were entered into multivariable models and sequentially eliminated in a stepwise backward manner. Potential prognostic factors considered in the analysis were recipient age, disease risk, hematopoietic cell transplantation-specific comorbidity index,[20] HLA disparity in the GVH direction in HLA-A, -B, -C, -DRB1 alleles, donor type (related or unrelated), donor-recipient sex-match, conditioning regimen, SC source, GVHD prophylaxis, total number of hemophagocytic cells in BM clot sections on day $+14\pm7$ and presence or absence of documented infections between day +7 and day +21. ES, sinusoidal obstruction syndrome, intestinal transplant-associated microangiopathy (intestinal TAM),[21,22] and posterior reversible encephalopathy syndrome were categorized as endothelial complications, as they are caused by vascular endothelial damage.[4,5,21–25] *P*-values of less than 0.05 were considered statistically significant.

Results

Patient characteristics

The median age of patients was 43 (range, 17–61) years. Diagnoses included AML ($n=24$), ALL ($n=13$), CML ($n=2$), myelodysplastic syndrome ($n=11$), malignant lymphoma ($n=9$), T-cell prolymphocytic leukemia ($n=1$), chronic active EB-virus infection ($n=1$), aplastic anemia ($n=7$) and paroxysmal nocturnal hemoglobinuria ($n=2$). Disease risk was standard in 40 patients and high in 30 patients. HLA was matched in the GVH direction in 47 patients, and matched in the host-vs-graft direction in 45 patients. Myeloablative conditioning regimens were used in 40 patients, and reduced-intensity conditioning regimens in 30. GVHD prophylaxis consisted of a combination of CYA and short-term MTX ($n=21$) or that of tacrolimus and short-term MTX ($n=49$). The median follow-up period of survivors was 556 (range, 236–1272) days.

The median total number of hemophagocytic cells in three fields was two (range, 0–30). The patients were divided into two groups on the basis of nearly bimodal distribution of total numbers of hemophagocytic cells (Figure 2): HP group (total number of hemophagocytic cells $>=5$, median 8, $n=23$) and non-HP group (total number of hemophagocytic cells <5, median 1, $n=47$). Patient characteristics are summarized in Table 1. There were no significant differences between the HP and non-HP groups.

Clinical and laboratory features

Clinical and laboratory features from day +7 to day +21 were compared between the HP and non-HP groups (Table 2). Compared with the non-HP group, fever, neurological symptoms, body weight gain, hypoxia, elevated total bilirubin, elevated serum creatinine and elevated C-reactive protein were more frequent in the HP group. In contrast, there were no significant differences in the incidence of skin rash and diarrhea between the two groups.

Among the 23 patients in the HP group, 14 (61%) developed infections between day +7 and +21. The causes

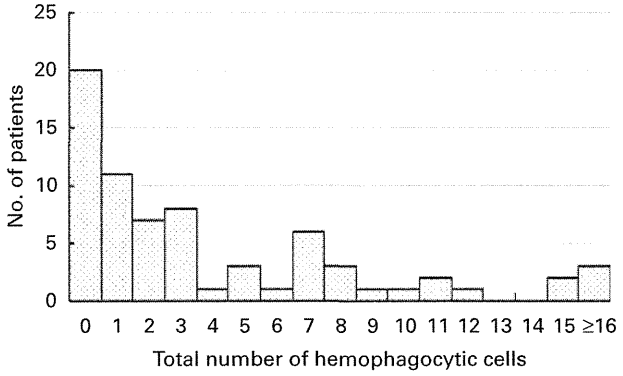

Figure 2 Distribution of numbers of hemophagocytic cells.

of these infections were bacteria ($n=9$), adenovirus and BK virus ($n=1$), CMV ($n=1$), human herpesvirus-6 ($n=1$), aspergillus ($n=1$) and bacteria and aspergillus ($n=1$). Of the 47 patients in the non-HP group, 8 (17%) developed infections. The causes of these infections were bacteria ($n=5$), adenovirus ($n=2$) and *Pneumocystis carinii* ($n=1$). The incidence of infections between day +7 and +21 was significantly higher in the HP group ($P<0.01$).

Hematopoietic recovery

Five patients (22%) in the HP group had graft failure, whereas one patient (2%) in the non-HP group had graft failure ($P=0.01$). Of these six patients, four had primary graft failure and two in the HP group had secondary graft failure. The median time to ANC 500/μL was 18 (range, 12–32) days in the HP group and 15 (range, 11–23) days in the non-HP group ($P<0.01$). Median time to platelet 50 000/μL was 31 (range, 18–214) days and 24.5 (range, 11–94) days, respectively ($P=0.04$), and the median time to reticulocyte 1% was 24.5 (range, 17–38) days and 20 (range, 13–38) days, respectively ($P<0.01$).

We next analyzed hematopoietic recovery after excluding 22 patients who had concomitant infections because the HP group included significantly more patients who had concomitant infections, and infections are known to interfere with effective and sustained reconstitution of hematopoiesis.[14] One (11%) out of nine patients in the HP group had graft failure, whereas one (3%) out of the 39 patients in the non-HP group had graft failure ($P=0.34$). The HP group was associated with significantly slower neutrophil, platelet and reticulocyte recovery than the non-HP group (18 days vs 14.5 days, $P<0.01$ for neutrophil recovery; 36 days vs 24 days, $P=0.01$ for platelet recovery; 24 days vs 19.5 days, $P=0.02$ for reticulocyte recovery). To eliminate the effect of PBSC use on hematopoietic recovery, we carried out subgroup analysis that included only those patients who received BMT. This subgroup analysis also showed that the HP group was associated with worse hematopoietic recovery than the non-HP group (data not shown).

