% (95% CI 27·7–47·7) (*P* = 0·539), and 39·1% (95% CI 27·5–50·8) and 44·5% (95% CI 34·3–54·6) (*P* = 0·555; Fig 5A,B). In the multivariate Cox analysis, the use of PBSCT was not a significant factor for both PFS and OS (Table V). We obtained the same result using the IPTW method. The following variables were significant adverse risk factors for both PFS and OS, respectively: high-risk disease (HR = 2·41, 95% CI 1·82–3·21, *P* < 0·001; HR = 2·45, 95% CI 1·79–3·34, *P* < 0·001), ECOG PS 2–4 (HR = 2·83, 95% CI 1·63–4·92, *P* < 0·001; HR = 3·31, 95% CI 1·88–5·84, *P* < 0·001), and grades II–IV acute GvHD (HR = 1·33, 95% CI 1·00–1·78, *P* = 0·05; HR = 1·57, 95% CI 1·15–2·13, *P* = 0·004).

Discussion

This is the first large comparative study from an Asian area on the outcome of allogeneic HSCT using different sources of stem cells (BMT or PBSCT). We analysed the outcome of allogeneic HSCT from related donors in 509 Japanese patients with leukaemia and MDS. All of the patients in our cohort were given G-CSF postgrafting and we confirmed the more rapid haematological recovery after PBSCT than in BMT, which is in line with many previous studies (Schmitz *et al*, 1998, 2002; Champlin *et al*, 2000; Heldal *et al*, 2000; Powles *et al*, 2000; Bensinger *et al*, 2001; Cutler *et al*, 2001; Couban *et al*, 2002; Ringden *et al*, 2002).

It has been suggested that the increased incidence of acute GvHD in PBSCT patients is a consequence of PBSC grafts containing 1 log more T cells compared with bone marrow grafts, although this may be counterbalanced by the decreased potential of type 1 cytokine secretion from donor T cells in PBSC grafts (Mielcarek *et al*, 1997). In clinical studies, a statistically significant increase in acute GvHD after PBSCT has been reported in an RCT (Schmitz *et al*, 2002) and a meta-analysis (Cutler *et al*, 2001). On the contrary, there was no difference in other RCTs (Heldal *et al*, 2000; Powles *et al*, 2000; Bensinger *et al*, 2001; Couban *et al*, 2002). We also found no increased incidence of grades II–IV acute GvHD after PBSCT in the current study. Another important point to be discussed is the dose of MTX that was used as prophylaxis for GvHD. The most common regimen for MTX in Japanese institutions in HLA-identical-related donor transplantation is

Table V. Multivariate Cox regression analysis and inverse probability of treatment weighted (IPTW) method analysis comparing transplant-related mortality (TRM), progression-free survival (PFS) and overall survival (OS) after peripheral blood stem cell transplantation (PBSCT) and bone marrow transplantation (BMT).

Outcomes	Analysis	Variables	HR (95% CI)	P-value
TRM at 100 d	Cox	Stem cell source: PBSCT	1·18 (0·66–2·12)	0·584
		Acute GvHD: grades II–IV	4·92 (2·57–9·42)	<0·001
	IPTW	Stem cell source: PBSCT	1·33 (0·84–2·10)	0·230
TRM at 1 year	Cox	Stem cell source: PBSCT	1·07 (0·69–1·66)	0·773
		Donor age: 40 years or older	1·98 (1·03–3·80)	0·040
		Acute GvHD: grades II–IV	2·58 (1·65–4·05)	<0·001
	IPTW	Stem cell source: PBSCT	1·17 (0·82–1·66)	0·381
Relapse	Cox	Stem cell source: PBSCT	0·95 (0·64–1·41)	0·806
		Disease risk: high	3·97 (2·66–5·94)	<0·001
		ECOG PS: 2–4	3·42 (1·73–6·77)	0·004
	IPTW	Stem cell source: PBSCT	0·95 (0·73–1·23)	0·676
PFS	Cox	Stem cell source: PBSCT	1·03 (0·77–1·37)	0·868
		Disease risk: high	2·41 (1·82–3·21)	<0·001
		ECOG PS: 2–4	2·83 (1·63–4·92)	<0·001
		Acute GvHD: grades II–IV	1·33 (1·00–1·78)	0·05
	IPTW	Stem cell source: PBSCT	1·05 (0·87–1·27)	0·589
OS	Cox	Stem cell source: PBSCT	0·99 (0·73–1·36)	0·972
		Disease risk: high	2·45 (1·79–3·34)	<0·001
		ECOG PS: 2–4	3·31 (1·88–5·84)	<0·001
		Acute GvHD: grades II–IV	1·57 (1·15–2·13)	0·004
	IPTW	Stem cell source: PBSCT	1·05 (0·85–1·29)	0·659

The following covariates were included in the Cox models as explanatory variables: patient and donor age (less than or more than 40 years), sex, sex matching, Eastern Cooperative Oncology Group performance status (ECOG PS), disease risk, cytomegalovirus (CMV) serology, stem cell source, conditioning regimen, doses of methotrexate (MTX), grades II–IV acute graft *versus* host disease (GvHD) and chronic GvHD. The values of stem cell source and significant covariates are shown in this table. Grades II–IV GvHD and chronic GvHD were included as time-dependent covariate (HR, hazard ratio).

