Table 1
Patient Characteristics

Characteristics	0MM (n = 2570)	1MM-Bi (n = 1020)	1MM-GVH (n = 83)	1MM-HVG (n = 83)	*P*
Median recipient age at transplant, yr (range)	43 (16-77)	41 (16-74)	43 (16-65)	43 (18-71)	.037
Recipient age at transplant					
16-39 yr	1061	457	32	31	.175
40 + yr	1509	563	51	52	
HLA mismatch					
A locus	0	119	11	21	
B locus	0	27	0	3	
C locus	0	521	47	38	
DR locus	0	353	25	21	
Recipient sex					
Female	1018	423	34	29	.573
Male	1552	597	49	54	
Sex mismatch between donor and recipient					
Match	1591	617	43	52	.017
Male donor–female recipient	580	207	17	16	
Female donor–male recipient	399	196	23	15	
Diagnosis					
AML	1329	513	33	49	.137
ALL	647	240	24	17	
CML	236	122	11	6	
MDS	358	145	15	11	
Disease risk at transplant					
Standard risk	1659	616	55	46	.188
High risk	832	373	26	33	
Missing	79	31	2	4	
GVHD prophylaxis					
Cyclosporine based	811	299	22	31	.177
Tacrolimus based	1701	704	59	48	
Others/missing	58	17	2	4	
Conditioning regimen					
Meyeloablative	2001	806	64	58	.444
Reduced intensity	486	176	15	22	
Missing	83	38	4	3	
Transplant year					
2000-2005	1147	548	40	47	<.001
2006-2011	1423	472	43	36	

Values are total number of cases, unless otherwise noted.

AML in 1924, ALL in 928, CML in 375, and MDS in 529. Two-thirds of the patients had standard-risk diseases. Tacrolimus-based GVHD prophylaxis was used in 67%. Transplantation was performed between 2006 and 2011 in 1974 cases (53%).

HLA matching was categorized as follows: HLA match in both the GVH and HVG directions (0MM, n = 2570, 68%), bidirectional 1-allele mismatch in the GVH and HVG directions (1MM-Bi, n = 1020, 27%), 1-allele mismatch in the GVH direction but 0 mismatches in the HVG direction (1MM-GVH, n = 83, 2%), and 1-allele mismatch in the HVG direction but 0 mismatches in the GVH direction (1MM-HVG, n = 83, 2%). More transplants using a matched unrelated donor with 0MM were performed between 2006 and 2011.

Overall Survival

The median follow-up period in survivors was 3.4 years (range, .7 to 12.6). The unadjusted 3-year overall survival rate was 55% (95% confidence interval [CI], 52% to 57%) in the 0MM group, 46% (95% CI, 43% to 49%) in the 1MM-Bi group, 62% (95% CI, 50% to 72%) in the 1MM-GVH group, and 52% (95% CI, 41% to 63%) in the 1MM-HVG group ($P < .001$, Figure 1). The risk of overall mortality was significantly higher in the 1MM-Bi group than in the 0MM group (hazard ratio [HR], 1.31; 95% CI, 1.19 to 1.46; $P < .001$), whereas there was no difference between the 0MM group and the 1MM-GVH group (HR, .97; 95% CI, .70 to 1.34; $P = .850$) or the 1MM-HVG group (HR, 1.13; 95% CI, .85 to 1.55; $P = .439$) (Table 2).

Nonrelapse Mortality and Relapse

The cumulative incidence of unadjusted 3-year nonrelapse mortality was 24% (95% CI, 22% to 25%) in the 0MM group, 30% (95% CI, 27% to 33%) in the 1MM-Bi group, 26% (95% CI, 17% to 36%) in the 1MM-GVH group, and 25% (95% CI, 16% to 35%) in the 1MM-HVG group ($P < .001$, Figure 2). The risk of nonrelapse mortality was significantly higher in the

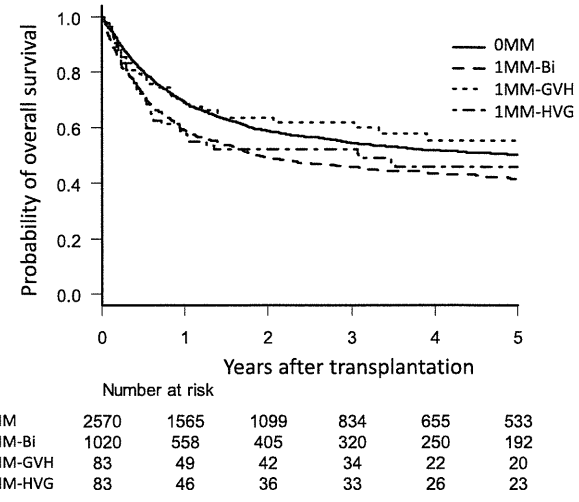

Figure 1. Overall survival. The unadjusted probability of overall survival is shown.

Table 2
Overall Mortality, Nonrelapse Mortality, and Relapse

	HR	95% CI	P
Overall mortality			
0MM	1.00		Reference
1MM-Bi	1.31	1.19-1.45	<.001
1MM-GVH	.97	.70-1.34	.850
1MM-HVG	1.13	.83-1.52	.439
Nonrelapse mortality			
0MM	1.00		Reference
1MM-Bi	1.38	1.21-1.59	<.001
1MM-GVH	1.22	.81-1.84	.334
1MM-HVG	1.12	.75-1.69	.575
Relapse[‡]			
0MM	1.00		Reference
1MM-Bi	.98	.85-1.14	.810
1MM-GVH	.78	.48-1.29	.338
1MM-HVG	.88	.55-1.43	.614

* Other significant variables were the recipient's age group, sex of the recipient, diagnosis, and disease risk.
† Other significant variables were the recipient's age group, sex of the recipient, diagnosis, disease risk, and transplant year.
‡ Other significant variables were diagnosis and disease risk.

1MM-Bi group than in the 0MM group (HR, 1.38; $P < .001$), whereas no difference was found between the 0MM group and the 1MM-GVH group (HR, 1.22; $P = .334$) or the 1MM-HVG group (HR, 1.12; $P = .575$) (Table 2). The cumulative incidence of unadjusted 3-year relapse was 26% (95% CI, 24% to 27%) in the 0MM group, 27% (95% CI, 24% to 29%) in the 1MM-Bi group, 21% (95% CI, 12% to 31%) in the 1MM-GVH group, and 24% (95% CI, 15% to 34%) in the 1MM-HVG group ($P = .635$, Figure 2). There was no significant difference between the 0MM group and the other groups in the multivariate analysis (Table 2).

Neutrophil Engraftment

The cumulative incidence of neutrophil engraftment at day 50 was 96% (95% CI, 95% to 96%) in the 0MM group, 94% (95% CI, 92% to 95%) in the 1MM-Bi group, 100% in the 1MM-GVH group, and 92% (95% CI, 83% to 96%) in the 1MM-HVG group ($P = .224$, Figure 3). There was no significant difference between the 0MM group and the other groups in the multivariate analysis (Table 3).

Acute and Chronic GVHD

The unadjusted cumulative incidence of grades III to IV acute GVHD was 12% (95% CI, 10% to 13%) in the 0MM group, 18% (95% CI, 16% to 20%) in the 1MM-Bi group, 18% (95% CI, 11% to 27%) in the 1MM-GVH group, and 15% (95% CI, 8% to 23%) in the 1MM-HVG group ($P < .001$, Figure 4). The risk of grades III to IV acute GVHD was significantly higher in the 1MM-Bi group (HR, 1.57; $P < .001$) and higher in the 1MM-GVH group with marginal significance (HR, 1.85; $P = .014$) than in the 0MM group (Table 3). There was no difference between the 0MM group and the 1MM-HVG group (HR, 1.25; $P = .468$). The unadjusted cumulative incidence of chronic GVHD was 37% (95% CI, 35% to 39%) in the 0MM group, 35% (95% CI, 32% to 38%) in the 1MM-Bi group, 41% (95% CI, 30% to 52%) in the 1MM-GVH group, and 30% (95% CI, 20% to 41%) in the 1MM-HVG group ($P = .584$, Figure 4). No significant difference was found between the 0MM group and the other groups in the multivariate analysis (Table 3).

DISCUSSION

Using Japanese registry data, we analyzed patients who received UBMT with either 1MM-GVH or 1MM-HVG and evaluated the impact of 1MM-GVH and 1MM-HVG on the clinical outcome. The risk of severe acute GVHD in the 1MM-GVH group tended to be higher than that in the 0MM group. However, there was no significant difference in overall survival or nonrelapse mortality between the 2 groups. The overall survival and nonrelapse mortality rates in the 1MM-HVG group were also comparable with those in the 0MM group. Unlike the conclusion of the CIBMTR study, there is no evidence in this study that an unrelated donor with 1MM-HVG should be prioritized over 1 with 1MM-GVH in a Japanese cohort.

Although the incidence of grades III to IV acute GVHD tended to be higher in the 1MM-GVH group than in the 0MM group, this did not translate into worse overall survival in this Japanese cohort. In interpreting this finding, several differences in patient background between the CIBMTR study [9] and the present study should be clarified. First, the CIBMTR study included transplants performed from 1988 to 2009, whereas our study included transplants performed from 2000 to 2011. Because treatment and supportive care for transplant-related complications such as GVHD and fungal or viral infections improved over this decade, the incidence of nonrelapse mortality was shown to be significantly decreased

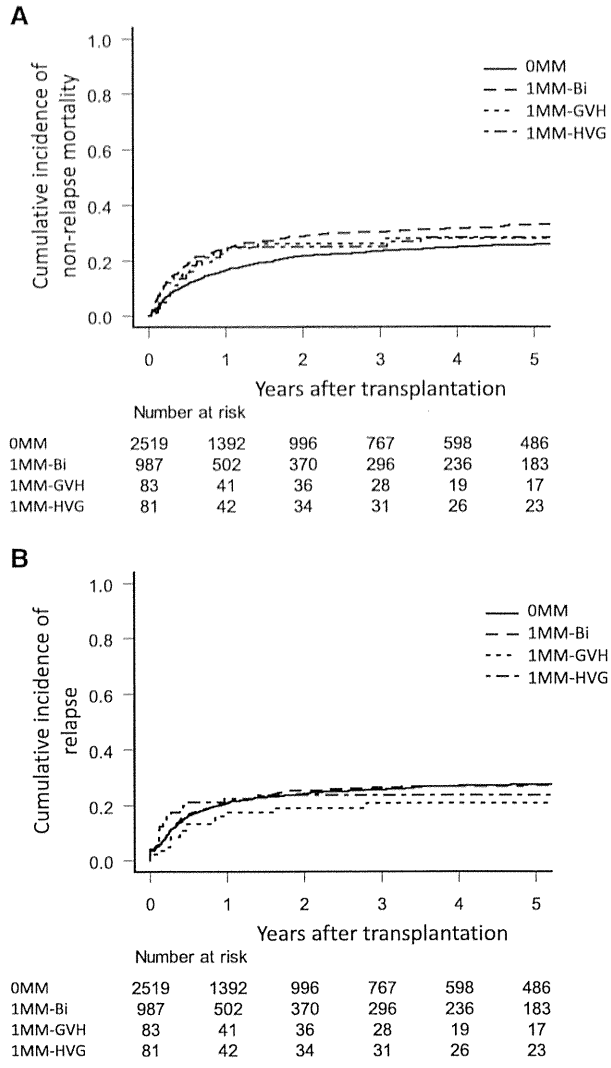

Figure 2. Nonrelapse mortality and relapse. The unadjusted incidences of nonrelapse mortality (A) and relapse (B) are shown.

