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# Continuing increased risk of oral/esophageal cancer after allogeneic hematopoietic stem cell transplantation in adults in association with chronic graft-versus-host disease

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**Background:** The number of long-term survivors after hematopoietic stem cell transplantation (HSCT) showed steady increase in the past two decades. Second malignancies after HSCT are a devastating late complication. We analyzed the incidence of, risk compared with that in the general population, and risk factors for secondary solid cancers.

**Patients and methods:** Patients were 17 545 adult recipients of a first allogeneic stem cell transplantation between 1990 and 2007 in Japan. Risks of developing secondary solid tumors were compared with general population by using standard incidence ratios (SIRs).

**Results:** Two-hundred sixty-nine secondary solid cancers were identified. The cumulative incidence was 0.7% [95% confidence interval (CI), 0.6%–0.9%] at 5 years and 1.7% (95% CI, 1.4%–1.9%) at 10 years after transplant. The risk was significantly higher than that in the general population (SIR = 1.8, 95% CI, 1.5–2.0). Risk was higher for oral cancer (SIR = 15.7, 95% CI, 12.1–20.1), esophageal cancer (SIR = 8.5, 95% CI, 6.1–11.5), colon cancer (SIR = 1.9, 95% CI, 1.2–2.7), skin cancer (SIR = 7.2, 95% CI, 3.9–12.4), and brain/nervous system cancer (SIR = 4.1, 95% CI, 1.6–8.4). The risk of developing oral, esophageal, or skin cancer was higher at all times after 1-year post-transplant. Extensive-type chronic graft-versus-host disease (GVHD) was a significant risk factor for the development of all solid tumors (RR = 1.8, $P < 0.001$), as well as for oral (RR = 2.9, $P < 0.001$) and esophageal (RR = 5.3, $P < 0.001$) cancers. Limited-type chronic GVHD was an independent risk factor for skin cancers (RR = 5.8, $P = 0.016$).

**Conclusion:** Recipients of allogeneic HSCT had a significantly higher ~2-fold risk of developing secondary solid cancers than the general population. Lifelong screening for high-risk organ sites, especially oral or esophageal cancers, is important for recipients with active, or a history of, chronic GVHD.

**Key words:** secondary solid cancers, late effect, hematopoietic stem cell transplantation

## introduction

Hematopoietic stem cell transplantation (HSCT) is a curative treatment of choice for malignant and non-malignant hematological disorders [1]. The annual number of allogeneic HSCT has increased steadily over the past three decades worldwide [2–6]. Progress in transplant procedures in addition to this steady increase in the number of HSCT procedures worldwide has contributed to an increase in the number of long-term survivors.

Secondary malignancies, including new solid cancers, are an important cause of late mortality. Several studies have reported that survivors of HSCT have a 2–3-fold increased risk of

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developing new solid cancers compared with an age-, sex-, region-, and calendar-year-adjusted population and the risk among long-term survivors ranges from 1% to 6% at 10 years after transplantation [7–14]. Identified risk factors include exposure to radiation as a part of the conditioning regimen and chronic graft-versus-host disease (GVHD), and the latter has been shown to be strongly correlated with the development of squamous cell carcinoma [8, 10, 12, 15–17]. However, a recent long-term follow-up analysis of patients who were transplanted after myeloablative doses of busulfan and cyclophosphamide without total body irradiation (TBI) found a similar increased incidence of 0.6% at 5 years and 1.2% at 10 years after transplantation [13]. We conducted a nationwide, retrospective cohort study with a large and different cohort from those used in previous reports from North America and Europe, to determine the incidence and risks of developing secondary solid cancers.

## methods

### data source and collection of data

The recipient clinical data were collected by the Japan Society for Hematopoietic Cell Transplantation (JSHCT) using the Transplant Registry Unified Management Program, as described previously [18]. The JSHCT collect recipients' baseline, disease, transplant, and transplant outcome information who received HSCT in the previous year. Patient information regarding survival, disease status, and long-term complications including chronic GVHD and second malignancies are renewed annually. This study was approved by the data management committee of the JSHCT, as well as the institutional review board of Nagoya University Graduate School of Medicine.

### patients

Adult patients (at least 16 years of age) who received a first HSCT between 1990 and 2007 were considered as subjects for the present study. Those who were inherently susceptible to developing cancer [Fanconi anemia ($N = 3$) and congenital immunodeficiency ($N = 12$)] were excluded. Three-hundred five recipients (1.7%) were excluded because of insufficient follow-up data. The study included 17 545 recipients; 5358 recipients of related bone marrow, 3587 recipients of related peripheral blood stem cells (including 134 bone marrow and peripheral blood stem cells combined), 6508 recipients of unrelated bone marrow, and 2092 recipients of unrelated cord blood.

### statistical analysis

Standard incidence ratios (SIRs) were calculated to determine whether the number of recipients in the present cohort who developed secondary solid tumor after receiving a HSCT was different than that in the general population (supplementary method, available at *Annals of Oncology* online). Cumulative incidences of solid cancer or GVHD were estimated by taking into account the competing risk of death among patients who did not develop a second malignancy or GVHD [19]. The influence of potential risk factors was estimated by using the Cox proportional hazard model [20]. A stepwise multivariate approach was used to identify the most important predictor with respect to the development of secondary solid cancers. The variables considered were age at transplant, patient sex, donor-type (related versus unrelated), graft source, TBI as part of the conditioning regimen, reduced-intensity conditioning, grade 2–4 acute GVHD, and chronic GVHD. The model was stratified into four categories according to the primary disease; acute myeloid leukemia, acute lymphoblastic leukemia, chronic myeloid leukemia, and others. Acute and chronic GVHD were considered as time-dependent covariates. TBI and chronic GVHD were frequent risk factors and were always kept in the model. Risk factors for high-risk cancer sites with adequate numbers of events for analyses were also analyzed: oral cavity/pharynx, esophagus, colon, and skin. The models for high-risk cancer sites were stratified according to the primary disease as described, and patient age at transplantation (<19, 20–29, 30–39, 40–49, 50–59, and >60), and also adjusted by patient age as a continuous variable. All *P*-values were two-sided.

## results

### patient and transplant characteristics

Table 1 shows the patient characteristics, their disease, and transplant regimens for 17 545 recipients of a first HSCT. The cumulative incidences of grade 2–4 acute GVHD at 150 days and chronic GVHD at 2 years post-transplant were 35% [95% confidence interval (CI), 35%–36%] and 41% (95% CI, 40%–41%), respectively. The observation period reached 69 465 person-years among the subjects for analyses. Of the 17 545 recipients, 5864 had survived for 5 or more years, and 2192 recipients had survived 10 or more years at the time of analysis (Table 2).

### incidence and types of secondary solid cancers

The cumulative incidence of solid cancers was 0.7% (95% CI, 0.6–0.9) at 5 years, 1.7% (95% CI, 1.4–1.9) at 10 years, and 2.9% (95% CI, 2.5–3.4) at 15 years after transplantation (Figure 1). Two-hundred sixty-nine solid cancers were identified. Multiple solid cancers were observed in 11 patients. Nineteen recipients were diagnosed within 1-year post-transplantation (Table 2).

### risk compared with the general population

HSCT recipients had a 1.8-fold higher risk of invasive solid cancers compared with the general population (95% CI, 1.5–2.0). SIR was significantly higher for cancers of the oral cavity/pharynx (SIR = 15.7), esophagus (SIR = 8.5), colon (SIR = 1.9), skin (SIR = 7.2), and brain/nervous system (SIR = 4.1; Table 2). The risks of developing secondary cancers of the oral cavity/pharynx, esophagus, and skin were significantly higher than those in the general population throughout all periods after 1 year (Figure 2). The risk for developing colon cancer was elevated during the period of 1–4 years (SIR = 2.7), whereas the risks for developing cancer of the pancreas (SIR = 4.5) were elevated during the period of 5–9 years. Recipients were at higher risk of developing cancers of the rectum (SIR = 3.6) and the brain/nervous system (SIR = 19.1) after 10 years post-transplantation. The risk of developing secondary solid cancers of all types compared with the general population increased with the time since transplantation. This trend was observed for oral/pharynx and esophageal cancer (Table 2; Figure 2).

### recipients' age at transplantation and risks for developing secondary solid cancers

SIRs were also analyzed according to the recipient's age at transplantation (Table 3). Compared with the general population in Japan, the SIRs were significantly increased for all solid cancers, oral/pharynx, esophagus, liver, bronchus/lung, and brain/nervous system for recipients who were 16–19 years of age at transplant, all solid cancers, oral/pharynx, and esophagus for recipients who

**Table 1.** Patient, disease, and transplant characteristics

| Characteristics | Number | Percent |
|---|---|---|
| Total number | 17 545 | |
| Year of transplant | | |
| 1990–1994 | 1630 | 9 |
| 1995–1999 | 3750 | 21 |
| 2000–2004 | 7078 | 40 |
| 2005–2007 | 5087 | 29 |
| Patient sex | | |
| Male | 10 386 | 59 |
| Female | 7149 | 41 |
| Missing | 10 | <1 |
| Patient age | | |
| Median (range) | 40 (16–85) | |
| 16–19 | 1399 | 8 |
| 20–29 | 3506 | 20 |
| 30–39 | 3787 | 22 |
| 40–49 | 4167 | 24 |
| 50–59 | 3549 | 20 |
| ≥60 | 1137 | 6 |
| Diagnosis | | |
| Acute myeloid leukemia | 6096 | 35 |
| Acute lymphoblastic leukemia | 3334 | 19 |
| Chronic myeloid leukemia | 2514 | 14 |
| Myelodysplastic syndromes | 1716 | 10 |
| Adult T-cell leukemia | 591 | 3 |
| Other leukemia | 130 | 1 |
| Myeloproliferative disorders | 224 | 1 |
| Non-Hodikin's lymphoma | 1652 | 9 |
| Hodikin's lymphoma | 46 | <1 |
| Other lymphoma/type missing | 54 | <1 |
| Multiple myeloma | 210 | 1 |
| Aplastic anemia | 745 | 4 |
| Pure red cell aplasia | 4 | <1 |
| Paroxysmal nocturnal hemoglobinuria | 20 | <1 |
| Solid tumor | 109 | 1 |
| Others | 86 | <1 |
| Data missing | 14 | <1 |
| Donor | | |
| Related, siblings | 7825 | 45 |
| Related, other relatives | 941 | 5 |
| Related, data missing | 179 | 1 |
| Unrelated | 8600 | 49 |
| Stem cell source | | |
| Bone marrow | 11 866 | 68 |
| Peripheral blood | 3453 | 20 |
| Bone marrow and peripheral blood | 134 | 1 |
| Cord blood | 2092 | 12 |
| Conditioning regimen | | |
| Myeloablative | | |
| Cyclophosphamide + TBI ± other | 8298 | 47 |
| Other TBI regimen | 1321 | 8 |
| Busulfan + cyclophosphamide ± other | 2798 | 16 |
| Other non-TBI regimen | 778 | 4 |
| Reduced intensity | | |
| Fludarabine + busulfan ± other | 1527 | 9 |
| Fludarabine + cyclophosphamide ± other | 503 | 3 |
| Fludarabine + melphalan ± other | 1480 | 8 |
| Other RIST | 631 | 4 |
| Data missing | 209 | 1 |
| GVHD prophylaxis | | |
| No | 85 | <1 |
| Cyclosporine A + sMTX | 10 091 | 58 |
| Cyclosporine A ± other | 1175 | 7 |
| Tacrolimus + sMTX | 4682 | 27 |
| Tacrolimus ± other | 876 | 5 |
| Other | 323 | 2 |
| Data missing | 312 | 2 |

TBI, total body irradiation; sMTX, short-term methotrexate.

were 20–29 years of age at transplant, all solid cancers, oral/pharynx, esophagus, and gallbladder for recipients who were 30–39 years of age at transplant, all solid cancers, oral/pharynx, esophagus, and skin for recipients who were 40–49 years of age at transplant, all solid cancers, oral/pharynx, esophagus, colon, and skin for recipients who were 50–59 years of age at transplant (Table 3).

### risk factors for the development of secondary solid cancers

Extensive-type chronic GVHD and age at transplantation were important risk factors for the development of secondary solid cancers (Table 4). The risk was not increased in recipients who received TBI for conditioning. The results were similar when subjects were limited to those who received myeloablative conditioning (RR = 1.5, $P = 0.069$ for limited-type chronic GVHD, RR = 1.9, $P < 0.001$ for extensive-type chronic GVHD, and RR = 0.9, $P = 0.751$ for TBI). Risk factor analyses for high-risk organs with more than 10 cancer cases revealed that extensive-type chronic GVHD was an independent risk factor for cancers in the oral cavity/pharynx and esophagus. Limited-type chronic GVHD was a risk factor for cancers of skin (Table 4). For secondary cancers which developed within 1-year post-transplant, the only risk factor identified was older age at transplant (age 60 years or older; supplementary Table, available at *Annals of Oncology* online).