Engraftment syndrome and GVHD

Among those patients who engrafted, there was no significant difference in the incidence of ES between the

Table 1 Patient characteristics

Characteristics	*HP group*	*Non-HP group*	P-*value*
No. of patients	23	47	
Median age, years (range)	49 (17–61)	41 (19–60)	0.33
Female, *n* (%)/male, *n* (%)	11 (48)/12 (52)	22 (47)/25 (53)	0.94
Donor/patient sex, *n* (%)			0.53
Femal/male	7 (30)	11 (23)	
Others	16 (70)	36 (77)	
Diagnosis, *n* (%)			0.25
Myeloid malignancy	12 (52)	25 (53)	
Lymphoid malignancy	6 (26)	18 (38)	
Benign hematologic disease	5 (22)	4 (9)	
Disease risk, *n* (%)			0.27
Standard	11 (48)	29 (62)	
High	12 (52)	18 (38)	
HCT-CI, *n* (%)			>0.99
<3	21 (91)	41 (87)	
> =3	2 (9)	6 (13)	
SC source, *n* (%)			0.35
BM	20 (87)	35 (74)	
PB	3 (13)	12 (26)	
Cell dose[a]			0.79
> =median	13 (57)	25 (53)	
<median	10 (43)	22 (47)	
Donor type, *n* (%)			0.68
Related	11 (48)	20 (43)	
Unrelated	12 (52)	27 (57)	
HLA disparity in GVH direction, *n* (%)			0.43
Match	14 (61)	33 (70)	
Mismatch	9 (39)	14 (30)	
HLA disparity in HVG direction, *n* (%)			0.68
Match	14 (61)	31 (66)	
Mismatch	9 (39)	16 (34)	
Conditioning regimen, *n* (%)			0.11
Myeloablative	10 (43)	30 (64)	
Reduced-intensity	13 (57)	17 (36)	
GVHD prophylaxis, *n* (%)			0.54
CsA + MTX	8 (35)	13 (28)	
FK + MTX	15 (65)	34 (72)	
ABO compatibility, *n* (%)			0.56
Match	11 (48)	26 (55)	
Mismatch	12 (52)	21 (45)	

Abbreviations: FK = tacrolimus; HCT-CI = hematopoietic cell transplantation-specific comorbidity index; HVG = host versus graft.
[a]The median nucleated cell dose was 2.83×10^8/kg (range, 1.21–5.03) in BM recipients, and the median CD34+ cell dose was 3.50×10^6/kg (range, 2.03–5.95) in PBSC recipients.

HP group and the non-HP group (20% in the HP group vs 20% in the non-HP group, $P>0.99$). In all, 10 patients (43%) in the HP group and 22 patients (47%) in the non-HP group developed aGVHD ($P=0.79$). Chronic GVHD developed in four of nine evaluable patients (44%) in the HP group, whereas it developed in 22 of 41 evaluable patients (54%) in the non-HP group ($P=0.72$).

OS, non-relapse mortality and relapse

When patients were divided into quartiles according to the total number of hemophagocytic cells, 20 patients were in the first quartile (no hemophagocytic cells), 18 were in the second quartile (1 to 2 hemophagocytic cells), 19 were in the third quartile (3 to 7 hemophagocytic cells) and 13 were in the fourth quartile (8 or more hemophagocytic cells). As the cut-off point of 5 or more hemophagocytic cells was used to define the HP group, the third quartile was further divided into two groups (3 to 4 and 5 to 7 hemophagocytic cells). A 2-year cumulative incidences of NRM for patients with 0 to 4 hemophagocytic cells were 18–33%, whereas those for patients with 5 or more hemophagocytic cells were as high as approximately 50% (Table 3).

OS rates for the HP group and non-HP group were 52 and 94% at day +100, and 30 and 65% at 2 years ($P<0.01$), respectively (Figure 3). Cumulative incidences of NRM for the HP group and non-HP group were 43 and 4% at day +100, and 48 and 27% at 2 years ($P<0.01$), respectively (Figure 4a). The cumulative incidences of relapse for the HP group and non-HP group were 22 and 13% at 2 years ($P=0.31$), respectively (Figure 4b).

Results of univariate and multivariate analysis of factors affecting post-transplantation outcomes are summarized in Table 4. Multivariate analysis showed that the prognostic factors for lower OS were HP group (hazard ratio (HR) = 2.3; 95% confidence interval (CI), 1.0–5.4; $P=0.048$), high-risk disease (HR = 3.8; 95% CI, 1.6–9.1; $P<0.01$), and presence of infections (HR = 3.2; 95% CI, 1.2–8.3; $P=0.02$). Similarly, the prognostic factors for higher NRM were HP group (HR = 4.0; 95% CI, 1.6–9.9; $P<0.01$), and patient age > = 50 years (HR = 4.5; 95% CI, 1.7–12; $P<0.01$). Furthermore, HP group was associated with an increased NRM at day +100 on multivariate analysis (HR = 11; 95% CI, 2.4–52; $P<0.01$). Multivariate analysis showed that the prognostic factors for higher relapse rates were HP group (HR = 3.6; 95% CI, 1.1–12; $P=0.04$), and high-risk disease (HR = 4.9; 95% CI, 1.0–23; $P=0.047$).