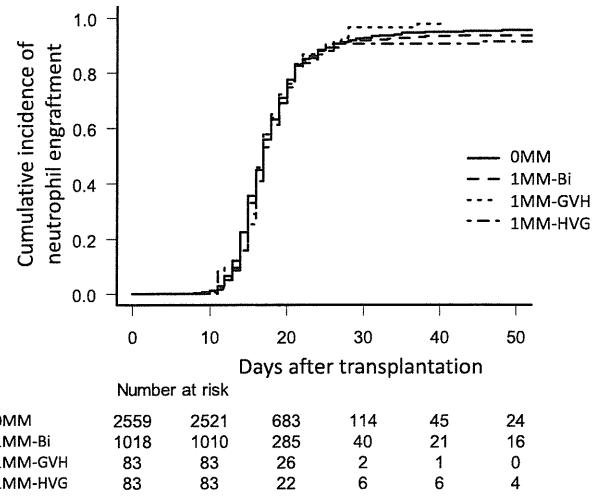

Figure 3. Neutrophil engraftment. The unadjusted incidence of neutrophil engraftment is shown.

in a recent cohort [20,21]; the 1-year survival in patients who developed grades III to IV acute GVHD after HLA 1-allele mismatched UBMT improved from 32.1% in the period from 1993 to 2001 to 44.4% in the period from 2002 to 2011 [21]. Including only a recent cohort in our study may have reduced the impact of acute GVHD on the nonrelapse mortality rate.

The second difference is the definition of allele mismatch. We included only patients who received UBMT from HLA-A, -B, or -DR antigen matched pairs, following the standard donor selection process of the JMDP, because such a donor can be found for more than 90% of patients in Japan. In this process, we start to search for an HLA-A, -B, -C, and -DRB1 matched unrelated donor; if one is not available, we then search for a 1-allele mismatched donor among HLA-A, -B, and -DR antigen matched unrelated donor pools. We generally do not extend the donor search to an HLA-A and -B antigen mismatched unrelated donor (an HLA-DR antigen mismatched donor is an exception [22]). Regarding HLA-C mismatch, 89% of the HLA-C allele mismatches were at the antigen level in this study. Another CIBMTR study showed no significant differences in overall survival or acute GVHD rates

Table 3
Neutrophil Engraftment, Acute GVHD, and Chronic GVHD

	HR	95% CI	*P*
Neutrophil engraftment*			
0MM	1.00		Reference
1MM-Bi	.94	.88-1.01	.108
1MM-GVH	1.01	.84-1.21	.956
1MM-HVG	.97	.78-1.21	.781
Grades III to IV acute GVHD†			
0MM	1.00		Reference
1MM-Bi	1.57	1.30-1.90	<.001
1MM-GVH	1.85	1.13-3.01	.014
1MM-HVG	1.25	.69-2.27	.468
Chronic GVHD‡			
0MM	1.00		Reference
1MM-Bi	.97	.85-1.11	.681
1MM-GVH	1.10	.76-1.59	.618
1MM-HVG	.88	.57-1.35	.558

* Other significant variables were the recipient's age group, sex of the recipient, sex mismatch, GVHD prophylaxis, and disease risk.

† Other significant variables were the recipient's age group, sex of the recipient, sex mismatch, disease risk, and transplant year.

‡ Another significant variable was transplant year.

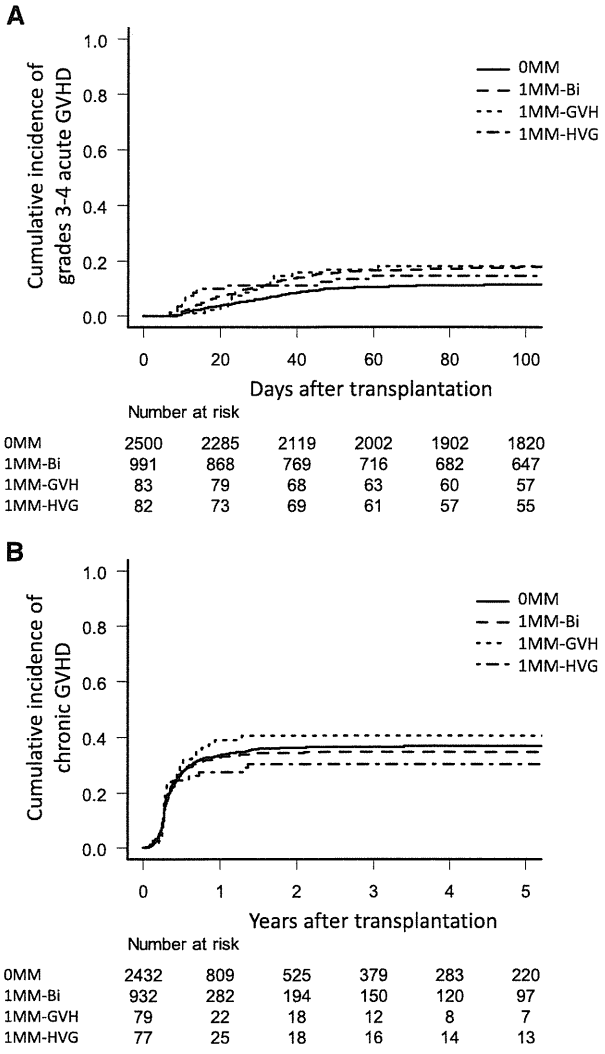

Figure 4. Acute and chronic GVHD. The unadjusted incidences of grades III to IV acute GVHD (A) and chronic GVHD (B) are shown.

between HLA-A, -B, or -DR 1-antigen and 1-allele mismatched transplants [4]. However, the possibility remains that acute GVHD may have less impact on nonrelapse mortality in 1-allele mismatch transplantation than in 1-antigen mismatched transplantation in a specific HLA mismatch status, such as 1 mismatch only in the GVH direction.

The third difference is in ethnicity. The incidence of severe acute GVHD is higher in White populations than in Japanese populations in HLA-matched related or unrelated BMT [23,24], although there was no difference in pediatric UCBT [25]. Ethnic differences may affect the treatment response for severe acute GVHD.

The fourth difference is the stem cell source. Both peripheral blood stem cells (PBSCs) and bone marrow were included in the CIBMTR study, whereas bone marrow was exclusively included in our study. Although there is no difference in the incidence of severe acute GVHD between unrelated PBSC transplantation and BMT [26], the use of PBSCs might be associated with a lower treatment response for acute GVHD, leading to a relatively higher incidence of nonrelapse mortality and overall mortality in a White cohort.

The impact of the HLA mismatch direction has also been evaluated in UCBT. In the New York Blood Center study, UCBT with a mismatch only in the GVH direction was associated

with a higher probability of overall survival compared with UCBT with 1MM-Bi [27], whereas a Japanese study showed the direction of the HLA mismatch does not significantly affect overall survival [28]. The different findings in the UCBT studies as compared with UBMT studies may be partly attributable to the difference in graft components, that is, a cord blood unit contains significantly fewer T cells and total nucleated cells than bone marrow or PBSCs and lower frequency of severe GVHD in the UCBT. The counting method of HLA mismatches was also different. Matching in HLA-A and HLA-B was counted as antigen level and HLA-C was not considered. In addition, 2 unidirectional mismatches were included in the UCBT studies.

We did not find any association between neutrophil engraftment and HLA mismatch in the HVG direction. One explanation for this observation is that our cohort included only HLA-A, -B, and -DR antigen-matched pairs, in which graft failure associated with HLA antibodies against donor-specific HLA antigens is less likely to occur [29-31]. However, even in the CIBMTR cohorts that included antigen-mismatched pairs, no association between graft failure and HLA mismatch direction was observed.

Each locus mismatch may have a different effect on the transplant outcome. For example, an HLA-C mismatch in the GVH direction can be a killer immunoglobulin-like receptor 2DL (KIR2DL) ligand mismatch in the HVG direction in some patients, and vice versa. In a Japanese population, a KIR2DL ligand mismatch in the GVH direction, but not that in the HVG direction, has been shown to be associated with a high risk of acute GVHD and overall mortality [32]. Therefore, the adverse impact of an HLA-C mismatch in the HVG direction may be increased by the presence of a KIR2DL ligand mismatch in the GVH direction in some patients. However, it is difficult to test any hypothesis regarding the impact of each locus mismatch and a KIR2DL ligand mismatch because of the small sample size in this study.

This study has several limitations inherent to a retrospective analysis. First, the heterogeneous backgrounds may have resulted in a statistical bias, although we tried to reduce this bias by adjusting the impact in multivariate analyses. Second, the number of subjects in the 1MM-GVH and 1MM-HVG groups was limited. Therefore, the results should be interpreted with caution. Finally, we did not find any differences in any of the outcomes among the 1MM-GVH, 1MM-HVG, and 1MM-Bi groups (data not shown), partly because of the small sample size in the 1MM-GVH and 1MM-HVG groups. Therefore, we could not make any conclusion regarding the comparison between the 1MM-GVH or 1MM-HVG and 1MM-Bi groups.