## discussion

Our main objective was to determine the incidence of, the risk compared with the general population, and risk factors for secondary solid tumors after allogeneic stem cell transplantation in a large cohort of adult recipients. Allogeneic HCT recipients were at higher risk of developing cancers of the oral cavity, esophagus, colon, and skin. The incidence and SIR of developing all solid cancers continued to increase with follow-up, which suggested a continuous increase as follow-up progressed. Our data are important since we included a large number of subjects and person-years of follow-up, in a transplant cohort that is different from those in previously reported large studies.

**Table 2.** Standard incidence ratio, ratio of observed versus expected number of secondary solid cancers according to duration post-transplant

| | Time since transplantation (years) | | | | | | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | <1 | | 1–4 | | 5–9 | | 10 or longer | | | | |
| Number of recipients | 17 545 | | 10 210 | | 5864 | | 2192 | | 17 545 | | |
| Person-years at risk | 12 803 | | 30 599 | | 18 845 | | 7218 | | 69 465 | | |
| Secondary cancer sites | O | SIR | O | SIR | O | SIR | O | SIR | O/E | SIR | 95% CI |
| All solid cancers | 19 | 0.7 | 97 | 1.5* | 90 | 2.0* | 63 | 3.1* | 269/153.6 | 1.8* | 1.5–2.0 |
| Oral/pharynx | 0 | 0.0 | 16 | 9.5* | 27 | 23.4* | 21 | 38.5* | 64/4.1 | 15.7* | 12.1–20.1 |
| Esophagus | 0 | 0.0 | 13 | 6.5* | 17 | 12.6* | 11 | 16.8* | 41/4.8 | 8.5* | 6.1–11.5 |
| Stomach | 2 | 0.4 | 7 | 0.6 | 6 | 0.8 | 1 | 0.3 | 16/26.0 | 0.6 | 0.4–1.0 |
| Colon | 2 | 0.8 | 16 | 2.7* | 5 | 1.2 | 4 | 2.2 | 27/14.3 | 1.9* | 1.2–2.7 |
| Rectum | 0 | 0.0 | 1 | 0.2 | 0 | 0.0 | 5 | 3.6* | 6/10.7 | 0.6 | 0.2–1.2 |
| Liver | 1 | 0.6 | 5 | 1.4 | 0 | 0.0 | 2 | 1.8 | 8/8.6 | 0.9 | 0.4–1.8 |
| Gallbladder | 2 | 5.1 | 2 | 2.1 | 2 | 3.0 | 0 | 0.0 | 6/2.3 | 2.6 | 1.0–5.7 |
| Pancreas | 0 | 0.0 | 2 | 1.0 | 6 | 4.5* | 1 | 1.6 | 9/4.7 | 1.9 | 0.9–3.7 |
| Broncus/lung | 3 | 1.2 | 4 | 0.6 | 9 | 2.1 | 3 | 1.5 | 19/15.1 | 1.3 | 0.8–2.0 |
| Skin | 2 | 7.0 | 6 | 8.1* | 3 | 5.7* | 2 | 8.4* | 13/1.8 | 7.2* | 3.9–12.4 |
| Female breast | 0 | 0.0 | 3 | 0.3 | 1 | 0.1 | 3 | 0.9 | 7/24.5 | 0.3 | 0.1–0.6 |
| Cervix uteri | 1 | 1.3 | 4 | 2.0 | 1 | 0.7 | 1 | 1.6 | 7/4.8 | 1.5 | 0.6–3.0 |
| Corpus uteri | 2 | 3.7 | 1 | 0.7 | 2 | 1.8 | 0 | 0.0 | 5/3.6 | 1.4 | 0.4–3.2 |
| Ovary | 0 | 0.0 | 1 | 0.7 | 1 | 1.0 | 1 | 2.2 | 3/3.6 | 0.8 | 0.2–2.4 |
| Prostate | 1 | 1.2 | 0 | 0.0 | 1 | 0.6 | 1 | 1.4 | 3/5.4 | 0.6 | 0.1–1.6 |
| Bladder | 1 | 1.9 | 3 | 2.4 | 0 | 0.0 | 0 | 0.0 | 4/2.9 | 1.4 | 0.4–3.5 |
| Kidney | 0 | 0.0 | 1 | 0.6 | 1 | 0.9 | 0 | 0.0 | 2/4.1 | 0.5 | 0.1–1.8 |
| Brain/nervous system | 1 | 3.4 | 1 | 1.4 | 1 | 2.1 | 4 | 19.1* | 7/1.7 | 4.1* | 1.6–8.5 |
| Thyroid | 0 | 0.0 | 2 | 1.1 | 2 | 1.5 | 0 | 0.0 | 4/4.5 | 0.9 | 0.2–2.3 |
| Other[a] | 1 | | 9 | | 4 | | 3 | | 17 | | |

[a]Other sites included two testicular cancers, four connective tissue cancers, four bone cancers, one larynx cancer, one malignant salivary gland tumor, one duodenum papilla cancer, one germ cell tumor, one carcinomatous pleurisy of origin unknown, and two squamous cell carcinomas of unknown origin.
*$P < 0.05$.

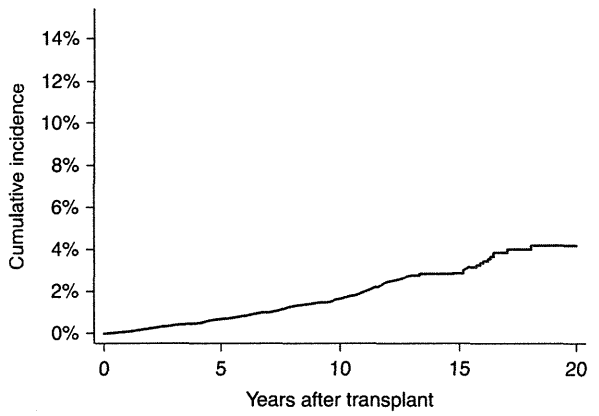


**Figure 1.** Cumulative incidence of developing a secondary solid cancer. The cumulative incidence of solid cancers was 0.7% [95% confidence interval (CI), 0.6–0.9] at 5 years, 1.7% (95% CI, 1.4–1.9) at 10 years, and 2.9% (95% CI, 2.5–3.4) at 15 years after transplantation.

Extensive-type chronic GVHD has repeatedly been shown to be a significant risk factor for the development of secondary solid tumor and is highly correlated with squamous cell carcinoma [8, 9, 12, 15, 16]. Extensive-type chronic GVHD was also shown to be a significant risk factor for oral cancer in our study. Extensive-type chronic GVHD was shown to be a significant risk factor for esophageal cancer, which was found to be increased in recipients compared with the general population in our study as well as in two other smaller Japanese cohorts in previous studies [11, 14]. Subjects were shown to be at a higher risk for the development of cancers of the oral cavity or esophagus at all time periods after 1 year. Data were not obtained for affected organ sites of chronic GVHD in JSHCT data collection prior to transplants in 2006. Therefore, we could not investigate whether oral or esophageal cancers were related to the chronic GVHD of the same organ. However, results of risk factor analyses for cancer sites of oral, esophagus, colon, and skin which showed high associations of extensive-type chronic GVHD and oral or esophagus cancer, limited-type chronic GVHD, and skin cancer showed that development of secondary solid tumors were likely to be influenced by GVHD-affected sites. Lifelong screening for oral, pharynx, or esophageal cancers for recipients with active or resolved chronic GVHD is important after 1-year post-transplant. The prognosis of solid cancers is highly influenced by the stage of the cancers when they are first detected. Our findings support recently published recommended screening guidelines [21, 22].

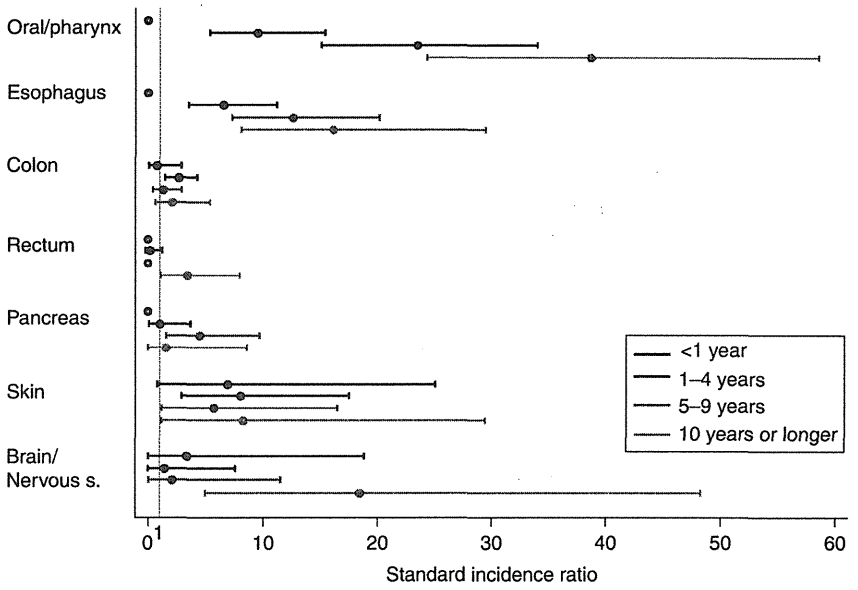


**Figure 2.** Trends of standard incidence ratios (SIRs) and its 95% confidence intervals (CIs) of high-risk secondary solid cancer sites according to time since transplant. The SIR and 95% CIs for <1, 1–4, 5–9, and 10 years or longer post-transplant were 0.0, 9.5 (5.4–15.4), 23.4 (15.4–34.0), and 38.5 (23.8–58.9) for oral/pharynx cancer, 0.0, 6.5 (3.5–11.2), 12.6 (7.3–20.2), and 16.8 (8.4–30.1) for esophageal cancer, 0.8 (0.1–2.9), 2.7 (1.5–4.3), 1.2 (0.4–2.9), and 2.2 (0.6–5.7) for colon cancer, 0.0, 0.2 (0.0–1.3), 0.0, and 3.6 (1.2–8.4) for rectum cancer, 0.0, 1.0 (0.1–3.7), 4.5 (1.6–9.7), and 1.6 (0.0–8.9) for pancreatic cancer, 7.0 (0.8–25.1), 8.1 (3.0–17.5), 5.7 (1.2–16.7), and 8.4 (1.0–30.3) for skin cancer, and 3.4 (0.1–19.0), 1.4 (0.0–7.7), 2.1 (0.1–11.6), and 19.1 (5.2–49.0) for cancers of brain/nervous system, respectively.