Causes of death

Of 23 patients in the HP group, 16 died. Their causes of death were relapse ($n=5$), graft failure ($n=4$), ES ($n=1$), posterior reversible encephalopathy syndrome ($n=1$), sinusoidal obstruction syndrome ($n=1$), intestinal TAM[21,22] ($n=2$), and infection ($n=2$). Of 47 patients in the non-HP group, 14 died. Their causes of death were relapse ($n=5$), interstitial pneumonia ($n=3$), infection ($n=2$), cryptogenic organizing pneumonia ($n=1$), chronic GVHD ($n=1$), intestinal TAM ($n=1$), and secondary cancer ($n=1$). The incidence of death due to graft failure was significantly higher in the HP group than in the non-HP group (17 vs 0%, $P<0.01$), and that of death due to endothelial complications (ie, ES, sinusoidal obstruction syndrome, intestinal TAM, and posterior reversible encephalopathy syndrome) was significantly higher in the HP group than in the non-HP group (22 vs 2%, $P=0.01$).

Discussion

This study demonstrated that activation of macrophages in the BM early in the post-transplant period was associated

Table 2 Features during day +7 to day +21

	HP group n = *23*	*Non-HP group* n = *47*	P-*value*
Fever (> = 38.3 °C for 3 consecutive days), *n* (%)	15 (65)	15 (32)	<0.01
Skin rash, *n* (%)	3 (13)	9 (19)	0.74
Diarrhea, *n* (%)[a]	6 (26)	8 (17)	0.37
Neurological symptoms, *n* (%)	3 (13)	0 (0)	0.03
Body weight gain (> = 5% of baseline), *n* (%)[b]	16 (73)	14 (30)	<0.01
Hypoxia (SpO2 < = 95%), *n* (%)	9 (39)	6 (13)	0.01
Total bilirubin > = 2 mg/dL, *n* (%)	17 (74)	5 (11)	<0.01
AST > = twice the UNL, *n* (%)	6 (26)	6 (13)	0.16
ALT > = twice the UNL, *n* (%)	12 (52)	22 (47)	0.67
LDH > = twice the UNL, *n* (%)	6 (26)	10 (21)	0.65
Creatinine > = twice the baseline, *n* (%)	5 (22)	1 (2)	0.01
CRP > = 10 mg/dL, *n* (%)	13 (57)	7 (15)	<0.01
Infection, *n* (%)	14 (61)	8 (17)	<0.01

Abbreviations: AST = aspartate aminotransferase; ALT = alanine aminotransferase; CRP = C-reactive protein; LDH = lactate dehydrogenase.
[a]Diarrhea, which is grade 3 or 4 according to the National Cancer Institute common toxicity criteria.
[b]Body weight of one patient in the HP group and one patient in the non-HP group was not evaluable because of poor performance status.

Table 3 Non-relapse mortality and OS according to the total number of hemophagocytic cells

Total no. of hemophagocytic cells	*2-year NRM*	*2-year OS*
0 (*n* = 20)	18	68
1–2 (*n* = 18)	30	64
3–4 (*n* = 9)	33	67
5–7 (*n* = 10)	50	20
> = 8 (*n* = 13)	46	38

Abbreviation: NRM = non-relapse mortality.

with impaired hematopoietic recovery, distinctive clinical and laboratory features, higher NRM rates and lower OS rates. The results of this study revealed that early macrophage activation is an important complication, which has a significant impact on outcomes of allo-HSCT.

In this study, 23 out of 70 patients (33%) were diagnosed as having hemophagocytosis. This suggests that early macrophage activation is a relatively common but unrecognized complication. Even if none of the 26 patients excluded from the analysis had hemophagocytosis, the incidence of hemophagocytosis would be still as high as 24% (23/96).

Factors known to influence hematopoietic recovery after allo-HSCT include intensity of conditioning, cell dose and HLA compatibility.[14] Although there were no significant differences in these factors between the HP group and the non-HP group, the incidence of graft failure was higher and hematopoietic recovery was slower in the HP group than in the non-HP group. The HP group was associated with slower hematopoietic recovery than the non-HP group when excluding from analysis those patients who had concomitant infections, which are known to interfere with reconstitution of hematopoiesis.[14] Furthermore, among those patients who received BM as a SC source, the HP group had inferior hematopoietic recovery to the non-HP

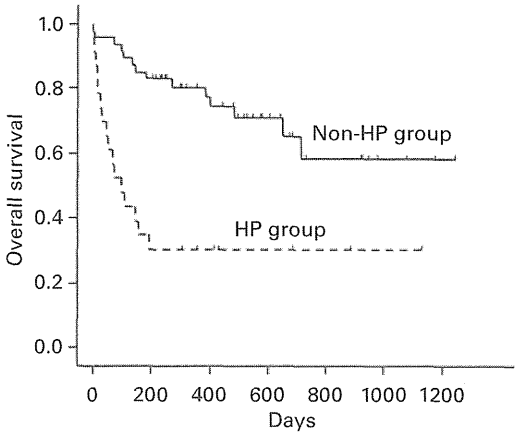

Figure 3 Probabilities of OS were significantly lower in the HP group than in the non-HP group (HP group: 52% at day +100 and 30% at 2 years; non-HP group: 94% at day +100 and 65% at 2 years; $P<0.01$).