In conclusion, the risk of severe acute GVHD in the 1MM-GVH group tended to be higher than that in the 0MM group. However, there were no significant differences in overall survival or nonrelapse mortality between the 0MM and 1MM-GVH or 1MM-HVG groups. Our results suggest that for patients without a matched sibling or matched unrelated donor, we can choose either an unrelated donor with 1MM-GVH or 1 with 1MM-HVG when available.

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Increasing Incidence of Chronic Graft-versus-Host Disease in Allogeneic Transplantation: A Report from the Center for International Blood and Marrow Transplant Research

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A B S T R A C T

Although transplant practices have changed over the last decades, no information is available on trends in incidence and outcome of chronic graft-versus-host disease (cGVHD) over time. This study used the central database of the Center for International Blood and Marrow Transplant Research (CIBMTR) to describe time trends for cGVHD incidence, nonrelapse mortality, and risk factors for cGVHD. The 12-year period was divided into 3 intervals, 1995 to 1999, 2000 to 2003, and 2004 to 2007, and included 26,563 patients with acute leukemia, chronic myeloid leukemia, and myelodysplastic syndrome. Multivariate analysis showed an increased incidence of cGVHD in more recent years (odds ratio = 1.19, $P < .0001$), and this trend was still seen when adjusting for donor type, graft type, or conditioning intensity. In patients with cGVHD, nonrelapse mortality has decreased over time, but at 5 years there were no significant differences among different time periods. Risk factors for cGVHD were in line with previous studies. This is the first comprehensive characterization of the trends in cGVHD incidence and underscores the mounting need for addressing this major late complication of transplantation in future research.

INTRODUCTION

Chronic graft-versus-host disease (cGVHD) remains a major complication after allogeneic hematopoietic cell transplantation (HCT) and is the leading cause of nonrelapse mortality (NRM) in patients surviving more than 2 years [1]. The incidence of cGVHD may be increasing despite the advances in transplantation practices [2]. Several studies have described risk factors associated with the potentially increasing risk of cGVHD, such as transplantation from donors other than matched sibling [3,4], the use of older recipients [5,6], and the use of peripheral blood graft [7-9]. In addition, better supportive care may have improved early NRM such that more patients are at risk to develop cGVHD and contribute to an increased incidence rate [10]. There is also a recent report of a GVHD-induced graft-versus-leukemia effect for myeloablative and reduced-intensity conditioning (RIC) transplants [11]. Donor cell infusions (DCIs) post-transplant have similarly contributed to cGVHD incidence [12]. However, there have been no reports on the trends in incidence and outcomes of cGVHD over time.

The objective of this study was to evaluate the possible differences in incidence and outcomes of cGVHD over critical time periods of practice change in allogeneic HCT, spanning from 1995 to 2007. Three time periods were chosen (1995 to 1999, 2000 to 2003, and 2004 to 2007) as best estimates of intervals of practice change. This study defines the time trends in cGVHD incidence, key clinical characteristics, NRM, and overall survival (OS).

METHODS

The data source for the study was the registry of the Center for International Blood and Marrow Transplant Research (CIBMTR), the voluntary working group of more than 500 transplantation centers that collaborates to share patient data and conduct scientific studies. The quality and compliance of data submission are monitored by computerized checks for errors, physician reviews, and on-site audits.

Observational studies conducted by the CIBMTR are performed with informed consent in accordance with the Declaration of Helsinki and in compliance with Health Insurance Portability and Accountability Act regulations as determined by the National Marrow Donor Program and Medical College of Wisconsin institutional review boards.

Table 1
Characteristics of Patients Who Underwent Allogeneic Transplant for AML, ALL, CML, and MDS by Time Period Reported to the CIBMTR from 1995 to 2007

Characteristics	1995-1999 n (%)	2000-2003 n (%)	2004-2007 n (%)	P
Number of patients	10,597	7472	8494	
Number of centers	318	274	255	
Median age, yr (range)	32 (<1-72)	35 (<1-79)	40 (<1-78)	<.001
Age at transplant, yr				<.001
0-9	1435 (14)	902 (12)	865 (10)	
10-19	1637 (15)	1079 (14)	1129 (13)	
20-29	1749 (17)	1151 (15)	1134 (13)	
30-39	2407 (23)	1269 (17)	1154 (14)	
40-49	2267 (21)	1420 (19)	1501 (18)	
50-59	1026 (10)	1227 (16)	1729 (20)	
60+	76 (1)	424 (6)	982 (12)	
Gender				.51
Male	6071 (57)	4297 (58)	4812 (57)	
Female	4526 (43)	3175 (42)	3682 (43)	
Race				<.001
White	8418 (79)	5743 (77)	6155 (72)	
African American	435 (4)	313 (4)	408 (5)	
Asian/Pacific Islander	915 (9)	752 (10)	610 (7)	
Hispanic	619 (6)	492 (7)	848 (10)	
Native American	31 (<1)	20 (<1)	26 (<1)	
Other	157 (1)	129 (2)	408 (5)	
Unknown/missing	22 (<1)	23 (<1)	39 (<1)	
Karnofsky score				<.001
<80%	1013 (10)	711 (10)	664 (8)	
≥80%	9478 (89)	6460 (86)	7410 (87)	
Missing	106 (1)	301 (4)	420 (5)	
Disease				<.001
AML	3383 (32)	3139 (42)	4215 (50)	
ALL	2662 (25)	1920 (26)	2174 (26)	
CML	3670 (35)	1576 (21)	1095 (13)	
MDS	882 (8)	837 (11)	1010 (12)	
Disease status at transplant*				<.001
Early	5452 (51)	3235 (43)	3959 (47)	
Intermediate	2636 (25)	2066 (28)	2311 (27)	
Advanced	2509 (24)	2171 (29)	2224 (26)	
Conditioning regimen				<.001
Myeloablative	10,409 (98)	6002 (80)	6234 (73)	
Nonmyeloablative	188 (2)	1470 (20)	2260 (27)	
Donor–recipient HLA match†				<.001
HLA-identical sibling	4880 (46)	2562 (34)	2339 (28)	
Other relative	794 (7)	383 (5)	247 (3)	
URD well matched	1265 (12)	2115 (28)	3453 (41)	
URD partially matched	2127 (20)	1234 (17)	1379 (16)	
URD mismatched	1259 (12)	620 (8)	319 (4)	
UCB matched (6/6)	12 (<1)	27 (<1)	57 (1)	
UCB 1 mismatched (5/6)	47 (<1)	96 (1)	123 (1)	
UCB ≥2 mismatch (≤4/6)	213 (2)	435 (6)	577 (7)	
Donor age, yr				
HLA-identical sibling				
0-9	446 (9)	182 (7)	112 (5)	
10-19	720 (15)	312 (12)	297 (13)	
20-29	858 (18)	376 (15)	346 (15)	
30-39	1153 (24)	484 (19)	368 (16)	
40-49	972 (20)	575 (22)	509 (22)	
50-59	497 (10)	398 (16)	433 (19)	
60+	187 (4)	213 (8)	252 (11)	
Missing	47 (1)	22 (1)	22 (1)	
Other relative				
0-9	50 (6)	19 (5)	12 (5)	
10-19	102 (13)	31 (8)	26 (11)	
20-29	138 (17)	68 (18)	41 (17)	
30-39	193 (24)	98 (26)	59 (24)	
40-49	159 (20)	92 (24)	50 (20)	
50-59	89 (11)	42 (11)	38 (15)	
60+	55 (7)	30 (8)	20 (8)	
Missing	8 (1)	3 (1)	1 (<1)	
URD				
18-19	20 (<1)	33 (1)	75 (1)	
20-29	1196 (26)	1141 (29)	1614 (31)	
30-39	1730 (37)	1463 (37)	1814 (35)	
40-49	1166 (25)	965 (24)	1190 (23)	
50-59	304 (7)	255 (6)	320 (6)	
60+	200 (4)	95 (2)	111 (2)	
Missing	35 (1)	17 (<1)	27 (1)	
Donor–recipient sex match				<.001
Female–male	2422 (23)	1639 (22)	1744 (21)	
Others	8120 (77)	5823 (78)	6705 (79)	
Missing	55 (1)	10 (<1)	45 (1)	
Donor–recipient CMV status				<.001
−/−	3313 (31)	2039 (27)	2294 (27)	
Others	7161 (68)	5392 (72)	6116 (72)	
Missing	123 (1)	41 (1)	84 (1)	
Graft type				<.001
Bone marrow	8479 (80)	3410 (46)	2383 (28)	
Peripheral blood	1846 (17)	3504 (47)	5354 (63)	
Cord blood	272 (3)	558 (7)	757 (9)	
Developed cGVHD				<.001
No	7387 (70)	5066 (68)	5403 (64)	
Yes	3210 (30)	2406 (32)	3091 (36)	
ATG and Campath usage (received in conditioning regimen or GVHD prophylaxis)				<.001
ATG and Campath	5 (<1)	1 (<1)	3 (<1)	
ATG only	2114 (20)	2167 (29)	2350 (28)	
Campath only	161 (2)	280 (4)	394 (5)	
No ATG or Campath	8317 (78)	5024 (67)	5747 (68)	
GVHD prophylaxis				<.001
Ex vivo T cell depletion alone	470 (4)	221 (3)	159 (2)	
Ex vivo T cell depletion + post-treatment immune suppression	757 (7)	302 (4)	168 (2)	
CD34 selection alone	39 (<1)	63 (1)	41 (<1)	
CD34 selection + post-treatment immune suppression	71 (1)	131 (2)	62 (1)	
Cyclophosphamide alone	0	0	17 (<1)	
FK506 + MMF ± others	20 (<1)	254 (3)	894 (11)	
FK506 + MTX ± others (except MMF)	647 (6)	1279 (17)	2611 (31)	
FK506 + others (except MTX, MMF)	167 (2)	120 (2)	220 (3)	
FK506 alone	35 (<1)	117 (2)	189 (2)	
CSA + MMF ± others (except FK506)	36 (<1)	536 (7)	743 (9)	
CSA + MTX ± others (except FK506, MMF)	7061 (67)	3471 (46)	2665 (31)	
CSA + others (except FK506, MTX, MMF)	698 (7)	500 (7)	376 (4)	
CSA alone	513 (5)	398 (5)	263 (3)	
Other GVHD prophylaxis	83 (1)	80 (1)	86 (1)	
Prior acute GVHD grades				<.001
0-II	7597 (72)	5388 (72)	6340 (75)	
III-IV	2956 (28)	2068 (28)	2136 (25)	
Missing	44 (<1)	16 (<1)	18 (<1)	

URD indicates unrelated donor; UCB, unrelated cord blood; CMV, cytomegalovirus; ATG, antithymocyte globulin; FK506, tacrolimus; MMF, mycophenolate mofetil; MTX, methotrexate; CSA, cyclosporine.