Table VI. Causes of mortality and time of death.

	BMT (n = 104)	PBSCT (n = 75)
Number of TRM	51 (49·0)	44 (58·7)
Causes of TRM		
GvHD	4 (3·8)	13 (17·3)
Non-infectious pneumonia	6 (5·8)	6 (8·0)
Veno-occlusive disease of the liver	5 (4·8)	1 (1·3)
Infection	25 (24·0)	14 (18·7)
Haemorrhage	1 (1·0)	3 (4·0)
Others	10 (9·6)	7 (9·3)
Time of TRM		
Days 0–30	7 (6·7)	4 (5·3)
Days 31–100	14 (13·5)	20 (26·7)
After day 100	30 (28·8)	20 (26·7)
Number of deaths in relapse	53 (51·0)	31 (41·3)

TRM, transplant-related mortality; BMT, bone marrow transplantation; PBSCT, peripheral blood stem cell transplantation; GvHD, graft *versus* host disease.
Values are given as n (%).

three doses of MTX (day +1: 10 mg/m²; day +3 and day +6: 7 mg/m²) rather than four doses of MTX routinely used in other countries, because of the lower frequency of GvHD in

Japan (Morishima *et al*, 1989). An RCT from the European Group for Blood and Marrow Transplantation (EBMT) study, in which increased incidence of acute and chronic GvHD was shown, also gave three doses of MTX (Schmitz *et al*, 2002). Omission of day +11, MTX may influence the incidence of acute and chronic GvHD (Nash *et al*, 1992; Cutler *et al*, 2001; Mehta & Singhal, 2002), although we did not find any difference among the different MTX dose groups. A recent report from the EBMT suggested that post-transplant G-CSF might increase the incidence of acute and chronic GvHD and TRM, resulting in lower leukaemia-free and OS rates after BMT (Ringden *et al*, 2004). Although the use of G-CSF postallografting is usually accepted as a standard care in Japan, we need to reconsider this indication, especially after BMT.

Notably, the observed cumulative incidence of grades II–IV acute GvHD in patients receiving HLA-identical transplants seemed lower in both groups (BMT 32·0%, PBSCT 37·4%) compared with rates reported from western countries (Powles *et al*, 2000; Bensinger *et al*, 2001; Couban *et al*, 2002; Schmitz *et al*, 2002). These data are consistent with previous reports on Japanese BMT patients (Morishima *et al*, 1989; Oh *et al*, 2002). Oh *et al* (2002) reported a multivariate analysis for adult allogeneic BMT patients showing that a Japanese cohort had a significantly lower risk of acute GvHD than white American, black American and Irish cohorts [relative risk
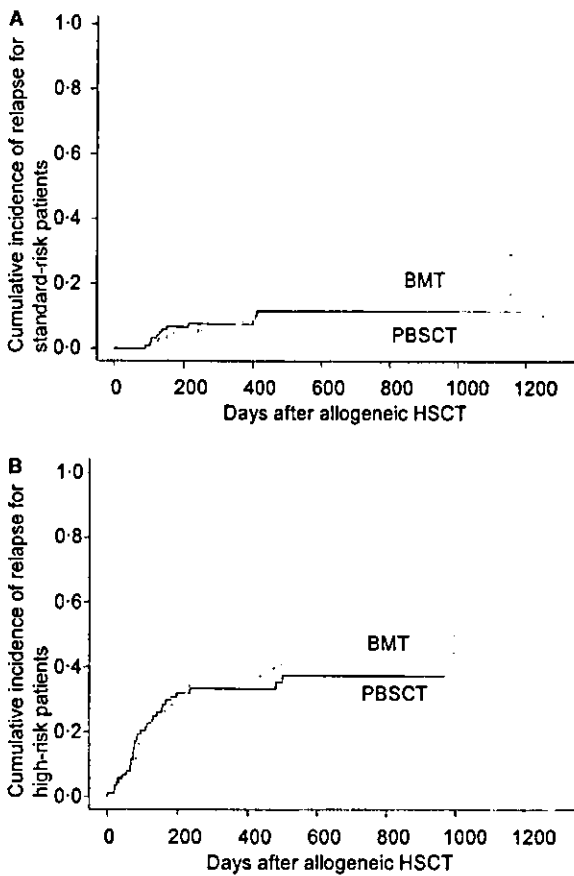
Fig 4. Cumulative incidences of relapse after allogeneic peripheral blood stem cell transplantation (PBSCT) compared with bone marrow transplantation (BMT). Cumulative incidence functions (A: standard-risk group; B: high-risk group) were predicted from the proportional subdistribution hazards model and adjusted for effects of significant covariates.
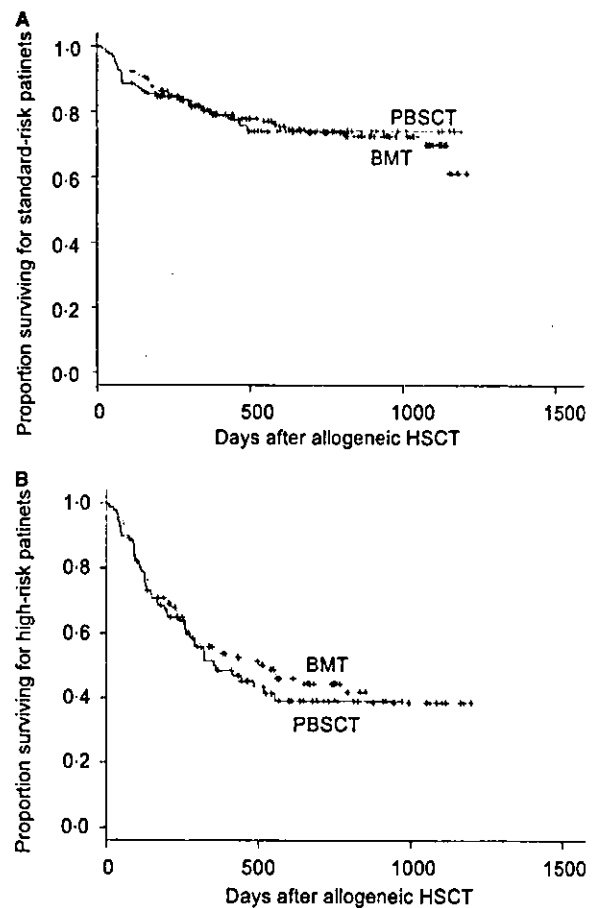