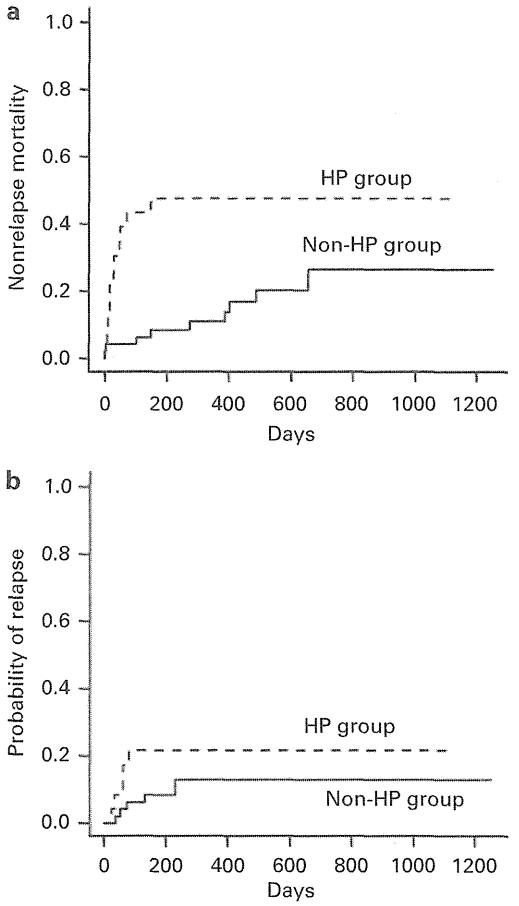

Figure 4 (**a**) Cumulative incidences of NRM were significantly higher in the HP group than in the non-HP group (HP group: 43% at day +100 and 48% at 2 years; non-HP group: 4% at day +100 and 27% at 2 years; $P<0.01$). (**b**) Cumulative incidences of relapse did not differ significantly between the HP group and the non-HP group (HP group: 22% at 2 years; non-HP group: 13% at 2 years; $P=0.31$).

group. Thus, the HP group is an independent factor affecting hematopoietic recovery.

Analysis of clinical features demonstrated that fever, neurological symptoms, weight gain, hypoxia, elevated

Table 4 Factors affecting (a) overall mortality, (b) non-relapse mortality and (c) relapse

Variables	*Adverse factors*	*Univariate*		*Multivariate*	
		HR (95% CI)	P-*value*	*HR (95% CI)*	P-*value*
(a)					
Hemophagocytosis	HP group	3.9 (1.9–8.2)	<0.01	2.3 (1.0–5.4)	0.048
Patients age	> = 50 years	2.4 (1.2–5.0)	0.02		
Disease risk	High	2.8 (1.3–5.8)	<0.01	3.8 (1.6–9.1)	<0.01
HCT-CI	> = 3	0.43 (0.10–1.8)	0.25		
HLA disparity in GVH direction	Mismatch	1.7 (0.80–3.4)	0.18		
Donor type	Related	1.9 (0.92–3.9)	0.08		
Donor/patient sex	Femal/male	2.1 (0.97–4.4)	0.06		
Conditioning regimen	RIC	2.2 (1.1–4.6)	0.03		
SC source	PB	1.8 (0.80–3.9)	0.16		
GVHD prophylaxis	FK + MTX	0.59 (0.28–1.2)	0.15		
Infections during day +7 ± 21	Yes	2.7 (1.3–5.6)	<0.01	3.2 (1.2–8.3)	0.02
(b)					
Hemophagocytosis	HP group	4.1 (1.7–10)	<0.01	4.0 (1.6–9.9)	<0.01
Patients age	> = 50 years	4.6 (1.8–12)	<0.01	4.5 (1.7–12)	<0.01
Disease risk	High	1.9 (0.80–4.7)	0.14		
HCT-CI	> = 3	0.66 (0.15–2.9)	0.58		
HLA disparity in GVH direction	Mismatch	1.6 (0.64–3.8)	0.33		
Donor type	Related	1.8 (0.73–4.3)	0.21		
Donor/patient sex	Femal/male	2.4 (0.98–6.0)	0.06		
Conditioning regimen	RIC	3.0 (1.2–7.4)	0.02		
SC source	PB	1.4 (0.50–3.9)	0.52		
GVHD prophylaxis	FK + MTX	0.48 (0.20–1.2)	0.10		
Infections during day +7 ± 21	Yes	4.0 (1.6–9.9)	<0.01		
(c)					
Hemophagocytosis	HP group	5.1 (1.5–17)	<0.01	3.6 (1.1–12)	0.04
Patients age	> = 50 years	0.41 (0.09–1.9)	0.26		
Disease risk	High	6.2 (1.3–29)	0.02	4.9 (1.0–23)	0.047
HCT-CI	> = 3	UE	0.23[a]		
HLA disparity in GVH direction	Mismatch	1.8 (0.51–6.0)	0.37		
Donor type	Related	2.0 (0.62–6.7)	0.24		
Donor/patient sex	Femal/male	1.3 (0.33–4.7)	0.74		
Conditioning regimen	RIC	1.5 (0.45–4.8)	0.53		
SC source	PB	2.4 (0.69–8.1)	0.17		
GVHD prophylaxis	FK + MTX	1.3 (0.34–4.9)	0.70		
Infections during day +7 ± 21	Yes	1.3 (0.28–6.1)	0.73		

Abbreviation: CI = confidence interval; FK = tacrolimus; HR = hazard ratio; HCT-CI = hematopoietic cell transplantation-specific comorbidity index; RIC = reduced-intensity conditioning; UE = unevaluable.
[a]None of the six patients with HCT-CI > = 3 relapsed ($P = 0.23$; log-rank test).

total bilirubin and elevated serum creatinine were more frequently observed among the HP group than the non-HP group. These abnormalities are similar to those observed in ES. Furthermore, macrophage activation, ES and aGVHD share the common feature of being associated with elevated levels of pro-inflammatory cytokines.[2,3,5] Therefore, we speculated that there might be an overlap between early macrophage activation following allo-HSCT, ES and aGVHD, but there were no statistically significant differences in the incidence of ES and that of aGVHD between the HP group and the non-HP group. These results suggest that early macrophage activation was rather an independent complication from ES and aGVHD, although we could not draw a definite conclusion because of insufficient statistical power of this analysis.