* Disease status is categorized as follows: Early = AML/ALL (CR1 [first complete remission]); CML (CP1 [first chronic phase]); MDS (RA/RARS [refractory anemia/refractory anemia with ring sideroblasts]/pre-HCT marrow blasts < 5%); Intermediate = AML/ALL (≥CR2); CML (AP [accelerated phase] or ≥ CP2 [second chronic phase]); Advanced = AML/ALL (REL [relapsed]/PIF [primary induction failure]); CML in BP; MDS (RAEB [refractory anemia with excess blasts]/RAEB-t [refractory anemia with excess blasts in transformation]/chronic myelomonocytic leukemia or marrow blasts ≥ 5%).

† D-R HLA match: Well-matched URD cases had either no identified HLA mismatch and informative data at 4 loci or allele matching at HLA-A, -B, and -DRB1. Partially matched URD pairs had a defined, single-locus mismatch and/or missing HLA data. Mismatched URD cases had ≥2 allele or antigen mismatches [32].

Table 2
cGVHD Characteristics

Characteristics	1995-1999 n (%)	2000-2003 n (%)	2004-2007 n (%)	P
For patients who developed cGVHD post-transplant (patients were censored at second transplant, DCI, or relapse)				
Time from transplant to cGVHD onset, mo (median, 5 mo)				.08
<5	1645 (51)	1267 (53)	1534 (50)	
≥5	1565 (49)	1139 (47)	1557 (50)	
Type of cGVHD onset				<.001
Progressive	1408 (44)	854 (35)	855 (28)	
Quiescent/interrupted	626 (20)	758 (32)	1171 (38)	
De novo	834 (26)	687 (29)	1011 (33)	
Missing/not collected on prior forms	342 (11)	107 (4)	54 (2)	
Maximum grade of cGVHD				<.001
Limited	1175 (37)	677 (28)	856 (28)	
Extensive	2019 (63)	1718 (71)	2227 (72)	
Unknown/missing	16 (<1)	11 (<1)	8 (<1)	
Maximum overall severity of cGVHD				<.001
Mild	1085 (34)	955 (40)	1297 (42)	
Moderate	852 (27)	817 (34)	1102 (36)	
Severe	466 (15)	470 (20)	641 (21)	
Unknown/missing	807 (25)	164 (7)	51 (2)	
Number of cGVHD organ involved at maximum severity				<.001
1	675 (21)	534 (22)	837 (27)	
2	677 (21)	478 (20)	708 (23)	
3	560 (17)	389 (16)	537 (17)	
4	411 (13)	330 (14)	371 (12)	
5+	604 (19)	528 (22)	429 (14)	
Missing	283 (9)	147 (6)	209 (7)	
Systemic immunosuppression given				<.001
Yes	2697 (84)	2253 (94)	2979 (96)	
No	428 (13)	133 (6)	100 (3)	
Missing	85 (3)	20 (1)	12 (<1)	
cGVHD organ involved at maximum severity				
Skin ± other	1650 (51)	1563 (65)	2192 (71)	
Eyes ± other	1145 (36)	811 (34)	657 (21)	
Mouth ± other	1384 (43)	1149 (48)	980 (32)	
Lung ± other	456 (14)	398 (17)	522 (17)	
GI/weight loss ± other	1261 (39)	995 (41)	1050 (34)	
Liver ± other	1525 (48)	1178 (49)	1399 (45)	
Other organ involvement ± other	1163 (36)	798 (33)	985 (32)	
Median follow-up, mo (range)	113 (3-196)	82 (3-135)	49 (3-89)	
DCI-associated cGVHD				
Total number patients with cGVHD after DCI	77	68	73	
Mild	19	27	29	
Moderate	22	26	28	
Severe	16	13	14	

GI indicates gastrointestinal.

Patient Selection

Adult and pediatric patients reported to the CIBMTR with acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML), and myelodysplastic syndrome (MDS) who had their first allogeneic transplant between 1995 and 2007 were included in the study. Recipients of all graft sources, donor types, and conditioning intensity were included.

Study Definitions

For this study, incidence was defined as the development of cGVHD within 1 year after transplant. The event was summarized by the cumulative incidence estimate. In analysis, death, second transplant, DCI, and relapse were considered competing risks.

NRM was defined as death in continuous complete remission. The event was summarized by the cumulative incidence estimate with relapse as the competing risk. OS was defined as death from any cause. Nonmyeloablative conditioning or RIC regimens were defined as busulfan dose <9 mg/kg, melphalan dose <150 mg/m^2, and total body irradiation dose ≤500 cGy (single or fractionated) or 500 to 800 cGy (fractionated).

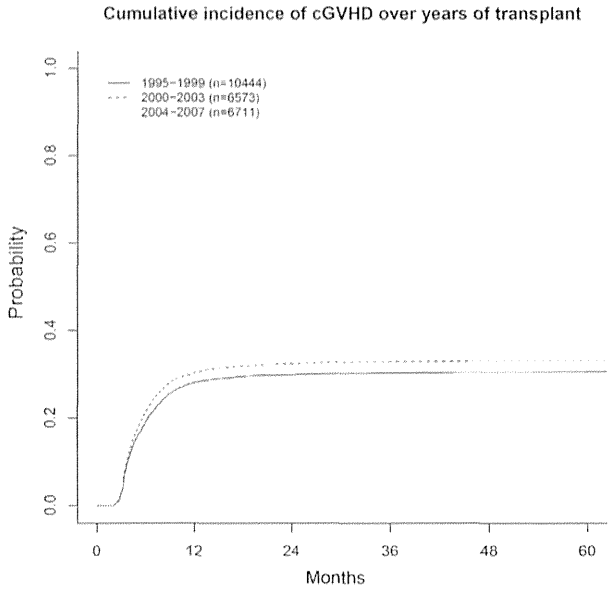

Figure 1. In the multivariate analysis, higher risk of cGVHD in the most recent time period (2004-2007 versus 1995-1999: OR = 1.19, $P < .0001$; and 2004-2007 versus 2000-2003: OR = 1.13, $P = .002$).

cGVHD was diagnosed according to Seattle criteria [13]. The new National Institutes of Health (NIH) consensus criteria had not yet been implemented on CIBMTR forms for this analysis [14]. The CIBMTR definition of mild, moderate, and severe categories of cGVHD was used as described before [15]. The CIBMTR definitions of cGVHD onset (progressive, quiescent, de novo) were used [2].

Statistical Analysis

The main objective of this study was to look at the cumulative incidence of any cGVHD (limited or extensive) as a time trend in transplants performed from 1995 to 2007. The main variable, year of transplant, was treated either as a categorical variable with groups 1995 to 1999, 2000 to 2003, 2004 to 2007 or as a continuous variable for testing the trend when a linear trend was reasonable. Descriptive analysis was performed to analyze maximum severity of the cGVHD within 1 year using chi-square tests. Descriptive analyses were performed to define cGVHD subsets (mild, moderate, severe or progressive, quiescent, de novo) and major organ and number of organ involvement (eye, mouth, skin, liver, lung, gastrointestinal).

Among all patients who developed cGVHD, 91.3% of patients developed cGVHD within 1 year after transplant. The remaining 8.7% of patients developing cGVHD after 1 year were censored and were not included in the analyses as having cGVHD.

The cumulative incidence estimator was used to calculate the probabilities of cGVHD [16]. The overall mortality trend was evaluated using the log-rank test and Kaplan-Meier estimator [17]. We also looked at NRM and OS only in patients who had cGVHD by left truncation from the time of diagnosis of cGVHD. Multivariate analysis on the cumulative incidence of cGVHD at 1 year after transplantation was performed with the pseudo-value approach [18] by using 2 methodologies: either treating only death as a competing risk or treating death, second transplant, DCI, and relapse as competing risks. Both demonstrated similar results; hence, the results from the second method (treating death, second transplant, DCI, and relapse as competing risks) are reported.

A stepwise model selection procedure was used to determine clinical variables affecting the incidence of cGVHD. The multivariate analysis evaluated the categorical year of transplant as the main variable and also assessed the possible interactions of the adjusted clinical variables with the year of transplant. To adjust for multiple testing, $P < .01$ was considered statistically significant for the main outcome variable of interest. SAS version 9.2 (SAS Institute, Cary, NC) was used for all analyses.

RESULTS

Patient Characteristics

Baseline characteristics are shown in Table 1. The study population included all patients (N = 26,563) who