Fig 5. Probabilities of overall survival after allogeneic peripheral blood stem cell transplantation (PBSCT) compared with bone marrow transplantation (BMT). Probabilities were derived from Kaplan–Meier estimates [A: overall survival (OS) for standard risk group; B: OS for high-risk group].

(RR) = 1·77, $P < 0.01$; RR = 1·84, $P < 0.01$; RR = 2·22, $P < 0.01$ respectively]. Our data suggest that this trend might also apply to PBSCT. This difference has been speculated to reflect a lower degree of diversity for HLA and minor histocompatibility antigens among Japanese. However, a recent report revealed the influence of an interleukin-10 promotor polymorphism after allogeneic HSCT (Lin *et al*, 2003). The interleukin-10-592A/A genotype was associated with a decreased risk of grade III or IV acute GvHD. The frequency of this genotype is 67% in the Japanese population (Tegoshi *et al*, 2002), which is much higher than the frequency of 23% and 24% in two white populations (Lin *et al*, 2003). This finding may account for the decreased incidence and severity of acute GvHD in Japanese population than in white populations.

We found a significantly increased cumulative incidence of chronic GvHD among PBSCT patients in accord with several previous studies (Champlin *et al*, 2000; Bensinger *et al*, 2001; Cutler *et al*, 2001; Schmitz *et al*, 2002; Heldal *et al*,

2003). In particular, the extensive form of chronic GvHD was increased in the PBSCT cohort, whereas the incidence of the limited form was similar in the two cohorts. There is now considerable evidence that the preferential expansion of T-helper 2 (Th2) cells after allogeneic HSCT is associated with the development of chronic GvHD in both murine models and human beings (Doutrelepont *et al*, 1991; Umland *et al*, 1992; Allen *et al*, 1993; De Wit *et al*, 1993; Garlisi *et al*, 1993; Tanaka *et al*, 1997). A G-CSF-induced Th2 cytokine profile of donor T cells may be associated with increased incidence and severity of chronic GvHD (Pan *et al*, 1995). G-CSF also mobilized type 2 dendritic cells, which promote Th2 responses (Arpinati *et al*, 2000). Thus, G-CSF may have an important role in the development of chronic GHVD among PBSCT patients.