**Table 3.** Standard incidence ratio according to recipient's age at transplant

| | Recipient's age at transplantation | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 16–19 | | 20–29 | | 30–39 | | 40–49 | | 50–59 | | 60 or older | |
| Number-of-recipients | 1399 | | 3506 | | 3787 | | 4167 | | 3549 | | 1137 | |
| Person-years at risk | 7083 | | 17 912 | | 17 303 | | 16 198 | | 9126 | | 1843 | |
| Secondary cancer sites | O | SIR | O | SIR | O | SIR | O | SIR | O | SIR | O | SIR |
| All solid cancers | 18 | 17.0* | 28 | 4.1* | 51 | 2.4* | 71 | 1.4* | 79 | 1.5* | 22 | 1.0 |
| Oral/pharynx | 7 | 140.0* | 11 | 50.7* | 19 | 36.5* | 13 | 10.1* | 12 | 8.1* | 2 | 3.9 |
| Esophagus | 1 | 350.0* | 3 | 131.0* | 13 | 48.5* | 10 | 7.0* | 13 | 5.9* | 1 | 1.1 |
| Stomach | 1 | 13.3 | 0 | 0.0 | 1 | 0.3 | 7 | 0.8 | 5 | 0.5 | 2 | 0.5 |
| Colon | 0 | 0.0 | 0 | 0.0 | 3 | 2.0 | 6 | 1.3 | 12 | 2.1* | 6 | 2.6 |
| Rectum | 1 | 33.1 | 0 | 0.0 | 0 | 0.0 | 1 | 0.3 | 4 | 0.9 | 0 | 0.0 |
| Liver | 1 | 66.4* | 1 | 8.1 | 0 | 0.0 | 2 | 0.8 | 3 | 0.8 | 1 | 0.6 |
| Gallbladder | 0 | 0.0 | 0 | 0.0 | 2 | 12.0* | 1 | 1.5 | 2 | 2.1 | 1 | 2.0 |
| Pancreas | 0 | 0.0 | 0 | 0.0 | 2 | 5.5 | 1 | 0.7 | 4 | 2.0 | 2 | 2.3 |
| Broncus/lung | 1 | 44.3* | 0 | 0.0 | 2 | 1.6 | 7 | 1.6 | 7 | 1.1 | 2 | 0.7 |
| Skin | 1 | 28.6 | 1 | 6.3 | 0 | 0.0 | 6 | 11.6* | 4 | 7.4* | 1 | 4.0 |
| Female breast | 0 | 0.0 | 1 | 0.7 | 1 | 0.2 | 1 | 0.1 | 3 | 0.5 | 1 | 0.9 |
| Cervix uteri | 0 | 0.0 | 1 | 1.2 | 3 | 1.9 | 2 | 1.4 | 1 | 1.4 | 0 | 0.0 |
| Corpus uteri | 0 | 0.0 | 1 | 5.2 | 0 | 0.0 | 2 | 1.4 | 2 | 1.6 | 0 | 0.0 |
| Ovary | 0 | 0.0 | 1 | 3.2 | 0 | 0.0 | 1 | 0.7 | 0 | 0.0 | 1 | 6.4 |
| Prostate | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 2.4 | 0 | 0.0 | 1 | 0.5 |
| Bladder | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 2.3 | 2 | 1.7 | 0 | 0.0 |
| Kidney | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 1.4 | 0 | 0.0 | 0 | 0.0 |
| Brain/nervous system | 2 | 23.9* | 1 | 3.8 | 1 | 2.7 | 1 | 2.0 | 1 | 2.6 | 1 | 9.1 |
| Thyroid | 0 | 0.0 | 2 | 3.9 | 0 | 0.0 | 1 | 0.7 | 1 | 0.9 | 0 | 0.0 |

*$P < 0.05$.

**Table 4.** Risk factors for second solid cancers among >1 year survivors after hematopoietic stem cell transplantation

| Solid cancer | Risk factor | Number of patients with second cancer | RR | 95% CI | *P*-value |
|---|---|---|---|---|---|
| All second solid cancers[a] | | 249 | | | |
| | Total body irradiation | 151 | 0.9 | 0.7–1.1 | 0.294 |
| | Chronic GVHD | | | | |
| | Limited type | 45 | 1.4 | 1.0–1.9 | 0.087 |
| | Extensive type | 93 | 1.8 | 1.4–2.4 | <0.001 |
| | Age at transplant (years) | | | | |
| | 16–29 | 45 | 1.0 | | |
| | 30–39 | 46 | 1.6 | 1.0–2.4 | 0.042 |
| | 40–49 | 68 | 2.5 | 1.7–3.7 | <0.001 |
| | 50–59 | 71 | 5.5 | 3.7–8.2 | <0.001 |
| | 60 or older | 19 | 7.9 | 4.4–14.1 | <0.001 |
| Oral cancer[b] | | 64 | | | |
| | Total body irradiation | 38 | 1.0 | 0.8–1.3 | 0.957 |
| | Chronic GVHD | | | | |
| | Limited type | 10 | 1.4 | 0.6–2.9 | 0.440 |
| | Extensive type | 29 | 2.9 | 1.6–5.1 | <0.001 |
| Esophageal cancer[b] | | 41 | | | |
| | Total body irradiation | 22 | 0.6 | 0.3–1.1 | 0.108 |
| | Chronic GVHD | | | | |
| | Limited type | 7 | 2.1 | 0.8–5.9 | 0.151 |
| | Extensive type | 25 | 5.3 | 2.4–11.8 | <0.001 |
| Colon cancer[b] | | 26 | | | |
| | Total body irradiation | 12 | 0.5 | 0.2–1.2 | 0.144 |
| | Chronic GVHD | | | | |
| | Limited type | 6 | 1.7 | 0.6–4.9 | 0.353 |
| | Extensive type | 10 | 1.6 | 0.6–4.2 | 0.329 |
| | Grade 2–4 acute GVHD | 12 | 2.0 | 0.9–4.4 | 0.101 |
| Skin cancer[b] | | 13 | | | |
| | Total body irradiation | 12 | 1.2 | 0.8–1.6 | 0.377 |
| | Chronic GVHD | | | | |
| | Limited type | 6 | 5.8 | 1.4–23.9 | 0.016 |
| | Extensive type | 2 | 1.8 | 0.3–8.9 | 0.500 |

RR, relative risk; CI, confidence interval; TBI, total body irradiation; GVHD, graft-versus-host disease.
[a]Stratified for primary disease (acute myeloid leukemia, acute lymphoblastic leukemia, chronic myeloid leukemia, and other).
[b]Stratified for primary disease (acute myeloid leukemia, acute lymphoblastic leukemia, chronic myeloid leukemia, and other) and patient age groups (<19, 20–29, 30–39, 40–49, 50–59, and >60). Adjusted for patient age as a continuous variable.

The incidence of secondary solid tumors in our study was similar to those in previously reported large studies [8, 9, 12, 13]. Rizzo et al. [12] reported that the incidence of secondary solid cancers among 28 874 transplant recipients and 85 583 person-years at risk was 1% at 10 years and 2.2% at 15 years, which were very similar to our results using the same statistical method for cumulative incidence, while treating death before secondary solid tumor as a competing risk. Majhail et al. [13] reported that the incidence of secondary solid cancers after HSCT using non-TBI, busulfan-cyclophosphamide conditioning was also ~1.2% at 10 years. The oral cavity was the most prominent high-risk cancer site compared with the general population, as in previous reports [8, 9, 12, 13]. Despite regional and racial differences in cancer incidence and cancer sites in the general population, the impact of HSCT on secondary cancer was similar.

In previous studies, TBI was reported to be a significant risk factor for the development of secondary cancer, but significant differences were not found in our study [7, 8, 10, 12, 23]. The subjects in this study were adult recipients, which may explain the different findings. Conditioning with radiation was reported to be associated with the development of secondary solid cancer in recipients at a younger age at transplant [12]. Moreover, a recent long-term follow-up analysis of patients who were transplanted after myeloablative doses of busulfan and cyclophosphamide without TBI found a similar increased incidence of secondary solid cancers as previous reports [13].

An older recipient age at transplant was a significant risk factor for the development of secondary solid tumor, as in previous studies [9, 13]. This result was not surprising since it is also the case in the general population. However, it is important to note that older patients are at higher risk of developing

secondary cancer and to promote patient education and preventive practices, since there has been a dramatic increase in the number of transplant recipients who are more than 50 years of age at transplant over the past decade. In comparison with the general population, younger patients were at a higher risk of developing a solid tumor. Several high-risk cancer sites (esophagus, liver, and bronchus/lung) in younger group did have only one observed cases, therefore, these results should not be emphasized and need to be confirmed in other studies. These sites were found to be significant because the expected numbers in general population for these sites were extremely small.

Although this study included a large number of recipients and a large number of person-years of follow-up, there are limitations. The follow-up years for older recipients were still limited, and therefore we may find a higher incidence of and risk of secondary solid cancers among recipients who are 50 years of age or older at transplant in the future. Second limitation involves possible under-reporting by recipients to transplant centers or by transplant centers to the registry. Until recently, transplant recipients have received only limited information regarding screening or the prevention of secondary solid cancers. Another limitation of this analysis was lack of central pathology review for secondary solid tumors. JSHCT data collection does not include the submission of specimen or pathology report. Since this study included transplants from 1990, central pathology review was difficult to perform at the time of analyses. In addition, limiting secondary tumors to centrally diagnosed tumors would decrease the number of identified secondary tumors; therefore, secondary solid tumors were identified as reported from transplant centers.

In conclusion, recipients of allogeneic hematopoietic stem cell transplant had a significantly higher risk of developing secondary solid cancers than the general population. Older recipients are at higher risk of developing secondary solid tumors, as in the general population. Lifelong screening is important for high-risk organ sites, especially for oral, pharynx, and esophageal cancers in recipients with active, or a history of, chronic GVHD.

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## disclosure

The authors have declared no conflicts of interest.

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ORIGINAL ARTICLE

# Risk factors and organ involvement of chronic GVHD in Japan

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Few studies have evaluated the risk factors for chronic GVHD and organ involvement associated with different graft types, including unrelated cord blood (U-CB). We retrospectively studied 4818 adult patients who received their first allogeneic transplantation and survived for at least 100 days. The incidence of chronic GVHD at 2 years was 37%. The following factors were associated with the development of chronic GVHD: female donor/male recipient, CMV-Ab seropositivity, matched related peripheral blood grafts vs matched related BM grafts, no *in vivo* T-cell depletion and the occurrence of grade II–IV acute GVHD. Among these factors, the association with acute GVHD occurrence was consistently significant across donor subtypes. The use of U-CB was not associated with chronic GVHD, but was associated with a low incidence of extensive chronic GVHD. Chronic GVHD patients who had received U-CB transplants showed less frequent involvement of the oral cavity (28% vs 55%), eye (12% vs 26%), liver (20% vs 44%), lung (11% vs 25%) and joint (0% vs 6%) than those with matched related BM grafts. In conclusion, we found that U-CB transplants were associated with a low incidence of extensive chronic GVHD and less frequent involvement of the oral cavity, eye, liver, lung and joints.

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**Keywords:** chronic GVHD; unrelated cord blood; acute GVHD; risk factors

## INTRODUCTION

Chronic GVHD is a serious complication that affects the survival and quality of life of long-term survivors after allogeneic hematopoietic SCT.[1–3] Various pre- and post-transplant risk factors associated with chronic GVHD have been identified, mostly in transplantations using BM and PBSC grafts from related or unrelated donors.[2,3] Several studies have reported a history of acute GVHD to be a strong risk factor that is consistently associated with chronic GVHD development.[4–8] Other identified risk factors include the following: female donor and male recipient,[4,6] use of PBSC grafts,[6,9–13] older patient,[4,6–8] older donor,[6,7] transplantation from a mismatched or unrelated donor,[5,6,14] diagnosis of CML[4,7,8] and absence of anti-thymocyte globulin (ATG) use.[15]

The number of unrelated cord blood (U-CB) transplantations performed has rapidly increased during the past decade. However, few studies have compared the incidences and risk factors of chronic GVHD and its organ-specific symptoms in adult patients receiving U-CB and other available grafts, including related or unrelated BM/PBSC grafts.[16,17] Therefore, we conducted a retrospective study using national registry data involving 4818 patients who underwent allogeneic transplantation. This study aimed to evaluate the incidence and risk factors of chronic GVHD, and the prevalence of chronic GVHD organ involvement in patients who received transplantation using various types of graft, including U-CB.

## MATERIALS AND METHODS

### Data collection

Data for 54 072 patients who had received auto-SCT or allo-SCT by December 31, 2009 were provided by the Transplant Registry Unified Management Program (TRUMP).[18] We included 4993 adult patients who had: (1) received allogeneic transplantation for hematologic malignancies; (2) received their first SCT; (3) used the same questionnaire form involving chronic GVHD organ involvement (skin, oral cavity, eye, liver, lung, joint, intestine/genitals and other manifestations; 2006–2009 for transplantations using BM or PBSC grafts and 2007–2009 for transplantations using U-CB units); (4) achieved neutrophil engraftment; (5) survived for at least 100 days; and (6) received the following: (a) a related BM or PBSC graft (R-BM/PB), (b) an unrelated BM (U-BM) or (c) a single U-CB unit. Donation of peripheral blood by unrelated volunteers was permitted for the first time in Japan in 2011. The following patients were excluded: (1) patients who received *ex vivo* T-cell-depleted grafts ($n = 26$) and (2) patients who lacked data on acute or chronic GVHD ($n = 149$). Thus, 4818 patients were included in this study, which was approved by the TRUMP Data Management Committees and by the institutional review board of the Nagoya University Graduate School of Medicine, where this study was performed.

### Histocompatibility

Histocompatibility data for the HLA-A, HLA-B and HLA-DR loci were obtained through reports acquired from the institution where the transplantation was performed or from the cord blood bank. HLA

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matching was assessed using serological data for the HLA-A, HLA-B and HLA-DR loci in R-BM/PB or U-CB transplantations, and using allelic data for the HLA-A, HLA-B and HLA-DRB1 loci in U-BM transplantations.