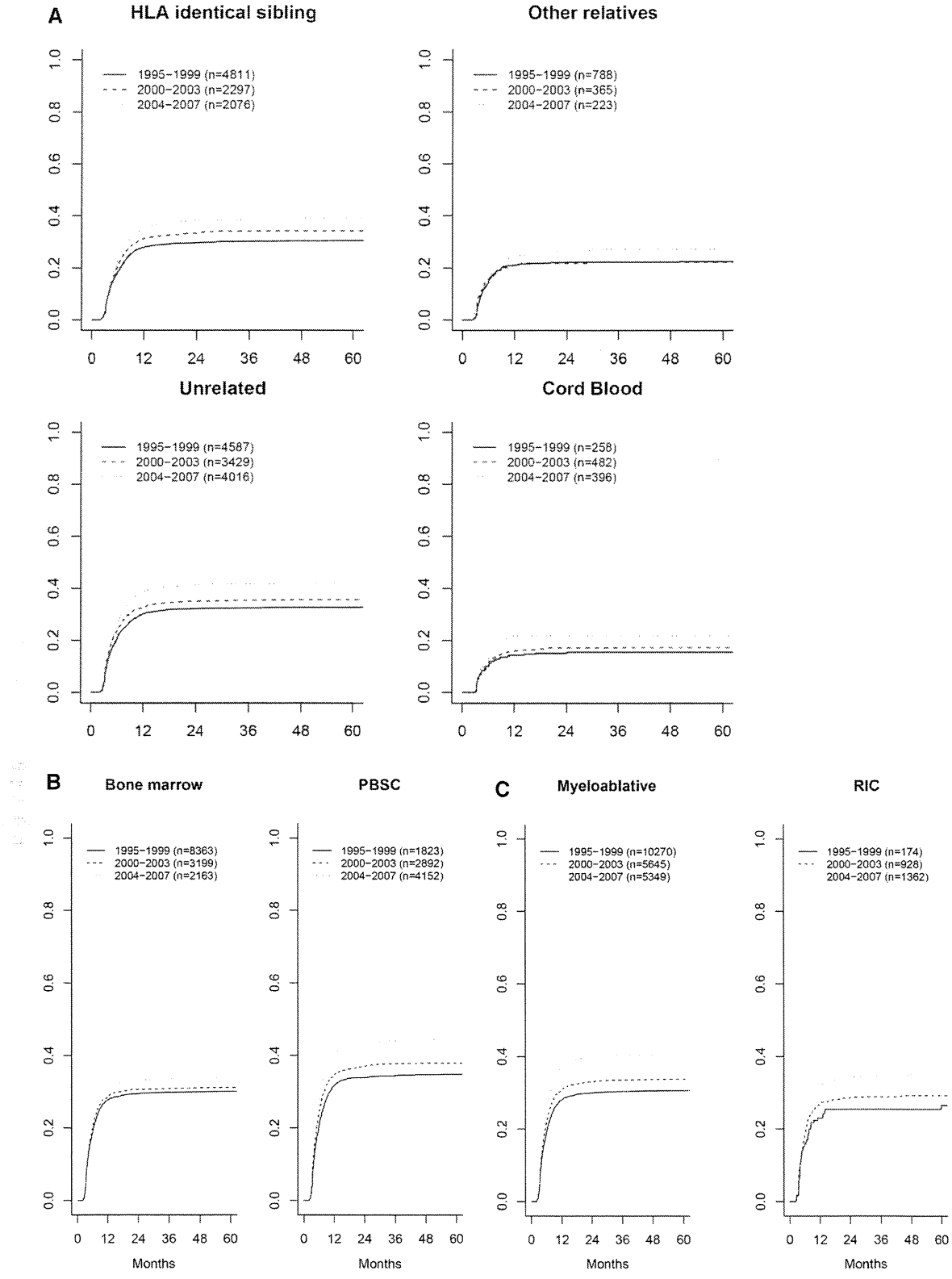

Figure 2. Increased cGVHD incidence when stratified by (A) donor type (HLA identical sibling: HR = 1.17; unrelated donor: HR = 1.07; cord blood: HR = 1.24, all $P < .01$), (B) graft type (PBSC: HR = 1.19; cord blood: HR = 1.24, $P < .01$), or (C) conditioning intensity (myeloablative: HR = 1.13; reduced intensity: HR = 1.16, $P < .01$).

underwent a first allogeneic HCT for acute leukemia (AML, n = 10,737; ALL, n = 6756), CML (n = 6341), and MDS (n = 2729) from 1995 to 2007. There were 10,597 patients transplanted between 1995 and 1999, 7472 patients transplanted between 2000 and 2003, and 8494 patients transplanted between 2004 and 2007. Over the course of time, transplantation for AML became more frequent, age at transplantation increased, and the use of nonmyeloablative conditioning/RIC, alternative donors, and peripheral blood stem cell (PBSC) grafts all increased.

Table 3
Univariate Analysis of NRM and OS of all Patients

Outcome Events	No. of Patients at Risk	1995-1999 Prob (95% CI)	No. of Patients at Risk	2000-2003 Prob (95% CI)	No. of Patients at Risk	2004-2007 Prob (95% CI)	*P*
NRM							
At 100 d	7334	21 (21-22)	5127	17 (16-18)	6255	11 (11-12)	<.001
At 1 yr	4908	32 (31-33)	3258	28 (27-29)	3947	21 (21-22)	<.001
At 3 yr	3742	36 (35-37)	2337	32 (31-33)	2430	26 (25-27)	<.001
At 5 yr	3114	37 (36-38)	1714	33 (32-34)	977	29 (28-30)	<.001
OS							
At 1 yr	5555	54 (53-55)	3842	53 (52-54)	4494	59 (58-60)	<.001
At 3 yr	4264	44 (43-45)	2744	42 (40-43)	2723	44 (43-46)	<.001
At 5 yr	3558	41 (40-42)	1993	38 (37-39)	1101	39 (38-40)	.002

Prob indicates probability; CI, confidence interval.

Incidence of cGVHD

cGVHD characteristics are shown in Table 2. Both univariate and multivariate analyses showed a significantly increased incidence of cGVHD in recent time periods. In univariate analysis, the cGVHD rates at 1 year by time period were 28% for 1995 to 1999, 31% for 2000 to 2003, and 37% for 2004 to 2007 ($P < .001$). In the multivariate analysis (Figure 1), the most recent time period (2004 to 2007) was associated with higher risk of cGVHD when compared with the 2 earlier time periods (2004 to 2007 versus 1995 to 1999: odds ratio [OR] = 1.19, $P < .0001$; and 2004 to 2007 versus 2000 to 2003: OR = 1.13, $P = .002$). This trend of increased cGVHD incidence was noted when stratified by donor type (HLA identical sibling: hazard ratio [HR] = 1.17; unrelated donor: HR = 1.07; cord blood: HR = 1.24; all $P < .01$; Figure 2A), graft type (PBSC: HR = 1.19; cord blood: HR = 1.24; $P < .01$; Figure 2B), or conditioning intensity (myeloablative: HR = 1.13; RIC: HR = 1.16; $P < .01$; Figure 2C). In mismatched related donors (HR = 1.08, $P = .24$) and bone marrow graft type (HR = 1.01, $P = .54$), there was no significant change in the incidence of cGVHD over time. An analysis of cGVHD incidence over time stratified by disease (AML, ALL, CML, MDS) showed a significant increase in incidence for all diseases except CML, which had no significant change.

Presenting Features of cGVHD

Progressive cGVHD (defined as acute GVHD progressed directly to cGVHD) [2] was found to be less frequently diagnosed over time as compared with quiescent or de novo cGVHD (Table 2). Because this trend might be from the recognition of the late acute classification in the 2005 NIH cGVHD consensus criteria [14], we attempted to capture the proportion of late acute GVHD patients within the group of early progressive onset patients. To do the calculation, we determined that 4756 patients developed cGVHD within 5 months of transplant. Within this group, 1635 patients were categorized as progressive onset cGVHD, and within these 1635 patients, 937 (57%) were diagnosed between 100 days and 5 months of HCT. We further examined the organ involvement of the 937 early progressive onset patients and determined that isolated skin, gut, or liver or combinations of these, suggesting late acute GVHD, was present in 628 patients. Thus, late acute GVHD might comprise about 13% (628/4756) of the overall early cGVHD patients. Although this number may not capture all late acute patients, our data suggest this as a possibility in this cohort. Overlap syndrome in the NIH criteria could also have been included under progressive onset and accounted for some of the decline in progressive onset reporting after 2005 [19].

Extensive, moderate, and severe categories of cGVHD were more frequent in the 2 most recent time periods (2000 to 2003 and 2004 to 2007) as compared with the earliest time period (1995 to 1999). Skin involvement at the maximum severity was more frequent in recent years, the greatest association with peripheral blood (33% in 2004 to 2007) compared with bone marrow (25% in 2004 to 2007, $P < .001$, data not shown).

NRM and OS Over Time

Univariate analyses of NRM and OS for all patients, patients without cGVHD, and patients with cGVHD are shown in Tables 3-5, respectively. NRM for all transplanted patients has decreased over time (Table 3). For patients without cGVHD, the NRM at 1 and 3 years went from 29% and 31%, respectively, in the 1995 to 1999 time period to 20% and 21%, respectively, in the 2004 to 2007 time period (Table 4). Similarly, for patients with cGVHD, the 1- and 3-year NRM was lower in more recent years; however, the trend has not continued in year 5, suggesting the risk of NRM persists over time for those who continue to have active cGVHD (Table 5).

Table 4
Univariate Analysis of NRM and OS of Patients without cGVHD (Patients Who Developed cGVHD Were Included and Censored at the Time When They Developed cGVHD)

Outcome Events	No. of Patients at Risk	1995-1999 Prob (95% CI)	No. of Patients at Risk	2000-2003 Prob (95% CI)	No. of Patients at Risk	2004-2007 Prob (95% CI)	*P*
NRM							
At 100 d	6888	21 (21-22)	4821	17 (16-18)	5910	12 (11-12)	<.001
At 1 yr	2737	29 (28-30)	1671	25 (24-26)	1762	20 (19-21)	<.001
At 3 yr	1909	31 (30-32)	1071	26 (25-27)	896	21 (20-22)	<.001
At 5 yr	1572	31 (30-32)	735	27 (26-28)	361	22 (21-23)	<.001
OS							
At 1 yr	3176	55 (54-56)	2016	54 (53-55)	2049	58 (57-59)	<.001
At 3 yr	2184	46 (45-48)	1254	44 (43-45)	1015	46 (45-48)	.018
At 5 yr	1807	44 (43-45)	854	41 (40-43)	411	43 (41-45)	.016

Table 5
Univariate Analysis of NRM and OS of Patients with cGVHD (Left Truncation)

Outcome Events	No. of Patients at Risk	1995-1999 Prob (95% CI)	No. of Patients at Risk	2000-2003 Prob (95% CI)	No. of Patients at Risk	2004-2007 Prob (95% CI)	*P*
NRM							
At 1 yr	2170	27 (25-29)	1590	25 (22-28)	2178	18 (16-20)	<.001
At 3 yr	1824	36 (34-39)	1266	36 (33-39)	1500	30 (28-32)	<.001
At 5 yr	1533	40 (38-42)	957	40 (37-42)	612	37 (34-39)	.11
OS							
At 1 yr	2379	67 (65-69)	1826	67 (65-70)	2446	73 (71-75)	.0002
At 3 yr	2081	53 (51-55)	1490	51 (48-53)	1676	53 (51-55)	.189
At 5 yr	1749	48 (46-50)	1129	46 (43-48)	690	45 (45-47)	.078

In general, the 3- and 5-year OS for patients with and without cGVHD has not changed in recent time periods.

There were 15,781 deaths for the entire cohort. The major cause of death for the entire patient cohort was relapse of the primary disease (n = 5263 [33%]), followed by infection (n = 2690 [17%]), organ failure (n = 2064 [13%]), and GVHD (n = 2039 [13%]); this trend was consistent across all 3 time periods. Death from disease relapse was 28% for the 1995 to 1999 time period (1827/6492), 34% for 2000 to 2003 (1577/4627), and 40% for 2004 to 2007 (1859/4662) (Supplemental Table 1). For patients who developed cGVHD, the major cause of death was also relapse of the primary disease. Thus, death from late relapse still persists in cGVHD patients. Relapse rate by severity grade of cGVHD was outside the focus of this study; however, a previous International Bone Marrow Transplant Registry/National Marrow Donor Program publication [15] has shown no association of relapse rate with cGVHD severity (mild, moderate, severe).