Another interesting point is the different distribution of organs affected by acute and chronic GvHD in BMT and PBSCT. Although previous reports demonstrated that skin and vaginal involvement (Bensinger *et al*, 2001; Flowers *et al*,

2002) or ocular involvement (Mohty et al, 2002) of chronic GvHD was more prevalent after PBSCT, the current study showed an increased incidence of skin, ocular sicca and oral mucositis, similar to Sjoegren syndrome. It is not well understood how selected organs become the targets of activated T cells. Inflammatory chemokines expressed in inflamed tissues upon stimulation by proinflammatory cytokines are specialized for the recruitment of effector cells (Moser & Loetscher, 2001). In mouse models, a comparative study of gene expression profiles of livers after experimental allogeneic and syngeneic BMT using oligonucleotide microarrays identified genes related to leucocyte trafficking that were upregulated at day 7 after allogeneic BMT when neither hepatic injury nor donor T-cell migration into the liver was evident (Ichiba et al, 2003). This study suggests that the interferon-γ produced by donor T cells in secondary lymphoid organs transactivates genes in target organs, stimulating the recruitment of effector cells to target organs and eventually rendering them vulnerable to effector cell attack. Thus, quantifiable and qualitative differences in immunological cells in PBSC grafts compared with bone marrow grafts may affect the chemokine environment, leading to the different distribution of affected organs. Alternately, increased numbers of affected organs in PBSC patients may simply reflect the increased severity of chronic GvHD.

Recent reports suggest that chronic GvHD with risk factors may negatively affect patients' survival (Akpek et al, 2001, 2003; Przepiorka et al, 2001). Long-term follow-up of an RCT showed that, although the cumulative incidence of chronic GvHD at 3 years was similar in BMT and PBSCT patients, chronic GvHD after PBSCT was more protracted and less responsive to treatment than after BMT (Bensinger et al, 2001; Flowers et al, 2002). With increasing numbers of long-term survivors, we need more information concerning the clinical characteristics of chronic GvHD after PBSCT (Przepiorka et al, 2001).

It has been postulated that a GVL effect may be observed, and the results of allogeneic HSCT may be improved in the presence of GvHD (Sullivan et al, 1989; Horowitz et al, 1990). However, the potential advantage of the GVL effect of allogeneic HSCT is often reduced by the GvHD-related morbidity and mortality (Weiden et al, 1981; Sullivan et al, 1989; Horowitz et al, 1990; Przepiorka et al, 2001; Lee et al, 2002). In most of the previous RCTs comparing BMT and PBSCT, the sample sizes were too small to detect meaningful survival increases (Schmitz et al, 1998; Blaise et al, 2000; Heldal et al, 2000; Powles et al, 2000). Even in the larger RCTs, survival was evaluated as a secondary end point (Bensinger et al, 2001; Couban et al, 2002; Schmitz et al, 2002). Bensinger et al (2001) and Couban et al (2002) have reported an OS benefit of PBSCT in patients with advanced disease. The former study included miscellaneous diseases and the observed advantage was derived from subgroup analysis, in which we were unable to draw reliable conclusions. The latter study, which involved 228 patients, included only myeloid

malignancy but the improved survival was due to lower TRM with similar relapse rates, suggesting that faster haematological recovery accounts for this benefit. A meta-analysis reported by Cutler et al (2001), which involved 16 studies, and a large RCT from the EBMT (Schmitz et al, 2002) included 350 patients, and showed an increased incidence of acute and chronic GvHD, with no significant difference in relapse (Cutler et al, 2001; Schmitz et al, 2002) and survival rate (Schmitz et al, 2002). A recent meta-analysis suggested that any survival advantage of PBSCT is limited to patients with advanced disease (Horan et al, 2003). Thus, allogeneic PBSCT offered the prospect of a better outcome, but evidence for a survival benefit has been inconclusive. We must explicitly state that caution is highly advisable when interpreting post hoc subgroup analyses. These cannot be used for recommendations on treatment selection for individual patients, although they can be used in the development of new, empirically based research hypotheses. In addition, there might be a different impact on patient outcome after allogeneic HSCT according to stem cell source in this particular ethnic group, if the incidence of acute GvHD is lower than western countries. In the present study, multivariate analyses revealed that differences in stem cell source was not a significant factor for acute GvHD, relapse, TRM, PFS and OS despite the increased incidence of chronic GvHD after PBSCT. Early mortality within day 100 of PBSCT could be reduced because of faster engraftment (Champlin et al, 2000; Couban et al, 2002) but we did not observe this advantage. Our data showed that grades II-IV acute GvHD were significant adverse prognostic factors for TRM. The advantages of PBSCT may thus be counterbalanced by the increased incidence of GvHD. Treatment of acute and chronic GvHD was performed at the physician's discretion and immunosuppressive treatment may hamper the GVL effect in some cases. This may indicate the difficulty of separating GVL effects from GvHD clinically. We analysed the data according to each disease category and risk status, although there were no apparent differences between the two groups (data not shown). Therefore, in contrast to general belief, whether the GVL effect will improve survival after PBSCT remains unknown. Assessment of the overall benefits of PBSCT compared with BMT will require long-term follow-up of the morbidity of patients associated with chronic GvHD.