### Statistical analysis

The physicians who performed the transplantations at each center diagnosed and classified acute and chronic GVHD according to traditional criteria.[1,19] The reported type of chronic GVHD was reclassified according to the information on its organ involvement. 'Progressive onset' of chronic GVHD was defined as chronic GVHD transitioned from active acute GVHD, 'quiescent onset' as chronic GVHD after remission of acute GVHD and '*de novo* onset' as chronic GVHD without history or acute GVHD. The intensity of conditioning regimen was classified as myeloablative or reduced intensity on the basis of the Center for International Blood and Marrow Transplant Research report and the information from the questionnaire, as previously described.[20–23] We defined the following as standard-risk diseases: AML and ALL in first or second remission; CML in the first or

**Table 1.** Patient characteristics

| *Variable* | *R-BM/PB* | | *U-BM* | | *U-CB* | | P-*value* |
|---|---|---|---|---|---|---|---|
| | n = *1859* | % | n = *2215* | % | n = *744* | % | |
| Recipient age, years, median (range) | 46 (16–74) | | 47 (16–73) | | 51 (16–82) | | <0.001 |
| Donor age, years, median (range) | 43 (10–79) | | 35 (20–55)[a] | | — | — | — |
| *Recipient sex* | | | | | | | |
| Female | 789 | 42 | 916 | 41 | 334 | 45 | 0.238 |
| Male | 1070 | 58 | 1299 | 59 | 410 | 55 | |
| *Sex match between recipient and donor* | | | | | | | |
| Match | 965 | 52 | 1251 | 56 | 227 | 31 | <0.001 |
| Male to female | 398 | 21 | 573 | 26 | 109 | 15 | |
| Female to male | 496 | 27 | 389 | 18 | 131 | 18 | |
| Missing | 0 | 0 | 2 | 0 | 277 | 37 | |
| *Disease* | | | | | | | |
| AML | 799 | 43 | 986 | 45 | 395 | 53 | 0.004 |
| MDS | 210 | 11 | 276 | 12 | 76 | 10 | |
| CML | 60 | 3 | 73 | 3 | 25 | 3 | |
| ALL | 385 | 21 | 439 | 20 | 123 | 17 | |
| ATL | 110 | 6 | 131 | 6 | 29 | 4 | |
| NHL | 206 | 11 | 214 | 10 | 70 | 9 | |
| Other diseases | 89 | 5 | 96 | 4 | 26 | 3 | |
| *Disease risk* | | | | | | | |
| Standard | 1058 | 57 | 1351 | 61 | 331 | 44 | <0.001 |
| High | 724 | 39 | 780 | 35 | 390 | 52 | |
| Missing | 77 | 4 | 84 | 4 | 23 | 3 | |
| *Source of stem cells* | | | | | | | |
| BM | 842 | 45 | 2215 | 100 | — | — | — |
| Peripheral blood | 1017 | 55 | — | — | — | — | |
| Cord blood | — | — | — | — | 744 | 100 | |
| *HLA compatibility*[b] | | | | | | | |
| Matched | 1486 | 80 | 1507 | 68 | 53 | 7 | <0.001 |
| Mismatched | 373 | 20 | 708 | 32 | 691 | 93 | |
| *Conditioning regimen* | | | | | | | |
| Myeloablative | 1202 | 65 | 1505 | 68 | 436 | 59 | <0.001 |
| Reduced intensity | 649 | 35 | 696 | 31 | 308 | 41 | |
| Missing | 8 | 1 | 14 | 1 | 0 | 0 | |
| *GVHD prophylaxis* | | | | | | | |
| CsA based | 1367 | 74 | 469 | 21 | 311 | 42 | <0.001 |
| Tac based | 449 | 24 | 1737 | 78 | 425 | 57 | |
| Others/missing | 43 | 2 | 9 | 1 | 8 | 1 | |
| *Use of* in vivo *T-cell depletion* | | | | | | | |
| No | 1741 | 94 | 2143 | 97 | 730 | 98 | <0.001 |
| Yes | 118 | 6 | 72 | 3 | 14 | 2 | |
| *CMV Ab (recipient and donor)* | | | | | | | |
| Both negative | 127 | 7 | 150 | 7 | 151 | 20 | <0.001 |
| Either positive | 1561 | 84 | 2003 | 90 | 535 | 72 | |
| Unknown | 171 | 9 | 62 | 3 | 58 | 8 | |
| *Acute GVHD* | | | | | | | |
| Grade II–IV | 665 | 36 | 897 | 41 | 338 | 45 | <0.001 |
| Grade III–IV | 217 | 12 | 236 | 11 | 81 | 11 | 0.578 |
| Follow-up of survivors (years), median (range) | 2.0 (0.3–4.7) | | 1.9 (0.3–4.8) | | 1.7 (0.3–3.9) | | <0.001 |

Abbreviations: ATL = adult T-cell leukemia; MDS = myelodysplastic syndrome; NHL = non-Hodgkin's lymphoma; R-BM/PB = related BM or PBSC; Tac = tacrolimus; U-BM = unrelated BM; U-CB = unrelated cord blood. [a]Data are missing in 20 patients [b]HLA matching was assessed by serological data for HLA-A, HLA-B and HLA-DR loci in transplantation using R-BM/PB or U-CB grafts, whereas it was assessed by allelic data for HLA-A, HLA-B and HLA-DRB1 loci in transplantation using U-BM grafts.

second chronic phase or in the accelerated phase; myelodysplastic syndrome (MDS) with refractory anemia or refractory anemia with ringed sideroblasts; adult T-cell leukemia (ATL) in CR; and Hodgkin's or non-Hodgkin's lymphoma (NHL) in CR or PR. Others were defined as high-risk diseases.

The probability of developing chronic GVHD was estimated on the basis of cumulative incidence curves.[24] Competing events for chronic GVHD were death or relapse without GVHD. Groups were compared using Gray's test.[25] The Cox proportional hazards model was used to evaluate the effect of confounding variables on chronic GVHD. The following possible confounding variables were considered: recipient age; recipient sex; sex mismatch between recipient and donor (match, male (donor)/female (recipient), or female (donor)/male (recipient)); disease (CML or others); disease risk before transplantation (standard or high risk); donor type (HLA-matched related BM (MR-BM), HLA-matched related PBSCs (MR-PB), HLA-mismatched related BM (MMR-BM), HLA-mismatched related PBSCs (MMR-PB), HLA-matched unrelated BM (MU-BM), HLA-mismatched unrelated BM (MMU-BM) and U-CB); type of conditioning regimen (myeloablative or reduced intensity); type of GVHD prophylaxis (CsA based or tacrolimus based); use of *in vivo* T-cell depletion (yes or no); anti-CMV Ab detection (negative for both recipient and donor, or positive for either recipient or donor), and presence of grade II–IV acute GVHD. Confounding factors were selected in a stepwise manner from the model with a variable retention criterion of $P<0.05$. Reported factors associated with chronic GVHD (recipient age, sex mismatch, donor type, use of *in vivo* T-cell depletion and the presence of grade II–IV acute GVHD) was additionally selected as confounding factors in the analysis of chronic GVHD risk. In the subset analysis, the same variables used in the analysis for the entire cohort were added to the final model. Furthermore, the following variables were also added for the specific group: donor age, presence of an HLA mismatch and the use of PBSCs for the R-BM/PB group; donor age and presence of an HLA mismatch for the U-BM group; and presence of an HLA mismatch for the U-CB group.

We also compared the prevalence of chronic GVHD presentation or organ involvement between MR-BM and other graft types using the $\chi^2$ test. We further evaluated chronic GVHD-specific survival, which is defined as the time from the day of chronic GVHD diagnosis to the day of death in the absence of relapse, among patients who developed chronic GVHD. We also evaluated OS among those who developed chronic GVHD. The probability of developing chronic GVHD-specific survival or OS from the onset of chronic GVHD was estimated using the Kaplan–Meier method, and univariate comparison between groups was performed using the log-rank test. In the analysis of chronic GVHD-specific survival, patients who were alive without disease recurrence were censored at the time of their last follow-up visit and those who experienced disease recurrence were censored at the time of diagnosis of recurrence. The Cox proportional hazards model was used to evaluate the effect of presentation or of each organ's manifestation of chronic GVHD on chronic GVHD-specific survival, after adjusting for donor type and other confounding factors that were selected from the model in a stepwise manner using a variable retention criterion of $P<0.05$. We also evaluated the effect of chronic GVHD on relapse, where the occurrence of chronic GVHD was treated as a time-varying covariate.

All tests were two-sided, and *P*-values $<0.05$ were considered statistically significant, except for the comparison of prevalence of chronic GVHD organ involvement between MR-BM and other graft types, where *P-values* $<0.008$ *was significant in consideration of multiple comparison.* All statistical analyses were performed using Stata version 12 (Stata Corp., College Station, TX, USA) and EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan),[26,27] which is a graphical user interface for R (R Foundation for Statistical Computing, version 2.13.0, Vienna, Austria).

## RESULTS

### Patient characteristics

Table 1 shows patient characteristics according to the stem cell source. The median age of recipients at the time of the transplant was 47 years (range, 16–82 years) for the entire cohort, and it was significantly higher for patients in the U-CB group. High-risk diseases were more prevalent in the U-CB group. The grafts used were MR-BM ($n=687$), MR-PB ($n=799$), MMR-BM ($n=155$), MMR-PB ($n=218$), MU-BM ($n=1507$), MMU-BM ($n=708$) and U-CB ($n=744$). CsA-based GVHD prophylaxis was received by 74% of the patients in the R-BM/PB group and by only 21% of the U-BM recipients. *In vivo* T-cell depletion was used for only 4% of the entire cohort (ATG, $n=197$; alemtuzumab, $n=7$). Grade II–IV and III–IV acute GVHD occurred in 39% and 11% of the cohort, respectively.

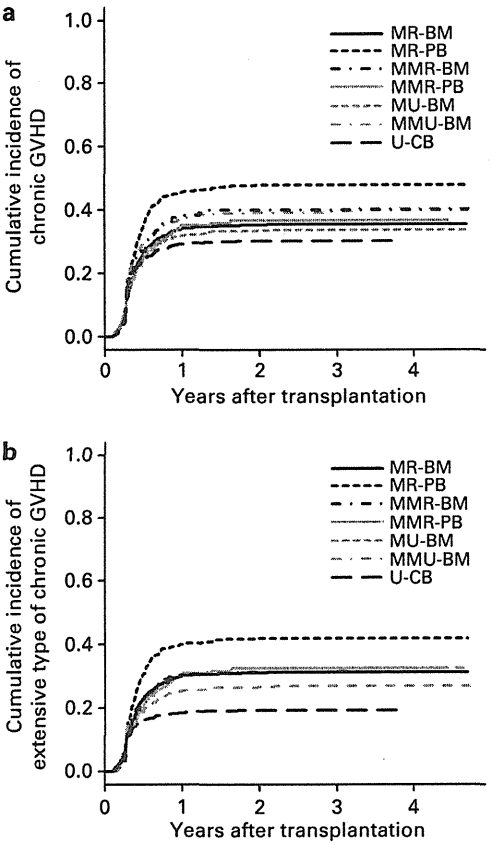


**Figure 1.** Cumulative incidence of chronic GVHD (**a**) and extensive type of chronic GVHD (**b**).

### Chronic GVHD

The incidence of chronic GVHD at 2 years was 37% (95% confidence interval (CI), 35–38%) for the entire cohort, with a median onset of 120 days (range, 30–1203 days), 36% (32–39%) for the MR-BM group, 48% (44–51%) for the MR-PB group, 40% (32–48%) for the MMR-BM group, 37% (30–44%) for the MMR-PB group, 34% (31–36%) for the MU-BM group, 40% (36–44%) for the MMU-BM group and 30% (27–34%) for the U-CB group (Gray's test for the whole group, $P<0.001$; Figure 1a). Female/male mismatch between recipient and donor (hazard ratio (HR), 1.29; $P<0.001$), CMV Ab detection (HR, 1.26; $P=0.015$), the use of MR-PB vs MR-BM graft (HR, 1.49; $P<0.001$), the use of *in vivo* T-cell depletion (HR, 0.48; $P<0.001$) and the occurrence of grade II–IV acute GVHD (HR, 1.62; $P<0.001$) were significantly associated with chronic GVHD development (Table 2). The use of PBSC grafts was significantly associated with chronic GVHD development in the R-BM/PB group (HR, 1.42; $P<0.001$). The impact of CMV Ab positivity on chronic GVHD development was significant only for the U-CB group, but HR was consistently high across donor subtypes. The effect of sex mismatch was significant for the R-BM/PB group, but was not significant for the U-CB group. The effect of grade II–IV acute GVHD occurrence on chronic GVHD development was consistently significant across donor subtypes.