Factors Affecting the Incidence of cGVHD

Results of the multivariate analysis are shown in Table 6. The use of bone marrow with an unrelated donor (matched or mismatched) and PBSC graft with all categories of donor group was associated with higher risk of cGVHD, as compared with the use of bone marrow with a matched sibling donor. The risk of cGVHD was lower for unrelated cord blood 5/6 or ≤4/6 mismatched compared with an unrelated PBSC graft (matched or mismatched) and was similar to a bone marrow graft with a matched sibling donor. Expected associations of higher risk of cGVHD with female-to-male transplants ($P < .0001$) and lower risk with T cell depletion (OR = .53, $P < .0001$) were also seen. Other combinations of GVHD prophylaxis did not affect the incidence of cGVHD over the time periods. Cytomegalovirus status of the donor–recipient pair also did not impact cGVHD incidence in our model. The analysis did demonstrate a statistically significant decrease in cGVHD risk after nonmyeloablative/RIC transplant (OR = .84, $P = .0021$).

DISCUSSION

In this large-scale analysis, we identify a clear increase in the incidence of cGVHD over the study time period from 1995 to 2007. This trend was confirmed despite controlling for factors related to donor, graft, and conditioning regimen associated with that trend. One possible explanation for this unfavorable trend in cGVHD is the steadily increasing number of long-term survivors because of lower early NRM [20]. However, in the analysis focused only on patients who survived beyond 100 days post-transplant, this trend was maintained. Multiple factors are thus influencing the increased cGVHD incidence trend besides long-term survivorship. Our study confirmed the increase over time in

Table 6
Multivariate Analysis of cGVHD at 1 Year after Transplant Treating Death, Second Transplant, DCI, and Relapse as Competing Risks

Variables	n	OR (95% CI)	*P*
Year of transplant			.0001
1995-1999	10,444	1.00	
2000-2003	6573	1.06 (.98, 1.14)	.1646
2004-2007	6711	1.19 (1.1, 1.3)	<.0001
Contrast comparison 2004-2007 vs. 2000-2003		1.13 (1.05, 1.22)	.002
Age at transplant			<.0001
0-9 yr	2965	1.00	
10-19 yr	3596	1.22 (1.07, 1.39)	0.0029
20-29 yr	3769	1.57 (1.38, 1.78)	<.0001
30-39 yr	4537	1.61 (1.41, 1.82)	<.0001
40-49 yr	4732	1.6 (1.41, 1.82)	<.0001
50-59 yr	3222	1.52 (1.32, 1.74)	<.0001
≥60 yr	907	1.19 (.97, 1.46)	.0999
ATG or Campath			<.0001
Yes	17,160	1.00	
No	6568	1.76 (1.63, 1.91)	<.0001
Disease			<.0001
AML	9200	1.00	
ALL	6217	.98 (.9, 1.06)	.6077
CML	6007	1.24 (1.15, 1.34)	<.0001
MDS	2304	1.44 (1.3, 1.6)	<.0001
Disease status at transplant			<.0001
Early	11,433	1.00	
Intermediate	6280	.88 (.82, .95)	.0005
Advanced	6015	.54 (.5, .59)	<.0001
Donor and graft type			<.0001
BM, HLA-identical sibling	4611	1.00	
BM, other relative	721	1.13 (.91, 1.4)	.281
BM, URD well matched	3414	1.83 (1.65, 2.04)	<.0001
BM, URD mismatched	1726	1.37 (1.19, 1.57)	<.0001
PBSC, HLA-identical sibling	4573	1.67 (1.51, 1.84)	<.0001
PBSC, other relative	655	1.53 (1.22, 1.9)	.0002
PBSC, URD well matched	2288	2.56 (2.26, 2.9)	<.0001
PBSC, URD partially matched	1018	2.75 (2.36, 3.21)	<.0001
PBSC, URD mismatched	333	2.19 (1.71, 2.8)	<.0001
5/6 UCB	204	1.16 (.73, 1.83)	.5308
4 or less/6 UCB	932	1.2 (.96, 1.51)	.1143
GVHD prophylaxis			<.0001
CSA + MTX ± others (except FK506, MMF)	13,178	1.00	
Ex vivo T cell depletion	2483	.53 (.46, .6)	<.0001
CSA ± others	2698	1.03 (.92, 1.15)	.6191
FK506 + MTX ± others (except MMF)	4528	1.05 (.97, 1.14)	.1979
FK506 ± others	841	1.01 (.87, 1.19)	.8693
Performance score			<.0001
<80%	2099	1.00	
≥80%	20,919	1.44 (1.28, 1.62)	<.0001
Unknown	710	1.39 (1.14, 1.68)	.001
Conditioning regimen			.0021
Myeloablative	21,264	1.00	
Nonmyeloablative/RIC	2464	.84 (.76, .94)	.0021
Donor–recipient sex match			<.0001
Female donor, male recipient	5218	1.00	
Other	18,424	.71 (.66, .76)	<.0001
Unknown	86	.92 (.58, 1.43)	.6996

BM indicates bone marrow.

number of older patients undergoing transplant, use of PBSC grafts, and use of alternative donors, all of which associate with increased cGVHD and have been previously described [21]. Moderate and severe categories of cGVHD were more frequently observed in recent years, further emphasizing the impact of more recent transplant strategies on cGVHD severity. A trend in cGVHD onset type with progressive less frequently diagnosed and quiescent or de novo cGVHD more frequently diagnosed over time may reflect more of a shift in definitions rather than an actual increase in quiescent and de novo onsets. One might speculate that improved early NRM suggests decreased mortality of acute GVHD and therefore more patients living to a quiescent or de novo onset.

Clearly, NRM at 1 and 3 years has improved significantly over the observed time periods for patients both with and without cGVHD. This trend is consistent with improvements in supportive care introduced in allogeneic HCT over the years [20]. The trend toward less severe (grades III to IV) acute GVHD (Table 1) and fewer deaths from infection (Supplemental Table 1) over time may have contributed to the observed decrease in early NRM. Possibly the increased use of PBSC grafts over time has impacted early NRM by resulting in faster neutrophil engraftment and earlier recovery of immunity to fungal and bacterial infections [20]. Long-term OS over the 3 time periods, however, has not significantly improved. These OS results are not unlike other reports on recent survival trends after allogeneic transplant [21,22] where improvements in day 100 survival did not translate into equally improved 1-year OS. This is because relapse remains the major cause of death over time. In support of this finding is the increased cumulative incidence of relapse for all patients on the study at years 3 and 5 over time (Supplemental Table 2). The relapse trend persists even when separating patients with and without cGVHD (Supplemental Table 3). Although our study did not focus on cGVHD impact on relapse, we can infer from the NRM and OS outcomes in our analysis that early 1-year survival has improved over time for cGVHD patients, perhaps from an early protective effect of cGVHD against relapse, but 3- and 5-year OS has not changed because of late relapses and from no greater protection from cGVHD after 1 year, especially for myeloablative transplants [11].

In the multivariate analysis, the identified risk factors for increased cGVHD incidence were not unexpected. Increased age at transplant [5,6], patients with CML and MDS [23,24], use of unrelated donors, female donor into male recipient [3,4] and use of PBSC grafts [7-9,25] are in accordance with previous reports on risk factors for cGVHD incidence. The reduced risk with antithymocyte globulin [26] or alemtuzumab [27] and ex vivo T cell depletion [28] is also in accordance with previous studies. Information on lower risk of cGVHD after nonmyeloablative conditioning and RIC transplants observed in this current study enhances our knowledge on this topic [29-31]. We recognize the limitations of this study as historical data collection via registry that did not include the recent NIH consensus criteria for cGVHD classification [13], which might impact some of the trends seen. Still, this information is obtained on a very large cohort of transplanted patients, and the characterization of the recent trends in cGVHD is the best available data to date. With cGVHD classification currently undergoing refined definitions from the 2005 NIH consensus, it is of value to comprehensively report our historical data because it may serve as a basis for future comparison.

In summary, these findings of cGVHD trends observed over a 12-year period provide convincing evidence of increasing cGVHD incidence in recent years and the factors associated with these trends. We see that newer transplant practices have also impacted early NRM in cGVHD patients but that 5-year NRM and OS have not significantly changed over time, suggesting adverse impact of protracted immunological derangements associated with cGVHD. These data provide the compelling epidemiological background on the current trends in cGVHD, which remains a major barrier for successful allogeneic HCT. They serve also as a helpful reference to guide future research efforts by the transplant and hematology community.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.bbmt.2014.10.021.

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bjh research paper

Allogeneic haematopoietic stem cell transplantation for infant acute lymphoblastic leukaemia with *KMT2A (MLL)* rearrangements: a retrospective study from the paediatric acute lymphoblastic leukaemia working group of the Japan Society for Haematopoietic Cell Transplantation

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Summary

Allogeneic haematopoietic stem cell transplantation (HSCT) is still considered to play an important role as a consolidation therapy for high-risk infants with acute lymphoblastic leukaemia (ALL). Here, we retrospectively analysed outcomes of HSCT in infants with ALL based on nationwide registry data of the Japan Society for Haematopoietic Cell Transplantation. A total of 132 allogeneic HSCT for infant ALL with *KMT2A (MLL)* gene rearrangements, which were performed in first complete remission (CR1), were analysed. The 5-year overall survival rate after transplantation was 67·4 ± 4·5%). Although recent HSCT (after 2004) had a trend toward better survival, no statistical correlation was observed between outcomes and each factor, including age at diagnosis, initial leucocyte count, cytogenetics, donor types or conditioning of HSCT. Myeloablative conditioning with total body irradiation did not provide a better survival (60·7 ± 9·2%) over that with busulfan (BU; 67·8 ± 5·7%). Two of the 28 patients treated with irradiation, but none of the 90 BU-treated patients, developed a secondary malignant neoplasm. In conclusion, allogeneic HSCT using BU was a valuable option for infant ALL with *KMT2A* rearrangements in CR1.

Keywords: infant, acute lymphoblastic leukaemia, stem cell transplantation, busulfan, total body irradiation.