The retrospective nature, the heterogeneity of the diagnoses and the relatively short follow-up limit the power of this analysis. We cannot exclude the possibility that there are unmeasured confounders that could cause a bias between two groups. Analysis of the CD34$^+$ and CD3$^+$ cell dose was not performed because these are generally dependent on the source of stem cells, and in addition, we could not obtain enough data, especially in the BMT group. In multicentre studies, there is likely to be a variation among centres in both baseline risks and treatment effects that cannot be explained by the known prognostic factors (Frassoni et al, 2000; Matsuo et al, 2000; Loberiza et al, 2003). To resolve the limitations described

above, we needed an RCT in Japan. We have therefore launched a prospective, open-label RCT comparing allogeneic BMT *versus* PBSCT for adult patients with leukaemia. The primary end point of this trial is leukaemia-free survival based on time-to-event analysis. We plan the sample size per one arm to be 160, in order to detect the difference of 1·6 to 1·7 in HR for leukaemia-free survival. If this study can be completed, the impact of stem cell source on survival will be defined more accurately than the previous studies.

In summary, we observed faster engraftment and increased incidence of chronic GvHD in PBSCT compared with BMT for Japanese patients. The incidence of GvHD was lower than the western populations, but there were no differences in relapse, TRM, PFS and OS between PBSCT and BMT. These results suggest that the choice of haematopoietic stem cell source should be considered based on the data for individual ethnic populations. More detailed analysis and future trials may reveal the differential applicability of stem cells from these different sources in each disease category and hence enable us to choose appropriately between BMT and PBSCT based on reliable evidence.

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Appendix A

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References

Akpek, G., Zahurak, M.L., Piantadosi, S., Margolis, J., Doherty, J., Davidson, R. & Vogelsang, G.B. (2001) Development of a prognostic model for grading chronic graft-versus-host disease. *Blood*, 97, 1219–1226.

Akpek, G., Lee, S.J., Flowers, M.E., Pavletic, S.Z., Arora, M., Lee, S., Piantadosi, S., Guthrie, K.A., Lynch, J.C., Takatu, A., Horowitz, M.M., Antin, J.H., Weisdorf, D.J., Martin, P.J. & Vogelsang, G.B. (2003) Performance of a new clinical grading system for chronic graft-versus-host disease: a multicenter study. *Blood*, 102, 802–809.

Allen, R.D., Staley, T.A. & Sidman, C.L. (1993) Differential cytokine expression in acute and chronic murine graft-versus-host-disease. *European Journal of Immunology*, 23, 333–337.

Arpinati, M., Green, C.L., Heimfeld, S., Heuser, J.E. & Anasetti, C. (2000) Granulocyte-colony stimulating factor mobilizes T helper 2-inducing dendritic cells. *Blood*, 95, 2484–2490.

Bensinger, W.I., Martin, P.J., Storer, B., Clift, R., Forman, S.J., Negrin, R., Kashyap, A., Flowers, M.E., Lilleby, K., Chauncey, T.R., Storb, R. & Appelbaum, F.R. (2001) Transplantation of bone marrow as compared with peripheral-blood cells from HLA-identical relatives in patients with hematologic cancers. *New England Journal of Medicine*, 344, 175–181.

Blaise, D., Kuentz, M., Fortanier, C., Bourhis, J.H., Milpied, N., Sutton, L., Jouet, J.P., Attal, M., Bordigoni, P., Cahn, J.Y., Boiron, J.M., Schuller, M.P., Moatti, J.P. & Michallet, M. (2000) Randomized trial of bone marrow versus lenograstim-primed blood cell allogeneic transplantation in patients with early-stage leukemia: a report from the Societe Francaise de Greffe de Moelle. *Journal of Clinical Oncology*, 18, 537–546.

Champlin, R.E., Schmitz, N., Horowitz, M.M., Chapuis, B., Chopra, R., Cornelissen, J.J., Gale, R.P., Goldman, J.M., Loberiza, Jr, F.R., Hertenstein, B., Klein, J.P., Montserrat, E., Zhang, M.J., Ringden, O., Tomany, S.C., Rowlings, P.A., Van Hoef, M.E. & Gratwohl, A. (2000) Blood stem cells compared with bone marrow as a source of hematopoietic cells for allogeneic transplantation. IBMTR Histocompatibility and Stem Cell Sources Working Committee and the European Group for Blood and Marrow Transplantation (EBMT). *Blood*, 95, 3702–3709.

Couban, S., Simpson, D.R., Barnett, M.J., Bredeson, C., Hubesch, L., Howson-Jan, K., Shore, T.B., Walker, I.R., Browett, P., Messner, H.A., Panzarella, T. & Lipton, J.H. (2002) A randomized multicenter comparison of bone marrow and peripheral blood in recipients of matched sibling allogeneic transplants for myeloid malignancies. *Blood*, 100, 1525–1531.