**Table 2.** Risk factors for chronic GVHD

| *Variable* | *Chronic GVHD (Total)* | | | *Chronic GVHD (R-BM/PB)* | | | *Chronic GVHD (U-BM)* | | | *Chronic GVHD (U-CB)* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *HR* | *95% CI* | P-*value* | *HR* | *95% CI* | P-*value* | *HR* | *95% CI* | P-*value* | *HR* | *95% CI* | P-*value* |
| Recipient age, per 10 years | 1.03 | (0.99–1.06) | 0.136 | 1.09 | (1.01–1.17) | 0.021 | 1.01 | (0.96–1.07) | 0.741 | 0.91 | (0.83–1.00) | 0.056 |
| Donor age, per 10 years | | | | 1.01 | (0.94–1.09) | 0.730 | 1.04 | (0.95–1.14) | 0.429 | | | |
| *Sex match between recipient and donor* | | | | | | | | | | | | |
| Match | 1.00 | | | 1.00 | | | | | | 1.00 | | |
| Male to female | 0.97 | (0.86–1.10) | 0.619 | 1.01 | (0.83–1.23) | 0.905 | 1.00 | (0.84–1.19) | 0.992 | 0.78 | (0.51–1.19) | 0.253 |
| Female to male | 1.29 | (1.14–1.44) | <0.001 | 1.45 | (1.23–1.71) | <0.001 | 1.16 | (0.96–1.41) | 0.127 | 1.12 | (0.78–1.62) | 0.535 |
| CMV Ab (donor and recipient) | | | | | | | | | | | | |
| Both negative | 1.00 | | | 1.00 | | | 1.00 | | | 1.00 | | |
| Either positive | 1.26 | (1.05–1.52) | 0.015 | 1.12 | (0.82–1.54) | 0.469 | 1.22 | (0.90–1.66) | 0.196 | 1.53 | (1.07–2.21) | 0.021 |
| Type of donor and stem cell source | | | | | | | | | | | | |
| MR-BM | 1.00 | | | | | | | | | | | |
| MR-PB | 1.49 | (1.26–1.75) | <0.001 | | | | | | | | | |
| MMR-BM | 1.21 | (0.91–1.60) | 0.187 | | | | | | | | | |
| MMR-PB | 1.31 | (1.00–1.72) | 0.054 | | | | | | | | | |
| MU-BM | 0.91 | (0.78–1.07) | 0.247 | | | | | | | | | |
| MMU-BM | 1.10 | (0.92–1.31) | 0.306 | | | | | | | | | |
| U-CB | 1.00 | (0.81–1.23) | 0.991 | | | | | | | | | |
| Type of stem cell source | | | | | | | | | | | | |
| BM | | | | 1.00 | | | | | | | | |
| PB | | | | 1.42 | (1.23–1.65) | <0.001 | | | | | | |
| HLA disparity | | | | | | | | | | | | |
| Match | | | | 1.00 | | | 1.00 | | | 1.00 | | |
| Mismatch | | | | 1.12 | (0.92–1.36) | 0.274 | 1.17 | (1.00–1.36) | 0.043 | 0.96 | (0.55–1.69) | 0.887 |
| *Use of* in vivo *T-cell depletion* | | | | | | | | | | | | |
| No | | 1.00 | | 1.00 | | | 1.00 | | | 1.00 | | |
| Yes | 0.48 | (0.34–0.66) | <0.001 | 0.29 | (0.18–0.45) | <0.001 | 0.85 | (0.55–1.34) | 0.490 | 0.35 | (0.05–2.50) | 0.293 |
| *Acute GVHD* | | | | | | | | | | | | |
| Grade 0–I | | 1.00 | | 1.00 | | | 1.00 | | | 1.00 | | |
| Grade II–IV | 1.62 | (1.47–1.78) | <0.001 | 1.44 | (1.24–1.66) | <0.001 | 1.73 | (1.50–2.00) | <0.001 | 1.76 | (1.34–2.31) | <0.001 |

Abbreviations: CI = confidence interval; HR = hazard ratio; MMR-BM = HLA-mismatched related BM; MMR-PB = HLA-mismatched related PBSCs; MMU-BM = HLA-mismatched unrelated BM; MR-BM = HLA-matched related BM; MR-PB = HLA-matched related PBSCs; MU-BM = HLA-matched unrelated BM; R-BM/PB = related BM or PBSC; U-BM; unrelated BM; U-CB = unrelated cord blood.

### Extensive chronic GVHD

The incidence of extensive chronic GVHD at 2 years was 30% (29–31%) for the entire cohort, 32% (28–35%) for the MR-BM group, 42% (39–46%) for the MR-PB group, 31% (24–39%) for the MMR-BM group, 33% (26–39%) for the MMR-PB group, 27% (25–29%) for the MU-BM group, 32% (28–36%) for the MMU-BM group and 19% (17–22%) for the U-CB group (Gray's test for the whole group, $P<0.001$; Figure 1b). In addition to being a significant variable in the analysis of chronic GVHD, the use of reduced-intensity conditioning (vs myeloablative conditioning) was inversely associated with the development of extensive chronic GVHD (HR, 0.86; $P=0.019$; Table 3). Compared with MR-BM, MR-PB and MMR-PB were associated with the development of extensive chronic GVHD, whereas MU-BM and U-CB grafts were inversely associated with its development. Grade II–IV acute GVHD occurrence was the only significant variable consistently observed across all donor types.

### Organ-specific chronic GVHD

Figure 2 shows the type of presentation and organ involvement associated with chronic GVHD. Among the 1716 patients who developed chronic GVHD, *de novo*, progressive and quiescent chronic GVHD presentations were observed in 467 (27%), 348 (20%) and 901 (53%) patients, respectively. Compared with the MR-BM group, progressive chronic GVHD was more frequently observed in the MMU-BM group (33% vs 15%), and quiescent chronic GVHD was more frequently observed in the U-CB group (62% vs 53%).

Limited type of skin involvement was more frequently observed in the U-CB group than in the MR-BM group (53% vs 29%). We examined the types of chronic GVHD (limited vs extensive) in patients with limited type of skin GVHD to evaluate the effect of limited type of skin GVHD on chronic GVHD type in the U-CB group. Accordingly, extensive chronic GVHD was observed in 73% of patients with limited type of skin GVHD in the MR-BM group, compared with 49% of patients in the U-CB group. Oral cavity (28% vs 55%), eye (12% vs 26%), liver (20% vs 44%), lung (11% vs 25%) and joint (0% vs 6%) involvement was less prevalent in the U-CB group than in the MR-BM group. There was no organ that was more frequently involved in the U-CB group than in the MR-BM group.

Progressive onset of chronic GVHD, extensive skin GVHD, intestinal or genital involvement and extensive type of chronic GVHD were significantly associated with lower chronic GVHD-specific survival rates in multivariate analysis, after adjusting for other confounders (Table 4). Lung involvement in GVHD was marginally significant. On the other hand, limited type of skin GVHD was associated with higher chronic GVHD-specific survival rates. Chronic GVHD-specific survival and OS curves showing a significant difference between the groups are shown in Figure 3 and Supplementary Figure 1. The impact of chronic GVHD on relapse is also an important issue. The occurrence of chronic GVHD

**Table 3.** Risk factors for extensive type of chronic GVHD

| *Variable* | *Extensive chronic GVHD (Total)* | | | *Extensive chronic GVHD (R-BM/PB)* | | | *Extensive chronic GVHD (U-BM)* | | | *Extensive chronic GVHD (U-CB)* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *HR* | *95% CI* | *P-value* | *HR* | *95% CI* | *P-value* | *HR* | *95% CI* | *P-value* | *HR* | *95% CI* | *P-value* |
| Recipient age, per 10 years | 1.10 | (1.05–1.15) | <0.001 | 1.12 | (1.03–1.21) | 0.010 | 1.07 | (1.00–1.15) | 0.049 | 1.10 | (0.96–1.26) | 0.180 |
| Donor age, per 10 years | | | | 1.02 | (0.94–1.10) | 0.662 | 1.08 | (0.98–1.20) | 0.136 | | | |
| *Sex match between recipient and donor* | | | | | | | | | | | | |
| Match | 1.00 | | | 1.00 | | | 1.00 | | | 1.00 | | |
| Male to female | 1.02 | (0.89–1.16) | 0.822 | 1.00 | (0.81–1.24) | 0.977 | 1.08 | (0.90–1.31) | 0.409 | 0.82 | (0.49–1.37) | 0.442 |
| Female to male | 1.32 | (1.16–1.50) | <0.001 | 1.49 | (1.25–1.77) | <0.001 | 1.25 | (1.01–1.55) | 0.042 | 0.88 | (0.55–1.41) | 0.608 |
| *CMV Ab (donor and recipient)* | | | | | | | | | | | | |
| Both negative | 1.00 | | | 1.00 | | | 1.00 | | | 1.00 | | |
| Either positive | 1.32 | (1.06–1.64) | 0.014 | 1.17 | (0.83–1.64) | 0.383 | 1.37 | (0.95–1.97) | 0.089 | 1.54 | (0.97–2.44) | 0.068 |
| *Type of donor and stem cell source* | | | | | | | | | | | | |
| MR-BM | 1.00 | | | | | | | | | | | |
| MR-PB | 1.41 | (1.19–1.58) | <0.001 | | | | | | | | | |
| MMR-BM | 1.08 | (0.79–1.49) | 0.614 | | | | | | | | | |
| MMR-PB | 1.35 | (1.01–1.81) | 0.042 | | | | | | | | | |
| MU-BM | 0.78 | (0.66–0.93) | 0.005 | | | | | | | | | |
| MMU-BM | 0.93 | (0.77–1.13) | 0.452 | | | | | | | | | |
| U-CB | 0.65 | (0.51–0.83) | 0.001 | | | | | | | | | |
| *Type of stem cell source* | | | | | | | | | | | | |
| BM | | | | 1.00 | | | | | | | | |
| PB | | | | 1.42 | (1.21–1.66) | <0.001 | | | | | | |
| *HLA disparity* | | | | | | | | | | | | |
| Match | | | | 1.00 | | | 1.00 | | | 1.00 | | |
| Mismatch | | | | 1.10 | (0.88–1.36) | 0.397 | 1.14 | (0.96–1.35) | 0.142 | 0.89 | (0.45–1.76) | 0.743 |
| *Conditioning* | | | | | | | | | | | | |
| Myeloablative | 1.00 | | | 1.00 | | | 1.00 | | | 1.00 | | |
| Reduced intensity | 0.86 | (0.75–0.97) | 0.019 | 0.90 | (0.74–1.08) | 0.255 | 0.88 | (0.72–1.07) | 0.206 | 0.64 | (0.42–0.96) | 0.031 |
| *Use of* in vivo *T-cell depletion* | | | | | | | | | | | | |
| No | 1.00 | | | 1.00 | | | 1.00 | | | | | |
| Yes | 0.39 | (0.26–0.58) | <0.001 | 0.23 | (0.13–0.41) | <0.001 | 0.80 | (0.46–1.37) | 0.407 | | | |
| *Acute GVHD* | | | | | | | | | | | | |
| Grade 0–I | 1.00 | | | 1.00 | | | 1.00 | | | 1.00 | | |
| Grade II–IV | 1.74 | (1.56–1.93) | <0.001 | 1.52 | (1.30–1.78) | <0.001 | 1.91 | (1.62–2.26) | <0.001 | 2.02 | (1.43–2.86) | <0.001 |

Abbreviations: CI = confidence interval; HR = hazard ratio; MMR-BM = HLA-mismatched related BM; MMR-PB = HLA-mismatched related PBSCs; MMU-BM = HLA-mismatched unrelated BM; MR-BM = HLA-matched related BM; MR-PB = HLA-matched related PBSCs; MU-BM = HLA-matched unrelated BM; R-BM/PB = related BM or PBSC; U-BM; unrelated BM; U-CB = unrelated cord blood.

was significantly associated with lower incidence of relapse than the absence of chronic GVHD for the total cohort (HR 0.88, $P = 0.018$). However, we did not find any significant different impact of type, onset and organ involvement of chronic GVHD on relapse among those with chronic GVHD.