The HP group had significantly higher NRM rates, resulting in significantly worse OS compared with the non-HP group. Although the incidence of concomitant infections was significantly higher in the HP group over the non-HP group, multivariate analysis demonstrated that the HP group and concomitant infections were independent risk factors for OS. Therefore, early macrophage activation seems to be an independent complication affecting transplant outcome. Of note, the incidence of death due to graft failure and endothelial complications was significantly higher in the HP group than in the non-HP group. Elevated levels of pro-inflammatory cytokines associated with activated macrophages such as TNF-α and macrophage inflammatory protein-1α might have contributed to the development of graft failure and exacerbation of endothelial complications, resulting in higher NRM rates in the HP group.[6,7,10,26,27] That C-reactive protein values were found to be higher in the HP group than in the non-HP group suggested that early macrophage activation was associated with a hyperinflammatory state. Future studies to measure the serum cytokine levels are warranted.

The results of our study suggest that early identification of patients at high risk of NRM might be possible by simply performing BM aspiration on day $+14\pm7$. This may have important implications for future therapeutic strategies because we could potentially lower the NRM rates by administering macrophage-targeted therapies in those patients who have an increased number of hemophagocytic cells in BM on day $+14\pm7$. The potential therapeutic options targeting macrophages could include anti-TNFα agents, etoposide or liposomal corticosteroids.[28–31] As we cannot rule out the possibility that early macrophage activation is the result rather than the cause of inflammatory processes, prospective trials are warranted to examine whether macrophage-targeted therapies for early macrophage activation can lower NRM rates.

Unexpectedly, the HP group was a risk factor for relapse in multivariate Cox regression analysis. However, this result should be interpreted with caution because of the relatively small sample size of our study. The impact of macrophage activation on relapse needs to be confirmed by larger studies.

Many types of cells decrease conspicuously in number following conditioning therapy, whereas macrophages do not.[32] Accordingly, the 'proportion' of hemophagocytic cells is likely to increase in hypocellular BM, but the 'absolute number' of hemophagocytic cells counted in BM clot sections would be little affected by the cellularity of BM. Additionally, it is difficult to distinguish between hemophagocytic cells and macrophages covered with other cells in BM smears, whereas overlapping of cells is virtually negligible in clot sections because clot sections are very thin (2 μm). For these reasons, we counted the absolute number of hemophagocytic cells in BM clot sections in this study.

We tested CD163 immunostaining in an effort to increase objectivity. CD163 is a specific marker for cells of the monocyte/macrophage lineage.[33] There was a good, but not perfect, correlation between the total number of $CD163^+$ macrophages and that of hemophagocytic cells identified by hematoxylin-eosin staining (Spearman $r=0.70$, $P<0.01$), which could be explained by the fact that hemophagocytic macrophages identified by hematoxylin-eosin staining are a sub-population of $CD163^+$ macrophages.[34–36] Although an increased number of $CD163^+$ macrophages was not a statistically significant factor for NRM (adjusted HR 2.1; 95% CI, 0.85–5.0; $P=0.11$), limited statistical power precluded us from excluding a clinically meaningful effect of it. Further studies are warranted.

In conclusion, the activation of macrophages in the BM early in the post-transplantation period is a relatively common but unrecognized complication with a negative impact on outcomes of allo-HSCT. The results of our study indicate the clinical usefulness of BM examination during the early post-transplantation period for the prediction of outcome.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This study was supported by a Ministry of Health, Labor and Welfare of Japan Grant-in-Aid (KM).