Cox, D.R. (1972) Regression models and life tables (with discussion). *Journal of the Royal Statistical Society, Series B*, 34, 187–220.

Cutler, C., Giri, S., Jeyapalan, S., Paniagua, D., Viswanathan, A. & Antin, J.H. (2001) Acute and chronic graft-versus-host disease after allogeneic peripheral-blood stem-cell and bone marrow transplantation: a meta-analysis. *Journal of Clinical Oncology*, 19, 3685–3691.

De Wit, D., Van Mechelen, M., Zanin, C., Doutrelepont, J.M., Velu, T., Gerard, C., Abramowicz, D., Scheerlinck, J.P., De Baetselier, P. &

Urbain, J. (1993) Preferential activation of Th2 cells in chronic graft-versus-host reaction. *Journal of Immunology*, 150, 361–366.

Doutrelepont, J.M., Moser, M., Leo, O., Abramowicz, D., Vanderhaegen, M.L., Urbain, J. & Goldman, M. (1991) Hyper IgE in stimulatory graft-versus-host disease: role of interleukin-4. *Clinical and Experimental Immunology*, 83, 133–136.

Fine, J.P. & Gray, R.J. (1999) A proportional hazards model for the subdistribution of a competing risk. *Journal of the American Statistical Association*, 94, 496–509.

Flowers, M.E., Parker, P.M., Johnston, L.J., Matos, A.V., Storer, B., Bensinger, W.I., Storb, R., Appelbaum, F.R., Forman, S.J., Blume, K.G. & Martin, P.J. (2002) Comparison of chronic graft-versus-host disease after transplantation of peripheral blood stem cells versus bone marrow in allogeneic recipients: long-term follow-up of a randomized trial. *Blood*, 100, 415–419.

Frassoni, F., Labopin, M., Powles, R., Mary, J.Y., Arcese, W., Bacigalupo, A., Bunjes, D., Gluckman, E., Ruutu, T., Schaefer, U.W., Sierra, J., Vernant, J.P., Willemze, R., de Witte, T. & Gorin, N.C. (2000) Effect of centre on outcome of bone-marrow transplantation for acute myeloid leukaemia. Acute Leukaemia Working Party of the European Group for Blood and Marrow Transplantation. *Lancet*, 355, 1393–1398.

Garlisi, C.G., Pennline, K.J., Smith, S.R., Siegel, M.I. & Umland, S.P. (1993) Cytokine gene expression in mice undergoing chronic graft-versus-host disease. *Molecular Immunology*, 30, 669–677.

Gooley, T.A., Leisenring, W., Crowley, J. & Storer, B.E. (1999) Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. *Statistics in Medicine*, 18, 695–706.

Heldal, D., Tjonnfjord, G., Brinch, L., Albrechtsen, D., Egeland, T., Steen, R., Solheim, B.G. & Evensen, S.A. (2000) A randomised study of allogeneic transplantation with stem cells from blood or bone marrow. *Bone Marrow Transplantation*, 25, 1129–1136.

Heldal, D., Brinch, L., Tjonnfjord, G., Solheim, B.G., Egeland, T., Albrechtsen, D., Aamodt, G. & Evensen, S.A. (2003) Fewer relapses and increased chronic GVHD in patients transplanted with blood stem cells: a 5-year follow-up in a single centre study. *Bone Marrow Transplantation*, 32, 257–264.

Horan, J.T., Liesveld, J.L., Fernandez, I.D., Lyman, G.H., Phillips, G.L., Lerner, N.B. & Fisher, S.G. (2003) Survival after HLA-identical allogeneic peripheral blood stem cell and bone marrow transplantation for hematologic malignancies: meta-analysis of randomized controlled trials. *Bone Marrow Transplantation*, 32, 293–298.

Horowitz, M.M., Gale, R.P., Sondel, P.M., Goldman, J.M., Kersey, J., Kolb, H.J., Rimm, A.A., Ringden, O., Rozman, C. & Speck, B. (1990) Graft-versus-leukemia reactions after bone marrow transplantation. *Blood*, 75, 555–562.

Ichiba, T., Teshima, T., Kuick, R., Misek, D.E., Liu, C., Takada, Y., Maeda, Y., Reddy, P., Williams, D.L., Hanash, S.M. & Ferrara, J.L. (2003) Early changes in gene expression profiles of hepatic GVHD uncovered by oligonucleotide microarrays. *Blood*, 102, 763–771.

Kaplan, E.L. & Meier, P. (1958) Nonparametric estimation from imcomplete observations. *Journal of American Statistical Association*, 53, 457–481.