## DISCUSSION

In the present study, we extensively analyzed the risk factors for chronic GVHD, particularly focusing on donor graft sources and organ involvement, using recently obtained national registry data that included a large number of U-CB transplantations. In addition to confirming previously reported chronic GVHD risk factors, we observed a lower incidence of extensive chronic GVHD in recipients of U-CB than in recipients of MR-BM. Moreover, in patients with chronic GVHD, oral cavity, eye, liver, lung and joint involvement was substantially lower in the U-CB group than in the MR-BM group.

Grade II–IV acute GVHD occurrence was a strong risk factor for chronic and extensive chronic GVHD, regardless of the donor type, which is consistent with previous findings.[4–7] The mechanism through which chronic GVHD develops is considered to be different from that of acute GVHD,[28] and the underlying mechanism by which acute GVHD strongly influences chronic GVHD development remains unknown. Acute GVHD causes thymic epithelial damage and functional deterioration, leading to a decrease in thymic output, represented by low T-cell receptor excision circle levels.[29] The association between low T-cell receptor excision circle levels and occurrence of chronic GVHD was reported in HLA-identical sibling transplantation,[30] which may partly explain the association between the history of acute GVHD and the development of chronic GVHD. The combination of female donor/male recipient was significantly associated with the development of chronic GVHD, which is also consistent with previous studies.[4,6] In the subset analysis, the combination of female donor/male recipient was significant for the R-BM/PB group, but not significant for the U-CB group. T cells transplanted from adult female donors can be activated by exposure to Y-chromosome-associated proteins and may cause chronic GVHD, but those from female U-CB units may be less activated against them.[31] Studies on the effect of the CMV Ab on chronic GVHD development have previously yielded controversial results.[2,32] In this study, we observed a significant impact of CMV seropositivity on the incidences of chronic GVHD and extensive chronic GVHD. However, the presence of antigenemia itself was not a significant factor in univariate analysis (data not shown); therefore, the mechanism through which CMV Ab affects chronic GVHD development remains unknown. We also confirmed that the use of a PBSC graft vs a BM graft constituted a strong risk factor for chronic and extensive chronic GVHD development in the R-BM/PB group. On the other

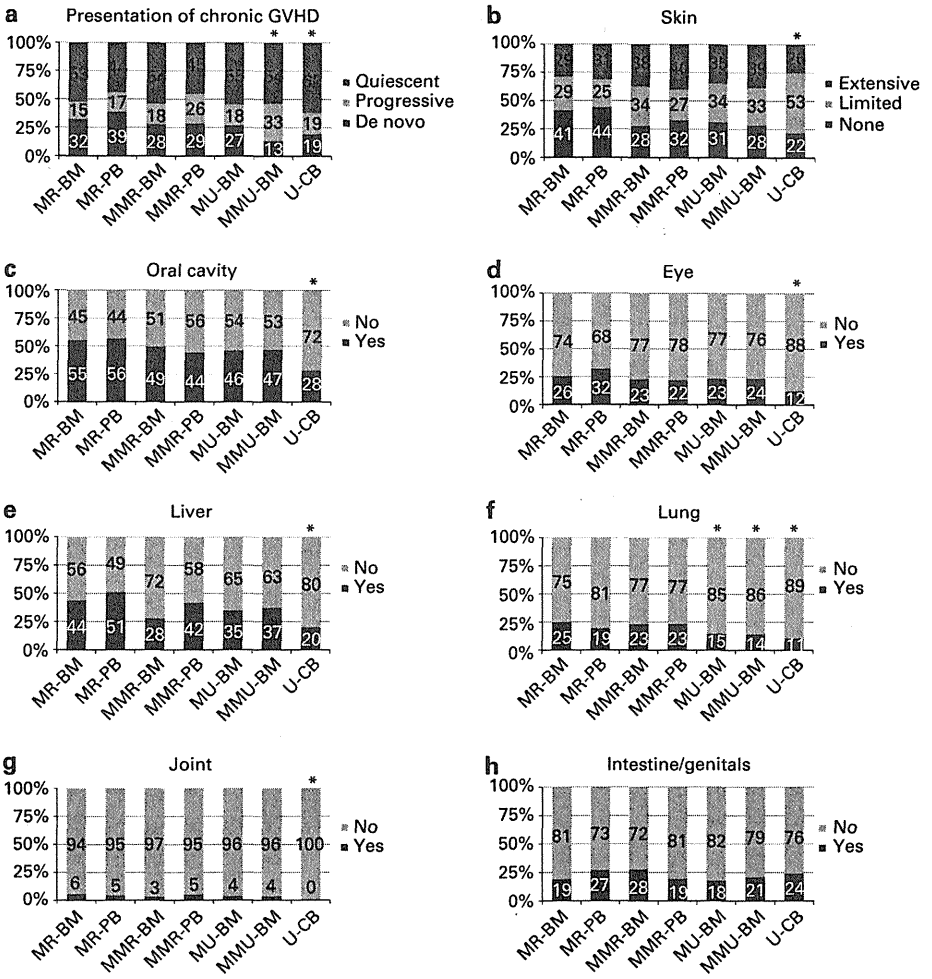


**Figure 2.** Presentation (**a**) and organ involvement (**b**–**h**) of chronic GVHD according to type of donor and stem cell source. Prevalence was compared between MR-BM and MR-PB, MMR-BM, MMR-PB, MU-BM, MMU-BM or U-CB. *$P < 0.008$.

hand, the use of ATG was associated with a lower incidence of chronic GVHD, particularly in the R-BM/PB group. Contrary to previous reports, HLA disparity did not have a strong effect on chronic GVHD development in the R-BM/PB group. In addition, the use of MU-BM grafts was significantly associated with a lower incidence of extensive chronic GVHD. These findings may indicate that GVHD prophylaxis was intensified according to the acknowledged risk of GVHD. Therefore, we performed the same analysis after excluding the use of ATG or in the subgroup of patients who used tacrolimus or CsA as GVHD prophylaxis. However, we obtained the same result, which suggests that some other factor, such as the timing of immunosuppressive agent tapering, may be affecting the results.

In the analysis of chronic GVHD-specific survival, extensive type (vs limited type), progressive onset (vs *de novo* onset), extensive skin involvement (vs none), no skin involvement (vs limited involvement), and intestinal or genital involvement were associated with lower chronic GVHD-specific survival rate. The impact of quiescent onset chronic GVHD has been controversial,[2,33] but chronic GVHD-specific survival in the patients showing quiescent onset chronic GVHD was almost comparable to those showing *de novo* onset in line with several recent reports.[5,34] Although oral involvement was not associated with lower chronic GVHD-specific survival, which is compatible with a previous report,[35] intestinal or genital involvement was associated with lower survival rate. The use of U-CB was not associated with chronic GVHD-specific survival, even when only patients with extensive chronic GVHD were considered (data not shown). This finding suggests that chronic GVHD, if it occurs, does not behave differently regardless of the stem cell source. On the other hand, oral cavity, eye, liver, lung and joint involvement were substantially lower in the U-CB group, which contributed to the significantly lower incidence of extensive GVHD in the U-CB than in the MR-BM group. The high incidence of early TRM, such as that involving graft failure and infection, is considered a disadvantage of U-CB transplantations. However, if a patient survives the first few months following U-CB transplantation without treatment-related complications, the risk of extensive GVHD and GVHD-associated treatment-related complications would then be lower than in other transplantations. The low incidence of chronic GVHD would also contribute to the early discontinuation of immunosuppressive agents, which would allow or even promote immune reconstitution in long-term survivors of U-CB transplantation. Therefore, the choice of using U-CB as an alternative graft source might be prioritized if early treatment-related complications can be avoided through new approaches to ensure engraftment and enhance early immune reconstitution.

**Table 4.** Impact of type, presentation and organ involvement of chronic GVHD on chronic GVHD-specific survival

| *Characteristics* | *Chronic GVHD-specific survival* | | |
|---|---|---|---|
| | *HR* | *95% CI* | *P-value* |
| *Type of chronic GVHD* | | | |
| Limited | 1.00 | | |
| Extensive | 2.60 | (1.67–4.05) | <0.001 |
| *Presentation of chronic GVHD* | | | |
| *de novo* | 1.00 | | |
| Progressive | 1.73 | (1.10–2.72) | 0.017 |
| Quiescent | 0.76 | (0.51–1.13) | 0.173 |
| *Skin* | | | |
| None | 1.00 | | |
| Limited | 0.58 | (0.41–0.83) | 0.002 |
| Extensive | 1.34 | (1.01–1.78) | 0.043 |
| *Oral cavity* | | | |
| No | 1.00 | | |
| Yes | 0.97 | (0.76–1.25) | 0.840 |
| *Eye* | | | |
| No | 1.00 | | |
| Yes | 1.03 | (0.78–1.35) | 0.859 |
| *Liver* | | | |
| No | 1.00 | | |
| Yes | 1.17 | (0.91–1.51) | 0.225 |
| *Lung* | | | |
| No | 1.00 | | |
| Yes | 1.29 | (0.96–1.74) | 0.091 |
| *Joint* | | | |
| No | 1.00 | | |
| Yes | 0.93 | (0.52–1.66) | 0.795 |
| *Intestine/genitals* | | | |
| No | 1.00 | | |
| Yes | 2.15 | (1.66–2.78) | <0.001 |
| *Others* | | | |
| No | 1.00 | | |
| Yes | 1.34 | (0.85–2.11) | 0.206 |

Abbreviations: CI = confidence interval; HR = hazard ratio. Hazard ratios were adjusted by type of stem cell source, recipient age, disease risk and grade II–IV acute GVHD.

Several limitations of this study should be noted. First, in this study, acute and chronic GVHD were diagnosed on the basis of traditional criteria, whereas chronic GVHD was diagnosed and classified on the basis of NIH criteria in recent studies.[36–39] Therefore, our results cannot be compared with those reported in other studies. In addition, it is possible that late onset acute GVHD was classified as chronic GVHD or early onset of chronic GVHD was defined as acute GVHD. This may bias the association between acute and chronic GVHD. Second, there is a possibility that chronic GVHD that developed a few years after SCT was not reported or was missed. Furthermore, detailed information on the clinical course of GVHD and on the onset of each chronic GVHD organ manifestation was not available; therefore, chronic GVHD-specific survival should be cautiously interpreted. Fourth, because organ involvement of chronic GVHD was not defined in detail in this large retrospective studies, there is a possibility of misclassification regarding organ involvement. Further, the information on intestinal or genital involvement was not separately collected in the questionnaire. Lastly, incidence of chronic GVHD in the present study was relatively low as compared with that in Caucasian cohorts, suggesting that the genetic differences between races may affect occurrence of chronic GVHD. Therefore, the results should be cautiously interpreted when the result is applied for non-Asian populations.

In conclusion, extensive chronic GVHD was less frequently observed in the U-CB group. In addition, among patients who developed chronic GVHD, oral cavity, eye, liver, lung and joint involvement were less frequently observed in the U-CB group. Although limited type of skin GVHD was frequently observed, it remains within the range of limited chronic GVHD. Therefore, the quality of life may be better for long-term survivors of the U-CB group than those of the MR-BM group or the other groups. Progressive onset, extensive chronic GVHD or intestinal or genital involvement was associated with lower chronic GVHD-specific survival, which suggests the need to intensify treatment for patients with these chronic GVHD characteristics. Finally, a prospective study using NIH criteria is needed to compare the

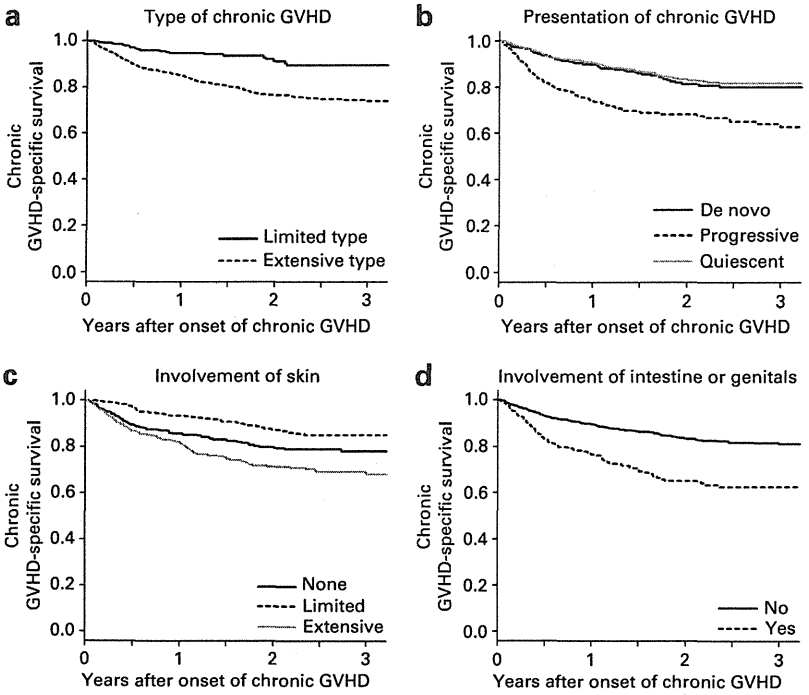


**Figure 3.** Chronic GVHD-specific survival stratified by type (**a**), presentation (**b**), involvement of skin (**c**) and involvement of intestine or genitals (**d**).

incidences of patients with chronic GVHD between Japan and other countries.