References

1 Parkin J, Cohen B. An overview of the immune system. *Lancet* 2001; **357**: 1777–1789.
2 Dale DC, Boxer L, Liles WC. The phagocytes: neutrophils and monocytes. *Blood* 2008; **112**: 935–945.
3 Ferrara JL, Levy R, Chao NJ. Pathophysiologic mechanisms of acute graft-vs-host disease. *Biol Blood Marrow Transplant* 1999; **5**: 347–356.
4 Kumar S, DeLeve LD, Kamath PS, Tefferi A. Hepatic veno-occlusive disease (sinusoidal obstruction syndrome) after hematopoietic stem cell transplantation. *Mayo Clin Proc* 2003; **78**: 589–598.
5 Spitzer TR. Engraftment syndrome following hematopoietic stem cell transplantation. *Bone Marrow Transplant* 2001; **27**: 893–898.
6 Holler E, Kolb HJ, Moller A, Kempeni J, Liesenfeld S, Pechumer H *et al.* Increased serum levels of tumor necrosis factor alpha precede major complications of bone marrow transplantation. *Blood* 1990; **75**: 1011–1016.
7 Schots R, Kaufman L, Van Riet I, Ben Othman T, De Waele M, Van Camp B *et al.* Proinflammatory cytokines and their role in the development of major transplant-related complications in the early phase after allogeneic bone marrow transplantation. *Leukemia* 2003; **17**: 1150–1156.
8 Abe Y, Choi I, Hara K, Matsushima T, Nishimura J, Inaba S *et al.* Hemophagocytic syndrome: a rare complication of allogeneic nonmyeloablative hematopoietic stem cell transplantation. *Bone Marrow Transplant* 2002; **29**: 799–801.
9 Nishiwaki S, Terakura S, Ito M, Goto T, Seto A, Watanabe K *et al.* Impact of macrophage infiltration of skin lesions on survival after allogeneic stem cell transplantation: a clue to refractory graft-versus-host disease. *Blood* 2009; **114**: 3113–3116.
10 Menten P, Wuyts A, Van Damme J. Macrophage inflammatory protein-1. *Cytokine Growth Factor Rev* 2002; **13**: 455–481.
11 Caligiuri MA. Human natural killer cells. *Blood* 2008; **112**: 461–469.
12 Borish LC, Steinke JW. Cytokines and chemokines. *J Allergy Clin Immunol* 2003; **111**(2 Suppl): S460–S475.
13 Morishima Y, Sasazuki T, Inoko H, Juji T, Akaza T, Yamamoto K *et al.* The clinical significance of human leukocyte antigen (HLA) allele compatibility in patients receiving a marrow transplant from serologically HLA-A, HLA-B, and HLA-DR matched unrelated donors. *Blood* 2002; **99**: 4200–4206.
14 Rondon G, Saliba RM, Khouri I, Giralt S, Chan K, Jabbour E *et al.* Long-term follow-up of patients who experienced graft failure postallogeneic progenitor cell transplantation. Results of a single institution analysis. *Biol Blood Marrow Transplant* 2008; **14**: 859–866.
15 Gorak E, Geller N, Srinivasan R, Espinoza-Delgado I, Donohue T, Barrett AJ *et al.* Engraftment syndrome after nonmyeloablative allogeneic hematopoietic stem cell transplantation: incidence and effects on survival. *Biol Blood Marrow Transplant* 2005; **11**: 542–550.
16 Schmid I, Stachel D, Pagel P, Albert MH. Incidence, predisposing factors, and outcome of engraftment syndrome in pediatric allogeneic stem cell transplant recipients. *Biol Blood Marrow Transplant* 2008; **14**: 438–444.

17 Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J *et al.* 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant* 1995; **15**: 825–828.
18 Sullivan KM, Agura E, Anasetti C, Appelbaum F, Badger C, Bearman S *et al.* Chronic graft-versus-host disease and other late complications of bone marrow transplantation. *Semin Hematol* 1991; **28**: 250–259.
19 Gooley TA, Leisenring W, Crowley J, Storer BE. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. *Stat Med* 1999; **18**: 695–706.
20 Sorror ML, Maris MB, Storb R, Baron F, Sandmaier BM, Maloney DG *et al.* Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. *Blood* 2005; **106**: 2912–2919.
21 Nishida T, Hamaguchi M, Hirabayashi N, Haneda M, Terakura S, Atsuta Y *et al.* Intestinal thrombotic microangiopathy after allogeneic bone marrow transplantation: a clinical imitator of acute enteric graft-versus-host disease. *Bone Marrow Transplant* 2004; **33**: 1143–1150.
22 Inamoto Y, Ito M, Suzuki R, Nishida T, Iida H, Kohno A *et al.* Clinicopathological manifestations and treatment of intestinal transplant-associated microangiopathy. *Bone Marrow Transplant* 2009; **44**: 43–49.
23 Woywodt A, Haubitz M, Buchholz S, Hertenstein B. Counting the cost: markers of endothelial damage in hematopoietic stem cell transplantation. *Bone Marrow Transplant* 2004; **34**: 1015–1023.
24 Reece DE, Frei-Lahr DA, Shepherd JD, Dorovini-Zis K, Gascoyne RD, Graeb DA *et al.* Neurologic complications in allogeneic bone marrow transplant patients receiving cyclosporin. *Bone Marrow Transplant* 1991; **8**: 393–401.
25 Hinchey J, Chaves C, Appignani B, Breen J, Pao L, Wang A *et al.* A reversible posterior leukoencephalopathy syndrome. *N Engl J Med* 1996; **334**: 494–500.
26 Parker AN, Pragnell IB. Inhibitors of haemopoiesis and their potential clinical relevance. *Blood Rev* 1995; **9**: 226–233.
27 Janka GE. Hemophagocytic syndromes. *Blood Rev* 2007; **21**: 245–253.
28 Couriel D, Saliba R, Hicks K, Ippoliti C, de Lima M, Hosing C *et al.* Tumor necrosis factor-alpha blockade for the treatment of acute GVHD. *Blood* 2004; **104**: 649–654.
29 Koyama M, Sawada A, Yasui M, Inoue M, Kawa K. Encouraging results of low-dose etoposide in the treatment of early-onset hemophagocytic syndrome following allogeneic hematopoietic stem cell transplantation. *Int J Hematol* 2007; **86**: 466–467.
30 Frankenberger M, Haussinger K, Ziegler-Heitbrock L. Liposomal methylprednisolone differentially regulates the expression of TNF and IL-10 in human alveolar macrophages. *Int Immunopharmacol* 2005; **5**: 289–299.
31 Ahsan F, Rivas IP, Khan MA, Torres Suarez AI. Targeting to macrophages: role of physicochemical properties of particulate carriers–liposomes and microspheres–on the phagocytosis by macrophages. *J Control Release* 2002; **79**: 29–40.
32 Thiele J, Kvasnicka HM, Beelen DW, Wenzel P, Koepke ML, Leder LD *et al.* Macrophages and their subpopulations following allogenic bone marrow transplantation for chronic myeloid leukaemia. *Virchows Arch* 2000; **437**: 160–166.
33 Lau SK, Chu PG, Weiss LM. CD163: a specific marker of macrophages in paraffin-embedded tissue samples. *Am J Clin Pathol* 2004; **122**: 794–801.
34 Avcin T, Tse SM, Schneider R, Ngan B, Silverman ED. Macrophage activation syndrome as the presenting manifestation of rheumatic diseases in childhood. *J Pediatr* 2006; **148**: 683–686.
35 Grom AA, Mellins ED. Macrophage activation syndrome: advances towards understanding pathogenesis. *Curr Opin Rheumatol* 2010; **22**: 561–566.
36 Van Gorp H, Delputte PL, Nauwynck HJ. Scavenger receptor CD163, a Jack-of-all-trades and potential target for cell-directed therapy. *Mol Immunol* 2010; **47**: 1650–1660.