Lee, S.J., Klein, J.P., Barrett, A.J., Ringden, O., Antin, J.H., Cahn, J.Y., Carabasi, M.H., Gale, R.P., Giralt, S., Hale, G.A., Ilhan, O., McCarthy, P.L., Socie, G., Verdonck, L.F., Weisdorf, D.J. & Horowitz, M.M. (2002) Severity of chronic graft-versus-host disease: association with treatment-related mortality and relapse. *Blood*, 100, 406–414.

Lin, D.Y. & Wei, L.J. (1989) The robust inference for the Cox proportional hazards model. *Journal of American Statistical Association*, 84, 1074–1078.

Lin, M.T., Storer, B., Martin, P.J., Tseng, L.H., Gooley, T., Chen, P.J. & Hansen, J.A. (2003) Relation of an interleukin-10 promoter polymorphism to graft-versus-host disease and survival after hematopoietic-cell transplantation. *New England Journal of Medicine*, 349, 2201–2210.

Loberiza, F.R., Serna, D.S., Horowitz, M.M. & Rizzo, J.D. (2003) Transplant center characteristics and clinical outcomes after hematopoietic stem cell transplantation: what do we know? *Bone Marrow Transplantation*, 31, 417–421.

Matsuo, K., Hamajima, N., Morishima, Y. & Harada, M. (2000) Hospital capacity and post-transplant survival after allogeneic bone marrow transplantation: analysis of data from the Japan Society for Hematopoietic Cell Transplantation. *Bone Marrow Transplantation*, 26, 1061–1067.

Mehta, J. & Singhal, S. (2002) Chronic graft-versus-host disease after allogeneic peripheral-blood stem-cell transplantation: a little methotrexate goes a long way. *Journal of Clinical Oncology*, 20, 603–606.

Mielcarek, M., Martin, P.J. & Torok-Storb, B. (1997) Suppression of alloantigen-induced T-cell proliferation by CD14+ cells derived from granulocyte colony-stimulating factor-mobilized peripheral blood mononuclear cells. *Blood*, 89, 1629–1634.

Mohty, M., Kuentz, M., Michallet, M., Bourhis, J.H., Milpied, N., Sutton, L., Jouet, J.P., Attal, M., Bordigoni, P., Cahn, J.Y., Boiron, J.M. & Blaise, D. (2002) Chronic graft-versus-host disease after allogeneic blood stem cell transplantation: long-term results of a randomized study. *Blood*, 100, 3128–3134.

Morishima, Y., Morishita, Y., Tanimoto, M., Ohno, R., Saito, H., Horibe, K., Hamajima, N., Naito, K., Yamada, K. & Yokomaku, S. (1989) Low incidence of acute graft-versus-host disease by the administration of methotrexate and cyclosporine in Japanese leukemia patients after bone marrow transplantation from human leukocyte antigen compatible siblings; possible role of genetic homogeneity. The Nagoya Bone Marrow Transplantation Group. *Blood*, 74, 2252–2256.

Moser, B. & Loetscher, P. (2001) Lymphocyte traffic control by chemokines. *Nature Immunology*, 2, 123–128.

Nash, R.A., Pepe, M.S., Storb, R., Longton, G., Pettinger, M., Anasetti, C., Appelbaum, F.R., Bowden, R.A., Deeg, H.J. & Doney, K. (1992) Acute graft-versus-host disease: analysis of risk factors after allogeneic marrow transplantation and prophylaxis with cyclosporine and methotrexate. *Blood*, 80, 1838–1845.

Oh, H., Zhang, M.J., Akiyama, H., Asai, T., Barrett, A.J., Loberiza, F.R., Miyawaki, S., Okamoto, S., Ringden, O. & Horowitz, M.M. (2002) Comparison of graft-versus-host disease (GVHD) and survival in different ethnic populations: Collaborative Study by the Japan Adult Leukemia Study Group (JALSG) and the International Bone Marrow Transplant Registry (IBMTR). *Blood*, 100, 418a.

Pan, L., Delmonte, Jr, J., Jalonen, C.K. & Ferrara, J.L. (1995) Pretreatment of donor mice with granulocyte colony-stimulating factor polarizes donor T lymphocytes toward type-2 cytokine production and reduces severity of experimental graft-versus-host disease. *Blood*, 86, 4422–4429.

Powles, R., Mehta, J., Kulkarni, S., Treleaven, J., Millar, B., Marsden, J., Shepherd, V., Rowland, A., Sirohi, B., Tait, D., Horton, C., Long, S. & Singhal, S. (2000) Allogeneic blood and bone-marrow stem-cell transplantation in haematological malignant diseases: a randomised trial. *Lancet*, 355, 1231–1237.

Przepiorka, D., Weisdorf, D., Martin, P., Klingemann, H.G., Beatty, P., Hows, J. & Thomas, E.D. (1995) 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplantation*, 15, 825–828.