## CONFLICT OF INTEREST

The authors declare no conflict interest.

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www.nature.com/bmt

ORIGINAL ARTICLE

# Recent decrease in non-relapse mortality due to GVHD and infection after allogeneic hematopoietic cell transplantation in non-remission acute leukemia

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Although recent improvements have been indicated in the outcome after allogeneic hematopoietic cell transplantation (allo-HCT), little information is available on how changes in transplant modalities have affected the outcomes after allo-HCT in non-remission, based on patient age, donor source and disease type. We compared the incidence and causes of non-relapse mortality (NRM) after allo-HCT in non-remission among three consecutive four-year periods using a nationwide transplant outcome registry database. A total of 3308 patients with acute leukemia in non-remission were analyzed. The risk of NRM decreased over the three periods, and the hazard ratios (HRs) in 2001–2004 and 2005–2008 compared with 1997–2000 were 0.86 (95% CI, 0.70–1.06; $P=0.16$) and 0.65 (95% CI, 0.53–0.80; $P<0.01$), respectively. A significant decrease in the HR for overall mortality was also observed in 2005–2008 (HR 0.85; 95% CI, 0.75–0.97; $P=0.02$). We found that a decrease in the incidences of death due to GVHD and infection contributed to the reduction in NRM, to which high-resolution donor-recipient HLA matching and other improvements may have contributed. As none of the subgroups showed improved survival without a reduction in NRM, the effective prevention of transplant-related complications appears to be necessary for improving outcomes after allo-HCT in non-remission.

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**Keywords:** allogeneic hematopoietic cell transplantation; acute leukemia; non-remission; non-relapse mortality; GVHD

## INTRODUCTION

Allogeneic hematopoietic cell transplantation (allo-HCT) is recognized as a potentially curative therapy for patients with high-risk hematologic malignancies, which can lower the risk of relapse. However, treatment-related mortality, which may offset the benefit of a reduced risk of relapse, has long been a major problem. Several changes have been made in modalities of allo-HCT, including patient-donor HLA matching, conditioning regimens, immunosuppressive therapy, and the prophylaxis, diagnosis and treatment of GVHD and infection. As a result, the risk of non-relapse mortality (NRM) after allo-HCT has decreased over the past few decades.[1–6]

AML and ALL account for the largest proportion of diseases indicated for allo-HCT. Furthermore, a substantial number of patients with AML or ALL receive allo-HCT in non-remission. Despite the fact that high-risk acute leukemia is definitely indicated for allo-HCT, patients with non-remission leukemia carry various factors that lead to a higher risk of treatment-related toxicity, including comorbidities due to prior chemotherapy and intensified conditioning regimens in need of an antitumor effect,[7–11] and a deteriorated general condition due to refractory disease. Although prior studies have shown improvements in the outcome after allo-HCT,[1–5] little information is available on how changes in transplant modalities have affected the outcomes after allo-HCT in non-remission, based on patient age, donor source and disease type. We recently reported changes in the incidence and causes of NRM after allo-HCT in remission in Japan.[6] Using the same nationwide transplant outcome registry database, we compared the incidence and causes of NRM in patients with AML or ALL in non-remission in three consecutive four-year periods.

## SUBJECTS AND METHODS

### Data source

Clinical data were extracted from a nationwide transplant outcome registry database provided by the Japan Society for Hematopoietic Cell Transplantation, the Japan Marrow Donor Program and the Japan Cord Blood Bank Network, to which 267 institutions/departments contributed. The clinical data were consecutively collected through Transplant Registry Unified Management Program as described previously.[12] This study was

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approved by the data management committees of the Japan Society for Hematopoietic Cell Transplantation, the Japan Marrow Donor Program and the Japan Cord Blood Bank Network, and by the Institutional Review Board at the National Cancer Center Hospital.

### Patients and definitions

We evaluated data on patients aged between 16 and 70 years who had AML or ALL and who received their first allo-HCT in non-remission between 1997 and 2008. Non-remission status was defined as any percentage of blasts in the peripheral blood, or a BM aspirate containing >5% blasts at the time of transplant. We compared the incidence of NRM after allo-HCT in three consecutive four-year periods (1997–2000, 2001–2004 and 2005–2008) for younger patients (16–49 years), and in the latter two periods for older patients (50–70 years). NRM was defined as death without the detection of recurrent disease after allo-HCT. In 154 patients who died without a confirmed hematological remission within 30 days from allo-HCT, the cause of death was defined as NRM. In 293 patients who died without a confirmed hematological remission after 31 days or later after allo-HCT, the cause of death was defined as refractory disease. A separate analysis that excluded these 447 patients who died without a confirmed remission was performed. We also changed the cutoffs from 30 days to 60 days or 90 days. Analyses were performed on the basis of patient's age (16–49 years and 50–70 years), disease (AML and ALL) and donor source (HLA-matched/1-Ag-mismatched related, unrelated BM and unrelated cord blood (CB)). In this study, matching of unrelated BM between recipient and donor were determined based on serum typing. In 2003, Japan Marrow Donor Program nationally recommended DNA typing of HLA-A and B, as well as HLA-DRB1. Since 2005, Japan Marrow Donor Program required all the candidates of unrelated allo-HCT to examine high-resolution typing of HLA-A, B and DRB1 and also recommended high-resolution typing of C-locus. In the era considered by this study, only BM was used from unrelated volunteer donors in Japan. Conditioning regimens were classified as indicated by Giralt *et al.*[13] The causes of death other than recurrent disease were obtained from the database and the incidences of mortality associated with GVHD, infection or organ failure were compared over the three time periods. In patients who had multiple causes among GVHD, infection and organ failure, information regarding the main cause of death was prioritized. The 447 patients who died without a confirmed hematological remission were excluded from the analyses regarding the causes of death.

### Statistical analysis

Data were retrospectively reviewed and analyzed as of March 2012. Among the three time periods, patient characteristics were compared using the $\chi^2$-test. The primary endpoint of the study was NRM after allo-HCT. Probabilities of NRM were estimated with the use of cumulative incidence curves, with relapse viewed as a competing risk of NRM. The Pepe and Mori test was used to evaluate the differences between groups. For the 337 patients (10%) who were known to have relapsed but whose date of relapse was unavailable, midpoint imputation was performed by substituting the midpoint from HCT to date of last contact as the date of relapse. The incidence of NRM was estimated as the probability at 2 years from allo-HCT. Multivariate analyses were performed for NRM and relapse using competing risk regression by the method of Fine and Gray, and for survival using a Cox proportional hazard regression model. The analyses were performed separately among younger patients aged 16–49 years and older patients aged 50–70 years. In the multivariate analyses, we considered the following factors as covariates: the year of allo-HCT (1997–2000 vs 2001–2004 or 2005–2008; because of the small number of HCT performed in 1997–2000, we considered 2001–2004 as reference vs 2005–2008 in subgroup analyses among older patients or those who received unrelated CB transplantation (UCBT)), disease type (AML vs ALL), patient age (16–29 years vs 30–39 or 40–49 among younger patients, 50–59 vs 60–70 among older patients), patient gender (male vs female), donor source (HLA-matched sibling vs other family donors, HLA-matched-unrelated BM, mismatched-unrelated BM or unrelated CB), and conditioning regimens (myeloablative vs reduce-intensity conditioning (RIC)). Multivariate analyses were also performed separately for patients who received related allo-HCT, patients who received unrelated BMT (UBMT), and patients who received UCBT. We considered two-sided *P*-values of $<0.05$ to be statistically significant. Statistical analyses were performed with SAS version 9.1.3 (SAS, Cary, NC, USA) and SPSS software version 11.0.1 (SPSS, Chicago, IL, USA).

**Table 1.** Patient characteristics according to the time period of transplant

| *Characteristics* | *1997–2000* N(%) | *2001–2004* N(%) | *2005–2008* N(%) | *P-value* |
|---|---|---|---|---|
| Total number of patients | 637 | 1165 | 1506 | |
| *Gender* | | | | *0.064* |
| Male | 355(56) | 674(58) | 793(53) | |
| Female | 281(44) | 489(42) | 505(34) | |
| *Age (years)* | | | | *<0.001* |
| 16–29 | 249(39) | 277(24) | 265(18) | |
| 30–39 | 139(22) | 240(21) | 278(18) | |
| 40–49 | 157(25) | 246(21) | 304(20) | |
| 50–59 | 83(13) | 296(25) | 430(29) | |
| 60–70 | 9(1) | 106(9) | 229(15) | |
| *Donor source* | | | | *<0.001* |
| HLA-matched sibling | 248(39) | 380(33) | 365(24) | |
| Related others | 94(15) | 165(14) | 196(13) | |
| Matched-unrelated BM | 213(33) | 288(25) | 461(31) | |
| Mismatched-unrelated BM | 34(5) | 83(7) | 85(6) | |
| Unrelated CB | 23(4) | 176(15) | 286(19) | |
| Others | 25(4) | 73(6) | 113(8) | |
| *Disease type* | | | | *<0.001* |
| AML | 388(61) | 840(72) | 1209(80) | |
| ALL | 249(39) | 325(28) | 297(20) | |
| Ph-positive ALL | 66(10) | 75(6) | 48(3) | |
| *Conditioning* | | | | *<0.001* |
| Myeloablative | 504(79) | 668(57) | 837(56) | |
| Reduced-intensity | 14(2) | 290(25) | 426(28) | |
| Not categorized | 119(19) | 207(18) | 243(16) | |
| *GVHD prophylaxis* | | | | *<0.001* |
| Cyclosporin-based | 472(74) | 679(58) | 618(41) | |
| Tacrolimus-based | 150(24) | 423(36) | 806(54) | |
| *Disease status at HCT* | | | | *<0.001* |
| No treatment | 20(3) | 43(4) | 115(8) | |
| Primary induction failure | 148(23) | 292(25) | 576(38) | |
| First relapse | 154(24) | 372(32) | 485(32) | |
| ⩾Second relapse | 55(9) | 159(14) | 157(10) | |
| Non-remission/no detailed data | 260(41) | 299(26) | 173(11) | |

Abbreviation: CB = cord blood.

## RESULTS

### Patients

A total of 3308 patients with a median age of 42 years and a median follow-up of 27 months (range, 0–150) was analyzed. The characteristics of the patients and transplantation procedures according to the time period are shown in Table 1. The number of allo-HCT procedures increased over time. The number and proportion of patients aged 50–70 years, allo-HCT from an unrelated CB donor and the use of a RIC regimen increased over the three periods. Most of the myeloablative regimens (96%) consisted of high-dose CY with TBI or BU. Tacrolimus-based GVHD prophylaxis increased, especially in allo-HCT from an unrelated BM and CB donor (BM: 1997–2000, $n=109$, 44%; 2001–2004, $n=231$, 62%; 2005–2008, $n=426$, 78%, CB: $n=5$, 22%; $n=50$, 28%; $n=174$, 61%). The proportion of allo-HCT given for ALL in non-remission decreased over the three periods with decreasing proportions of both Ph-positive ALL and Ph-negative

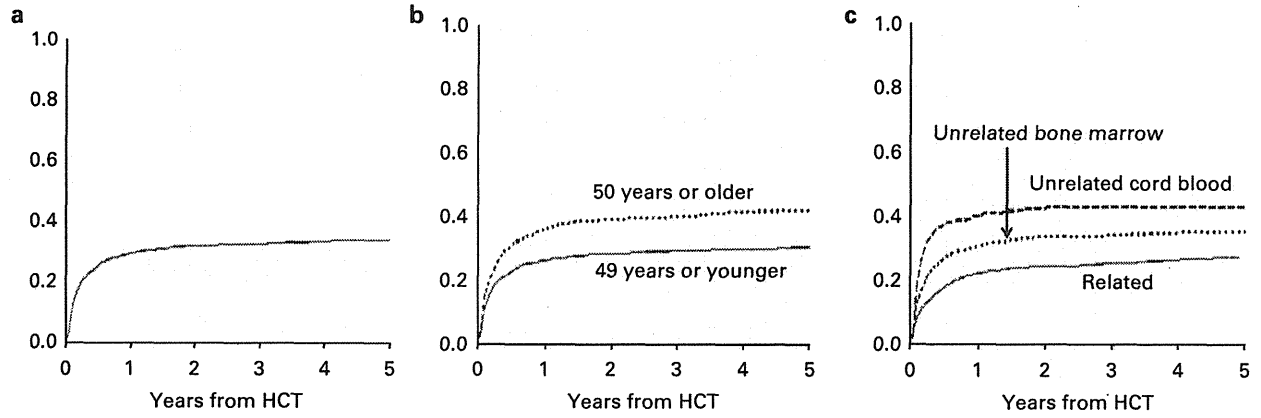


**Figure 1.** Cumulative incidence curves of NRM over the past 12 years among patients who received allo-HCT in non-remission are shown for the entire population (**a**), and subgroups based on age (**b**) and donor (**c**).