Int J Hematol (2011) 93:375–382
DOI 10.1007/s12185-011-0809-8

ORIGINAL ARTICLE

Incidence, risk factors and outcomes of bronchiolitis obliterans after allogeneic stem cell transplantation

Chiaki Nakaseko · Shinichi Ozawa · Emiko Sakaida · Miwa Sakai · Yoshinobu Kanda · Kumi Oshima · Mineo Kurokawa · Satoshi Takahashi · Jun Ooi · Takayuki Shimizu · Akira Yokota · Fumiaki Yoshiba · Katsumichi Fujimaki · Heiwa Kanamori · Rika Sakai · Takayuki Saitoh · Tohru Sakura · Atsuo Maruta · Hisashi Sakamaki · Shinichiro Okamoto

Received: 27 June 2010 / Revised: 15 November 2010 / Accepted: 15 December 2010

Abstract Bronchiolitis obliterans (BO) after allogeneic stem cell transplantation (allo-SCT) is a late-onset, life-threatening respiratory complication that significantly reduces a patient's quality of life. We retrospectively analysed the incidence of and risk factors for BO in allo-SCT recipients. In 2087 patients who underwent allo-SCT between January 1994 and June 2005 and survived >90 days after transplantation, 57 patients developed BO with a 5-year cumulative incidence of 2.8%. The median time interval from transplantation to BO diagnosis was 335 days (range 83–907 days). The 5-year cumulative incidence of BO was 1.62% in bone marrow transplantation (BMT) from related donors, 3.83% in peripheral blood stem cell transplantation (PBSCT) from related donors (R-PBSCT), 2.91% in BMT from unrelated donors and 2.65% in unrelated cord blood

For the Kanto Study Group for Cell Therapy (KSGCT).

C. Nakaseko (✉) · S. Ozawa · E. Sakaida
Department of Hematology,
Chiba University Graduate School of Medicine,
1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan
e-mail: chiaki-nakaseko@faculty.chiba-u.jp

M. Sakai · H. Sakamaki
Department of Hematology,
Tokyo Metropolitan Komagome Hospital,
Tokyo, Japan

Y. Kanda · K. Oshima
Division of Hematology, Saitama Medical Center,
Jichi Medical University, Saitama, Japan

M. Kurokawa
Department of Hematology and Oncology,
The University of Tokyo, Tokyo, Japan

S. Takahashi · J. Ooi
Department of Hematology and Oncology,
The Institute of Medical Science,
The University of Tokyo, Tokyo, Japan

T. Shimizu · S. Okamoto
Division of Hematology, Department of Medicine,
Keio University School of Medicine, Tokyo, Japan

A. Yokota
Department of Internal Medicine,
Chiba Aoba Municipal Hospital, Chiba, Japan

F. Yoshiba
Division of Hematology and Oncology,
Department of Medicine, Tokai University School of Medicine,
Isehara, Japan

K. Fujimaki
Department of Internal Medicine and Clinical Immunology,
Yokohama City University, Graduate School of Medicine,
Yokohama, Japan

H. Kanamori · R. Sakai · A. Maruta
Department of Hematology, Kanagawa Cancer Center,
Yokohama, Japan

R. Sakai
Department of Hematology,
Yokohama City University Medical Center,
Yokohama, Japan

T. Saitoh
Department of Medicine and Clinical Science,
Gunma University Graduate School of Medicine,
Maebashi, Japan

T. Sakura
Division of Hematology, Saiseikai Maebashi Hospital,
Maebashi, Japan

transplantation. The incidence of BO after R-PBSCT was significantly higher than that after any other type of allo-SCT ($p = 0.02$). R-PBSCT ($p = 0.019$) and preceding chronic graft-versus-host disease (GVHD) ($p < 0.001$) were BO-associated risk factors. Overall 5-year survival of patients with BO from the time of diagnosis was 45.4%, significantly less than those without (77.5% from day 335, $p < 0.001$). R-PBSCT recipients with existent chronic GVHD have a high risk of developing BO, and need extensive care and repeated pulmonary function tests.