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Although recent advances have achieved excellent cure rates in most cases of paediatric acute lymphoblastic leukaemia (ALL) (Inaba *et al*, 2013), infants with *KMT2A* (*MLL*) rearrangements have worse outcomes than older children (Rubnitz *et al*, 1999; Pui *et al*, 2002; Hilden *et al*, 2006) or infants without *KMT2A* rearrangements (Nagayama *et al*, 2006). Previous clinical studies have reported improvements in the outcomes of infants with ALL characterized by *KMT2A* rearrangements using intensified treatments and allogeneic haematopoietic stem cell transplantation (HSCT) (Silverman *et al*, 1997; Kosaka *et al*, 2004; Jacobsohn *et al*, 2005; Sanders *et al*, 2005; Tomizawa *et al*, 2007), and recent international studies revealed that low-risk infants with ALL could be treated without HSCT, whereas high-risk infants still require allogeneic HSCT as a consolidation therapy (Pieters *et al*, 2007; Mann *et al*, 2010; Dreyer *et al*, 2011). However, optimal allogeneic HSCT strategies, such as the best stem cell source or conditioning regimen, have yet to be determined mainly because of the rarity of infants with ALL.

The high relapse risk of infant ALL with *KMT2A* rearrangements is well known; therefore, allogeneic HSCT at first complete remission (CR1) was indicated for these patients from the second half of 1990s in Japan (Kosaka *et al*, 2004; Tomizawa *et al*, 2007). In the present study, we retrospectively analysed HSCT for infants with ALL based on nationwide registry data of the Japan Society for Haematopoietic Cell Transplantation (JSHCT) in order to obtain fundamental information for establishing a standard approach for infants with ALL.

Patients and methods

This study was approved by the Institutional Ethics Committee of the University of Tokyo Hospital. A total of 132 patients were analysed based on data reported to the JSHCT registry (Atsuta *et al*, 2007). The patients were selected according to the following criteria: (i) diagnosed as ALL with *KMT2A* rearrangements when aged < 1 year old; (ii) allogeneic HSCT was performed in CR1; (iii) HSCT was performed between 1996 and 2011.

The overall survival (OS) probability was calculated using Kaplan–Meier estimates. The duration of event-free survival (EFS) was defined as the time from HSCT to either treatment failure (relapse, death, or the diagnosis of secondary cancer) or to the latest day that the patient was confirmed to be alive. Cumulative incidence curves were used in a competing-risk setting to calculate the probability of engraftment, graft-*versus*-host disease (GVHD) and non-relapse mortality (NRM). Univariate analyses of OS were performed using the log-rank test, and Gray's test was used for group comparisons of cumulative incidences. Engraftment was defined as the first day of three consecutive days with an absolute neutrophil count $\geq 0{\cdot}5 \times 10^9$/l. Myeloablative conditioning was defined as total body irradiation (TBI) of 8 Gy or more, or the administration of busulfan (BU) at a dose higher than 8 mg/kg. All other regimens were analysed as non-myeloablative conditioning (Bacigalupo *et al*, 2009). Multivariate analysis was performed using the Cox proportional-hazard regression model. Univariate analysis did not find any statistical significance ($P < 0{\cdot}2$) between survival outcome and each factor except transplantation period, and the variables considered as clinically important were the patient's age at diagnosis, leucocyte count at diagnosis, the partner gene of the *KMT2A* fusion, donor type and conditioning regimen.

All statistical analyses were performed using R software 2·13·0 (The R Foundation for Statistical Computing, Vienna, Austria).

Results

Patients

All patients and transplantation characteristics are listed in Table I. The median age at diagnosis was 4 months. The median time from the diagnosis to HSCT was 148 days. The median follow-up period after HSCT was 4·9 years (range, 0–16·6 years).

The estimated OS and standard error (±SD) at 5 years after HSCT was 67·4 ± 4·5%. For the 132 patients who underwent HSCT in CR1, the EFS, relapse incidence and NRM were 53·9 ± 4·6%, 34·1 ± 4·4% and 12·0 ± 2·9%, respectively. Fifteen patients died without relapse from various causes: pulmonary complications ($n = 6$), infections ($n = 5$), GVHD ($n = 3$) and sinusoidal obstruction syndrome (SOS) ($n = 1$).

Outcomes of HSCT

The relationships between the outcomes of HSCT according to risk factor are shown in Table II. NRM of HSCT in the recent period (after 2004) was lower than that before 2003 (5·6 ± 2·8% and 20·8 ± 5·6%, respectively), but relapse of the surviving patients minimized the difference in OS (70·8 ± 6·3% and 60·3 ± 6·7%, respectively). Age at diagnosis, initial leucocyte count and partner genes of *KMT2A* rearrangements did not have a prognostic impact on OS, relapse rate or NRM (Figure S1). Thirty-two patients had an initial leucocyte count of 300×10^9/l or more, and the OS and EFS of these patients (74·3 ± 8·6% and 51·9 ± 9·9% at 5 years, respectively) were not inferior to those of the other patients.

Conditioning of HSCT

The OS following myeloablative conditioning with BU was 67·8 ± 5·7% ($n = 90$), and the OS with myeloablative TBI was 60·7 ± 9·2% ($n = 28$) (Table II, Fig 1B). Most patients received a combination of etoposide (VP16) and cyclophosphamide (CY) in these myeloablative regimens. Hepatic Sinusoidal obstruction syndrome was observed in 16 of 90 (17·8%) BU patients and three of 28 (10·7%) TBI patients.

Table I. Patient and transplantation characteristics.

Characteristics	Disease status at transplantation First remission
All patients, *n*	132
Transplantation period	
1996–2003	53
2004–2011	79
Age at diagnosis, months	
<3	28
3–5	56
6–12	48
Median initial leucocyte count, × 10^9/l	
<100	59
100–299	28
≥300 000	32
Not known	13
Cytogenetics, *n*	
KMT2A rearrangements	132
t(4;11)/*KMT2A-AFF1*	79
t(9;11)/*KMT2A-MLLT3*	10
t(11;19)/*KMT2A-MLLT1*	10
Other *KMT2A*	33
Others/not known	–
Transplantation donor, *n*	
Related	30
HLA-matched	15
HLA-mismatched	15
Unrelated	13
HLA-matched	12
HLA-mismatched	1
Cord blood	89
HLA-matched	35
HLA-mismatched	54
Transplantation conditioning, *n*	
Myeloablative busulfan	90
VP16+CY	85
Others/not known	5
Myeloablative TBI	28
VP16+CY	19
Others/not known	9
Non-myeloablative	3
Not known	11

HLA, human leucocyte antigen; TBI, total body irradiation; VP16, etoposide; CY, cyclophosphamide.

Although two out of 28 (7·1%) patients who received HSCT with TBI developed thyroid carcinoma as a secondary neoplasm (9·6 and 11·7 years after HSCT), these patients were alive 1·3 and 4·3 years after this diagnosis. In contrast, no secondary neoplasm occurred in the 90 patients that received myeloablative BU or non-myeloablative HSCT (P = 0·05, Fisher's exact test).

Stem cell sources of HSCT in CR1

The stem cell sources of HSCT did not have a significant impact on OS (Table II, Fig 2A). All related donors and unrelated donors achieved engraftment, with a median of 14·5 and 18 days after HSCT. Of 54 cord blood (CB) transplants, 48 achieved engraftment in a median of 18 days, and the engraftment probability was 93·3 ± 2·8% at day 60. The incidence of acute GVHD was slightly higher with unrelated donors (53·8 ± 14·7% at day 100, Fig 2B) than with related donors (28·6 ± 8·7%) and cord blood (29·4 ± 5·0%) (P-value by the log-rank test between unrelated donor and others was 0·05). Among 123 patients who were alive at 100 days after HSCT, chronic GVHD was observed in 6 (21·4%) of 28 transplanted from a related donor, 5 (41·7%) of 12 transplanted from a unrelated donor, and 15 (18·1%) of 83 transplanted with cord blood.

A total of 30 HSCT performed in CR1 was from a related donor, 15 of which were human leucocyte antigen (HLA)-mismatched. Of the 15 mismatched donors, 3 donors were 2- or 3-antigen-mismatched related donors in the graft-*versus*-host (GVH) direction. The HLA disparity among HSCT from related donors did not have a significant difference on outcomes. The 5-year OS of matched related donors was 50·0 ± 13·7%, whereas that of mismatched related donors was 78·0 ± 11·4% (P = 0·20).

Of 89 HLA-mismatched CB, 48 were 1-antigen-mismatched, while 6 were 2- or 3-antigen mismatched. However, mismatched CB was not associated with OS (76·6 ± 8·8% at 5 years for matched CB, 64·6 ± 7·8% for 1-antigen-mismatched CB, and 83·3 ± 15·2% for 2- or 3-antigen-mismatched CB, P = 0·68) (Fig 2C). Status of killer immunoglobulin-like receptor (KIR) ligand incompatibility was identified in 74 HSCT, including 9 KIR ligand mismatches; however, no significant differences were observed when the survival curve of the mismatched group was superimposed on the matched group (P = 0·70, Fig 2D).

The results of multivariate analysis were consistent with those of univariate analysis. Age at diagnosis, initial leucocyte count, partner of the *KMT2A* gene, conditioning regimen and stem cell source did not show significant correlation with survival (Table III). However, recent SCT (after 2004) had a trend toward lower mortality risk although the difference did not reach statistical significance.

Discussion

Although recent large studies reported that intensified chemotherapy without HSCT could provide non-inferior outcomes for relatively low-risk infants with ALL (Pieters *et al*, 2007; Dreyer *et al*, 2011), allogeneic HSCT is still a valuable option for infants with ALL; therefore, an optimal allogeneic HSCT treatment strategy needs to be established. In the present study, in which an analysis of the registry data of the JSCHT was conducted, disease status was the only prognostic factor for OS that was identified in allogeneic HSCT for infants with ALL, and allogeneic HSCT in CR1 could provide similar outcomes independent of the age at diagnosis, initial leucocyte count, partner genes of *KMT2A* rearrangements, stem cell source or conditioning regimen.

Table II. Outcome of HSCT in CR1.