Przepiorka, D., Anderlini, P., Saliba, R., Cleary, K., Mehra, R., Khouri, I., Huh, Y.O., Giralt, S., Braunschweig, I., van Besien, K. & Champlin, R. (2001) Chronic graft-versus-host disease after allogeneic blood stem cell transplantation. *Blood*, 98, 1695–1700.

Ringden, O., Labopin, M., Bacigalupo, A., Arcese, W., Schaefer, U.W., Willemze, R., Koc, H., Bunjes, D., Gluckman, E., Rocha, V., Schattenberg, A. & Frassoni, F. (2002) Transplantation of peripheral blood stem cells as compared with bone marrow from HLA-identical siblings in adult patients with acute myeloid leukemia and acute lymphoblastic leukemia. *Journal of Clinical Oncology*, 20, 4655–4664.

Ringden, O., Labopin, M., Gorin, N.C., Le Blanc, K., Rocha, V., Gluckman, E., Reiffers, J., Arcese, W., Vossen, J.M., Jouet, J.P., Cordonnier, C. & Frassoni, F. (2004) Treatment with granulocyte colony-stimulating factor after allogeneic bone marrow transplantation for acute leukemia increases the risk of graft-versus-host disease and death: a study from the Acute Leukemia Working Party of the European Group for Blood and Marrow Transplantation. *Journal of Clinical Oncology*, 22, 416–423.

Robins, J.M., Hernan, M.A. & Brumback, B. (2000) Marginal structural models and causal inference in epidemiology. *Epidemiology*, 11, 550–560.

Schmitz, N., Bacigalupo, A., Hasenclever, D., Nagler, A., Gluckman, E., Clark, P., Bourquelot, P., Greinix, H., Frickhofen, N., Ringden, O., Zander, A., Apperley, J.F., Gorin, C., Borkett, K., Schwab, G., Goebel, M., Russell, N.H. & Gratwohl, A. (1998) Allogeneic bone marrow transplantation vs filgrastim-mobilised peripheral blood progenitor cell transplantation in patients with early leukaemia: first results of a randomised multicentre trial of the European Group for Blood and Marrow Transplantation. *Bone Marrow Transplantation*, 21, 995–1003.

Schmitz, N., Beksac, M., Hasenclever, D., Bacigalupo, A., Ruutu, T., Nagler, A., Gluckman, E., Russell, N., Apperley, J.F., Gorin, N.C., Szer, J., Bradstock, K., Buzyn, A., Clark, P., Borkett, K. & Gratwohl, A. (2002) Transplantation of mobilized peripheral blood cells to HLA-identical siblings with standard-risk leukemia. *Blood*, 100, 761–767.

Shulman, H.M., Sullivan, K.M., Weiden, P.L., McDonald, G.B., Striker, G.E., Sale, G.E., Hackman, R., Tsoi, M.S., Storb, R. & Thomas, E.D. (1980) Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. *American Journal of Medicine*, 69, 204–217.

Sullivan, K.M., Weiden, P.L., Storb, R., Witherspoon, R.P., Fefer, A., Fisher, L., Buckner, C.D., Anasetti, C., Appelbaum, F.R. & Badger, C. (1989) Influence of acute and chronic graft-versus-host disease on relapse and survival after bone marrow transplantation from HLA-identical siblings as treatment of acute and chronic leukemia. *Blood*, 73, 1720–1728.

Tanaka, J., Imamura, M., Kasai, M., Hashino, S., Kobayashi, S., Noto, S., Higa, T., Sakurada, K. & Asaka, M. (1997) The important balance between cytokines derived from type 1 and type 2 helper T cells in the control of graft-versus-host disease. *Bone Marrow Transplantation*, 19, 571–576.

Tegoshi, H., Hasegawa, G., Obayashi, H., Nakano, K., Kitagawa, Y., Fukui, M., Matsuo, S., Deguchi, M., Ohta, M., Nishimura, M., Nakamura, N. & Yoshikawa, T. (2002) Polymorphisms of interferon-gamma gene CA-repeat and interleukin-10 promoter region (-592A/C) in Japanese type I diabetes. *Human Immunology,* **63,** 121–128.

Umland, S.P., Razac, S., Nahrebne, D.K. & Seymour, B.W. (1992) Effects of in vivo administration of interferon (IFN)-gamma, anti-IFN-gamma, or anti-interleukin-4 monoclonal antibodies in chronic autoimmune graft-versus-host disease. *Clinical Immunology and Immunopathology,* **63,** 66–73.

Weiden, P.L., Sullivan, K.M., Flournoy, N., Storb, R. & Thomas, E.D. (1981) Antileukemic effect of chronic graft-versus-host disease: contribution to improved survival after allogeneic marrow transplantation. *New England Journal of Medicine,* **304,** 1529–1533.