ALL. We categorized patients by detailed disease status; however, about 40% of allo-HCT performed in the earliest time period lacked the necessary information.

### Transplant outcomes

Overall, the incidence of NRM was 31% at 2 years after allo-HCT (Figure 1a). Patients who were 50 years or older had a significantly higher incidence of NRM than patients who were 49 years or younger (39% vs 28%, $P<0.001$, Figure 1b). The donor source significantly affected the incidence of NRM, and unrelated CB had the highest risk of NRM (related, 23%; unrelated BM, 33%; unrelated CB, 42%, $P<0.001$, Figure 1c).

Hazard ratios (HRs) for NRM, relapse and overall mortality in 2001–2004 and 2005–2008 compared with 1997–2000, after adjusting for disease type, patient age, patient gender, donor source and conditioning regimens, are shown in Table 2. In the overall 3308 patients, HRs for NRM in 2001–2004 and 2005–2008 were reduced, with a significant decrease in 2005–2008. A significant decrease in the HR for overall mortality was also observed in 2005–2008. The HR for relapse did not change among the three periods. Other factors that were significantly associated with increased NRM were older age (HR 1.43; 95% CI, 1.19–1.71; $P<0.01$), male gender (HR 1.20; 95% CI, 1.04–1.41; $P=0.01$) and donor other than HLA-matched sibling (other family donors, HR 1.55; 95% CI, 1.22–1.97; $P<0.01$; HLA-matched-unrelated BM, HR 1.57; 95% CI, 1.30–1.90; $P<0.01$; HLA-mismatched-unrelated BM, HR 1.82; 95% CI, 1.35–2.47; $P<0.01$; unrelated CB, HR 2.45; 95% CI, 1.96–3.08; $P<0.01$). Younger age and HLA-matched sibling donor were also significantly associated with reduced overall mortality. Although the HR for NRM in the RIC group tended to be higher than that in the myeloablative group (HR 1.20; 95% CI, 0.99–1.47; $P=0.07$), this difference was NS. An analysis according to disease type showed that the HRs for NRM and overall mortality were reduced in AML patients, but not in ALL patients (Table 2). The incidences of NRM and OS are presented as Supplementary Figures 1a–c.

### Transplant outcomes based on patient age

As the transplantation modality may vary according to the patient's age, HRs in comparison to those in the reference era were investigated separately for patients aged 49 years or younger (reference era: 1997–2000) and those aged 50 years or older (reference era: 2001–2004). As shown in Table 3, in patients aged 16–49, HRs for NRM and overall mortality in 2005–2008 were significantly reduced. In contrast, in patients aged 50–70, there were no remarkable changes in the HRs for NRM and overall mortality between 2001–2004 and 2005–2008. The incidences of NRM and OS are presented as Supplementary Figures 1d and e. RIC was used in 47% of patients aged 50 years or older (50–59: 36%, 60–70: 72%). There was no remarkable difference in the HR for NRM between the myeloablative and RIC groups (RIC: HR 0.97; 95% CI, 0.74–1.28; $P=0.85$).

### Transplant outcomes based on donor

We also performed analyses based on the donor source separately among younger and older patients (Table 3). In related donor transplantation, there were no differences in the HRs for NRM, relapse and OS among the time periods in both younger and older patients. In younger patients who received UBMT, there were significant reductions in the HRs for NRM in 2001–2004 and 2005–2008. The HR for overall mortality was also significantly reduced in 2005–2008. In younger patients who received UCBT, there were significant reductions in the HRs for NRM and overall mortality in 2005–2008. The incidences of NRM and OS are presented as Supplemental Figures 1f–k. The HRs for relapse among younger patients who received UBMT were significantly higher in recent periods. In patients aged 50 years or older, no significant changes in HRs for NRM, relapse or overall mortality were observed among the different time periods in either of the donor subgroups.

### Causes of death that accounted for changes in NRM

The causes of death were obtained in 98% of patients who died without recurrent disease. In 17% of patients for whom multiple causes of death were provided, GVHD, infection, or organ failure given as a main cause of death was prioritized. Overall, 151 patients died of acute or chronic GVHD (median OS: 101 days, range: 12–1979), 337 died of infection (median OS: 63 days, range: 1–2700), and 251 died of organ failure (median OS: 88 days, range: 0–2283). In the overall population, no remarkable decrease in the incidences of mortality due to these three causes was observed although the HRs for NRM and overall mortality decreased (Table 2). Meanwhile, significant reductions in the incidences of GVHD-related and infection-related mortality were observed among younger patients who received UBMT (Figure 2a) and UCBT (Figure 2b). In older patients or allo-HCT from a related donor, no remarkable differences were observed in the incidences of mortality due to GVHD, infection or organ failure among the time periods. The incidence of organ failure-related mortality did not decrease in any of the subgroups.

## DISCUSSION

We evaluated the changes in NRM after allo-HCT for acute leukemia in non-remission over the last 12 years. Overall, we found higher NRM rates compared with those after allo-HCT in

**Table 2.** Multivariate analyses for NRM, relapse and overall mortality after allogeneic HCT

| | *All patients* | | | *AML* | | | *ALL* | | |
|---|---|---|---|---|---|---|---|---|---|
| | *HR* | *N = 3308 95% CI* | *P value* | *HR* | *N = 2437 95% CI* | *P value* | *HR* | *N = 871 95% CI* | *P value* |
| *NRM* | | | | | | | | | |
| 1997–2000 | 1.00 | | | 1.00 | | | 1.00 | | |
| 2001–2004 | 0.86 | (0.70–1.06) | 0.16 | 0.82 | (0.64–1.05) | 0.12 | 0.96 | (0.67–1.38) | 0.83 |
| 2005–2008 | 0.65 | (0.52–0.80) | <0.01 | 0.59 | (0.46–0.75) | <0.01 | 0.85 | (0.58–1.25) | 0.42 |
| *Relapse* | | | | | | | | | |
| 1997–2000 | 1.00 | | | 1.00 | | | 1.00 | | |
| 2001–2004 | 1.01 | (0.87–1.18) | 0.88 | 1.05 | (0.86–1.27) | 0.64 | 0.92 | (0.70–1.20) | 0.53 |
| 2005–2008 | 1.07 | (0.92–1.25) | 0.38 | 1.08 | (0.89–1.30) | 0.43 | 1.07 | (0.80–1.43) | 0.65 |
| *Overall mortality* | | | | | | | | | |
| 1997–2000 | 1.00 | | | 1.00 | | | 1.00 | | |
| 2001–2004 | 0.94 | (0.82–1.07) | 0.32 | 0.91 | (0.78–1.07) | 0.26 | 0.97 | (0.77–1.21) | 0.76 |
| 2005–2008 | 0.85 | (0.75–0.97) | 0.02 | 0.79 | (0.67–0.92) | <0.01 | 1.07 | (0.85–1.36) | 0.56 |

Abbreviations: CI = confidence interval; HCT = hematopoietic cell transplantation; HR = hazard ratio; NRM = non-relapse mortality
Year of allo-HCT (1997–2000 versus 2001–2004 or 2005–2008), disease type (AML versus ALL), patient age (16–29 years versus 30–39, 40–49, 50–59 or 60–70), patient gender (male versus female), donor source (HLA-matched sibling versus other family donors, HLA-matched unrelated bone marrow, mismatched unrelated bone marrow or unrelated cord blood), and conditioning regimens (myeloablative versus reduced-intensity) were considered as covariates. In the analysis for AML and ALL, the 5 covariates were considered other than disease type.

remission (31 vs 22% at 2 years after HCT).[6] The HRs for NRM and overall mortality were lower in more recent time periods. Although several studies have shown changes in outcomes after allo-HCT,[1–6] this is the first analysis restricted to allo-HCT in non-remission, based on the patient age, donor source and disease type. The reduction in the HR for NRM was reflected in the reduced HR for overall mortality, and none of the subgroups showed a reduced risk for overall mortality without an improvement in NRM. This may indicate that lowering the risk of treatment-related mortality is, so far, an absolute requirement for improving outcomes after allo-HCT in non-remission, where a high-risk of relapse has always been an obstacle.

The reductions in the HRs for NRM and overall mortality in the overall population were accounted for by the reductions in HRs in patients with AML, and there was no improvement in NRM or overall mortality in those with ALL in non-remission over the three time periods. We also found that the number and proportion of patients who received allo-HCT for ALL in non-remission decreased over the three time periods despite an increase in the total number of allo-HCT. The proportions of both Ph-positive ALL and Ph-negative ALL decreased and, interestingly, more patients with Ph-positive ALL have received allo-HCT in remission after 2000. The introduction of imatinib may have helped more patients with Ph-positive ALL to receive allo-HCT in a controlled disease status.[14–16] In addition, a lowered expectation for the effect of allo-HCT in ALL in non-remission may have also impacted the indication. In patients who receive allo-HCT in non-remission, strategies that can provide intensified preparative regimens and a GVL effect without increasing toxicity need to be pursued.

No improvement in the HRs for NRM and overall mortality was observed in patients aged 50–70 who received allo-HCT in non-remission. Older patients with acute leukemia have been reported to have a worse prognosis because of more unfavorable disease profiles, deteriorated general conditions and an increased risk of comorbidities.[17] As the eradication of residual disease by provoking GVHD may increase toxicity and become unbearable for elderly patients, it may be necessary to reduce the tumor burden before transplantation. We previously demonstrated a significant reduction in NRM in patients aged 50 years or older who received allo-HCT in remission.[6] The safety and efficacy of modified induction chemotherapy or preparative regimen for elderly patients need to be validated.[18–25]

We found that decreases in GVHD-related and infection-related mortality contributed to the reduced risk of NRM. These findings are consistent with prior reports.[2,3] Based on an analysis of 14 403 patients with leukemia in the first CR who received allo-HCT from a matched sibling donor, Gratwohl *et al.*[3] showed that the rate of mortality due to infection decreased. In a detailed analysis in a single-center study, Gooley *et al.*[2] showed that the rates of severe GVHD and infection were recently reduced. There have been substantial improvements in HLA typing over the period of 1997–2008, with more accuracy in defining HLA haplotypes at high-resolution.[26,27] In addition to high-resolution donor-recipient HLA matching, the more frequent use of tacrolimus,[28–30] the prompt initiation of treatment after a more thorough examination to diagnose GVHD,[31] and supportive care and nutritional management[32] may have contributed to the reduced risk of GVHD-related mortality as did in allo-HCT in remission. Alternatively, the unique HLA epidemiological genetics of Japanese patients may have affected the results.[33,34] As GVHD and infection have been reported to be associated with each other's development and exacerbation,[35–37] an improved control of severe GVHD, along with the introduction of new antifungal drugs, may have led to the reduction of the risk of infection-related mortality. We did not find a reduction in the risk of organ failure-related mortality in any of the subgroups. Although intensified antitumor treatment may be required in allo-HCT in non-remission, continuous effort is needed for monitoring, prevention and intervention with regard to regimen-related toxicity, including late effects.[38–40]

As this analysis is based on a retrospectively collected multicenter database, our results may be susceptible to the disadvantages of any retrospective study using a multicenter registry database. In patients who died without a confirmed hematological remission, we assumed the disease status from the survival time. The impact on transplant outcome of detailed disease status in non-remission patients[41] was not assessed because of the lack of information. In addition, detailed data regarding the incidences of infection or other complications were not available. While we acknowledge these limitations, our data showed that the risks of NRM have decreased after allo-HCT for patients with acute