Characteristics	CI of relapse (at 5 years)	*P*	CI of NRM (at 5 years)	*P*	OS (at 5 years)	*P*
All patients	34·1 ± 4·4		12·0 ± 2·9		67·4 ± 4·5	
Transplantation period						
1996–2003	24·5 ± 6·0	0·08	20·8 ± 5·6	0·02	60·3 ± 6·7	0·05
2004–2011	41·9 ± 6·2		5·6 ± 2·8		70·8 ± 6·3	
Age at diagnosis (months)						
<3	39·4 ± 10·1	0·91	11·5 ± 6·4	0·92	74·1 ± 9·3	0·75
3–5	29·4 ± 6·5		13·4 ± 4·8		64·6 ± 6·9	
6–12	36·5 ± 7·5		10·7 ± 4·6		66·9 ± 7·4	
Initial leucocyte count ($\times 10^9$/l)						
<100	30·9 ± 6·4	0·42	7·0 ± 3·4	0·26	75·2 ± 6·1	0·25
≥100	39·9 ± 6·9		14·3 ± 4·7		64·2 ± 7·0	
Cytogenetics						
t(4;11)	29·8 ± 5·5	0·34	13·7 ± 5·5	0·68	64·1 ± 5·7	0·66
t(9;11)	41·6 ± 17·3		0·0 ± 0·0		65·6 ± 20·9	
t(11;19)	38·0 ± 19·6		20·0 ± 13·4		80·0 ± 12·7	
Other *KMT2As*	41·0 ± 9·0		9·4 ± 5·2		70·2 ± 9·3	
Transplantation donor						
Related	40·0 ± 9·7	0·95	13·8 ± 6·5	0·88	63·6 ± 9·3	0·71
Unrelated	15·4 ± 10·5		7·7 ± 7·7		76·9 ± 11·7	
Cord blood	35·2 ± 5·4		12·0 ± 3·6		66·7 ± 5·7	
Transplantation conditioning						
Myeloablative BU	38·9 ± 5·6	0·17	9·6 ± 3·2	0·09	67·8 ± 5·7	0·26
Myeloablative TBI	21·4 ± 7·9		21·4 ± 7·9		60·7 ± 9·2	

HSCT, haematopoietic stem cell transplantation; CR1, first complete remission; CI, cumulative incidence; NRM, non-relapse mortality; OS, overall survival; BU, busulfan; TBI, total body irradiation.

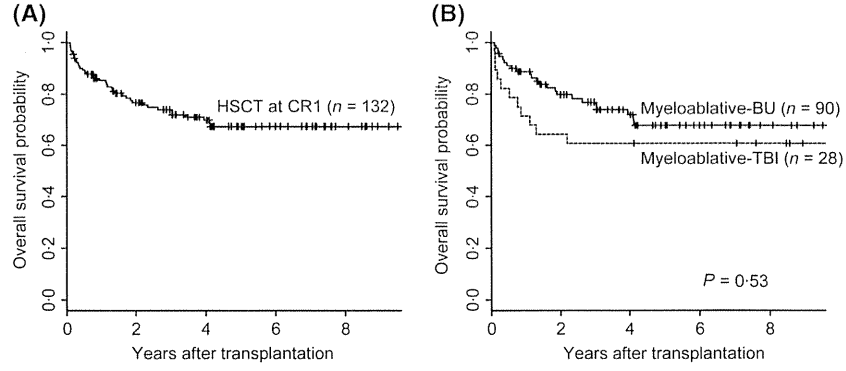

Fig 1. Overall survival following haematopoietic stem cell transplantation. Overall survival probability of (A) all the 132 patients and (B) according to conditioning regimen. BU, busulfan; TBI, total body irradiation.

Recent large studies demonstrated that younger age at diagnosis and higher initial leucocyte count were risk factors for relapse when patients were treated with intensified chemotherapy (Hilden *et al*, 2006; Pieters *et al*, 2007; Dreyer *et al*, 2011). Our results showed that the outcome of HSCT in CR1 for the high-risk group was not inferior to the other group, which suggested that age and leucocyte count influence outcomes only when HSCT could be performed during CR1. Based on the finding that the outcome of HSCT at non-remission was very poor (Tomizawa *et al*, 2009), we confirmed that intensified chemotherapy, which can achieve and maintain CR1 until HSCT, is essential in the treatment strategy for infants with high-risk ALL.

It is well recognized that recent progress in supportive therapy has resulted in a substantial reduction of the mortality rate (Gooley *et al*, 2010), and this was also reproduced in our cohort. Recent HCST was associated with a trend toward better outcomes in our cohort, although indication of HSCT did not differ during this study period.

TBI-based conditioning is the most potent and standard regimen for paediatric ALL (Davies *et al*, 2000; Bunin *et al*, 2003), but is associated with a higher incidence of late complications, especially in infants (Dvorak *et al*, 2011). Our results demonstrated that BU-based conditioning could be used as an alternative regimen and provided potentially better survival outcomes than TBI-based conditioning, with fewer late complications, such as secondary neoplasm (Curtis *et al*, 1997; Cohen *et al*, 2007; Schmiegelow *et al*, 2013). Although gonadal dysfunction is more problematic (Sarafoglou *et al*, 1997; Somali *et al*, 2005), the BU-based

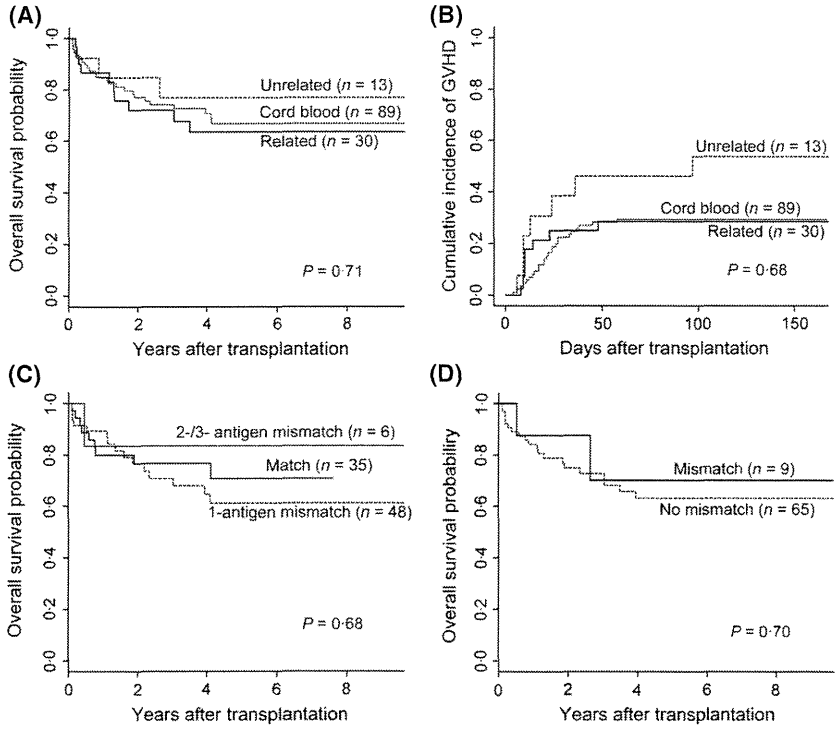

Fig 2. Stem cell sources and outcomes. (A) Overall survival according to stem cell source. (B) Cumulative incidence of grade II to IV acute graft-*versus*-host disease (GVHD). (C) Overall survival according to human leucocyte antigen (HLA) mismatches in cord blood transplantation. HLA disparities were defined as the number of serological mismatches. (D) Overall survival according to KIR ligand incompatibility.

Table III. Multivariate analysis of the risk factors for overall mortality (HSCT at CR1).

	Overall mortality	
Characteristic	Hazard ratio (95% CI)	*P*-value
Transplantation period		
1996–2003	1	0·07
2004–2011	0·40 (0·15–1·08)	
Age at transplantation (months)		
<3	1	0·93
3–5	1·05 (0·36–3·09)	
7–12	0·88 (0·28–2·80)	0·83
Initial leucocyte count ($\times 10^9$/l)		
<100	1	0·24
≥100	1·60 (0·73–3·50)	
Cytogenetics		
t(4;11)	1	0·25
Other *KMT2A*s	0·61 (0·26–1·43)	
Transplantation donor		
Related	1	0·73
Unrelated	1·32 (0·27–6·40)	
Cord blood	1·49 (0·51–4·37)	0·46
Transplantation conditioning		
Myeloablative BU	1	0·38
Myeloablative TBI	0·61 (0·20–1·86)	

95% CI, 95% confidence interval; BU, busulfan; TBI, total body irradiation.

regimen is assumed to be standard conditioning for infants with ALL.

In our cohort, CB was the main stem cell source, probably because of small body size of infants, and the types of stem cell sources and HLA disparities were not associated with survival. Previous studies reported that HLA mismatches could be a risk factor for paediatric leukaemia (Eapen *et al*, 2007); however, CB transplantation results in a large number of haematopoietic stem cells in infants due to their small body size, which could overcome the possible disadvantages associated with HLA disparities. The graft-*versus*-leukaemia (GVL) effect induced by a KIR ligand incompatibility could suppress the relapse of leukaemia (Willemze *et al*, 2009), and is more prominent in infants with ALL (Leung *et al*, 2004); however, we failed to confirm this finding in the present study.

This study retrospectively analysed registry data and naturally has some limitations. For example, data regarding late complications other than secondary malignancies, such as hormonal, pulmonary or neurocognitive dysfunction, is insufficient and inconsistencies were observed in the selection criteria for the stem cell source and conditioning regimen (BU or TBI), even though HSCT at CR1 had been principally indicated for infants with ALL during this period. Although this is one of the largest studies conducted on HSCT in infants with ALL, all of these subgroup analyses were underpowered due to the small sample size and wide confidence intervals, and the results obtained should be carefully interpreted. Therefore, further international studies in a large cohort are required to improve the treatment of infants with ALL with or without HSCT.

In conclusion, allogeneic HSCT with myeloablative BU conditioning is an important option for infants with high-risk ALL in CR1, and could provide similar survival probabilities regardless of the age at diagnosis, initial leucocyte count, *KMT2A* fusion partner of, and stem cell sources.

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Author contributions

M.K is the principal investigator and takes primary responsibility for the paper. M.K, D.H. and K.Kato designed the research; K.Koh, K.Kato, J.T, J.I, H.Y, H.G, S.A, A.H, Y.T, A.S, and Y.A recruited the patients and collected the data. M.K analysed the data, and M.K, D.H, A.Yand K.Kato wrote the manuscript. All authors discussed the results and commented on the manuscript.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Overall survival probability in each subgroups.

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