Keywords Bronchiolitis obliterans · Chronic GVHD · Late onset non-infectious pulmonary complications · PBSC

1 Introduction

Allogeneic stem cell transplantation (allo-SCT) has been established as a therapeutic approach for haematological malignancies, aplastic anaemia and rare immunodeficiency disorders. Half of the patients who undergo allo-SCT achieve long-term disease-free survival, but a similar number develop significant transplant-related complications. Both infectious and non-infectious pulmonary complications occur in 40–60% of allo-SCT recipients, which significantly affect prognosis as well as cause 10–40% of transplant-related death and decrease in the quality of life (QOL) [1]. Detection of cytomegalovirus (CMV) antigenemia or quantitative PCR and pre-emptive treatment with ganciclovir have been used widely to decrease the incidence of severe pneumonia in these recipients. However, the pathogenesis, prevention and treatment of non-infectious pulmonary complications remain unclear, which severely affects a patient's QOL and survival.

Late-onset non-infectious pulmonary complications (LONIPC) occurring beyond 90 days after allo-SCT include bronchiolitis obliterans (BO), bronchiolitis obliterans organizing pneumonia (BOOP) and interstitial pneumonia [2–5]. BO after allo-SCT was first described by Roca et al. [6] in a patient with chronic graft-versus-host disease (GVHD). The incidence of BO varies widely from 1.7 to 26% in different reports, in part, due to lack of a standard definition [7–12].

Despite different therapeutic protocols, BO mortality remains high and most patients die of respiratory failure or subsequent infections. In recent practice, different stem cell sources and conditioning regimens for allo-SCT have become more varied, but their influence on the incidence of BO is not established. In this situation, the risk factors for developing BO might be different among different hematopoietic stem cell transplantation (HSCT) recipient groups. Here, we retrospectively analysed the incidence of and risk factors for BO in allo-SCT protocols.

2 Materials and methods

2.1 Patient characteristics

Between January 1994 and June 2005, 2087 adult patients who underwent allo-SCT in 13 facilities of the Kanto Study Group for Cell Therapy (KSGCT) and survived at least 90 days post-transplantation were retrospectively evaluated using the KSGCT patient database and chart review. Patient characteristics are summarized in Table 1. The median age at transplantation was 38 years (range 15–66 years). The conditioning regimen consisted of a TBI-based regimen in 67.3% patients and a non-TBI regimen in 32.7%, a full-dose busulfan (BU) regimen in 21.2% and non-full dose BU regimen in 78.8%, respectively. Conventional stem cell transplantation (CST) using a myeloablative conditioning regimen was performed in 88.3% patients and reduced-intensity conditioning stem cell transplantation (RIST) in 11.7%. Donor sources included bone marrow from related donors (R-BMT, $n = 653$, 31.3%), peripheral blood from related donors (R-PBSCT, $n = 444$, 21.3%), BMT from unrelated donors (UR-BMT, $n = 793$, 38.0%) through the Japan Marrow Donor Program (JMDP) and unrelated cord blood transplantation (CBT, $n = 197$, 9.4%). No PBSCT from unrelated donors (UR-PBSCT) was performed through JMDP. Diagnosis and grading of chronic GVHD were made by classical Seattle criteria of limited and extensive. NIH consensus criteria [13] were not used.

2.2 Diagnosis of BO

The clinical diagnosis of BO was made by pulmonary function tests (PFTs) revealing a forced expiratory volume for 1 s (FEV1) <75% of the predicted value and an FEV1/forced vital capacity (FEV1/FVC) of less than 70%, along with typical changes on high-resolution computed tomography [13]. These include decreased attenuation of lung parenchyma, expiratory air trapping and sub-segmental or segmental bronchial dilatation [14, 15]. Bronchoscopy and bronchoalveolar lavage were performed to exclude infections. A pathological diagnosis was made by transbronchial lung biopsy (TBLB) or video-assisted thoracic surgery (VATS) in some patients. Clinical improvement (response) was defined by a 10% improvement in FEV1 and sustained reduction in shortness of breath and cough.

2.3 Statistical analysis

To evaluate potential risk factors for developing BO, the time-dependent Cox proportional hazard regression model was used for univariate and multivariate analyses [16]. Factors identified as significant ($p < 0.05$) in the univariate

Table 1 Patient characteristics

Clinical characteristics	No. of patients (>90 days) (n = 2087)	%	No. of patients with BO (n = 57)
Median age at SCT (range)	37 (15–66)		38 (17–61)
Sex			
Male	1271	60.9	28
Female	816	39.1	29
Diagnosis			
AML	683	32.7	22
ALL	430	20.6	10
CML	391	18.7	7
MDS	264	12.6	9
ML	152	7.3	7
MM	44	2.1	1
SAA	88	4.2	0
Others	35	1.7	1
Stem cell source			
R-BMT	653	31.3	13
R-PBSCT	444	21.3	16
UR-BMT	793	38	23
CBT	197	9.4	5
TBI			
Yes	1395	67.3	38
No	677	32.7	19
Full dose BU			
Yes	441	21.2	14
No	1637	78.8	43
Conditioning			
CST	1843	88.3	51
RIST	244	11.7	6
GVHD prophylaxis			
CsA	1595	79.9	48
FK506	401	20.1	9
Acute GVHD			
0	581	30	13
I	501	25.8	15
II	612	31.5	25
III	162	8.3	2
IV	86	4.4	2
Chronic GVHD			
(−)	926	45.7	3
Limited	396	19.5	7
Extensive	705	34.8	47

BO bronchiolitis obliterans, *SCT* stem cell transplantation, *AML* acute myelogenous leukaemia, *ALL* acute lymphoblastic leukaemia, *CML* chronic myeloid leukaemia, *MDS* myelodysplastic syndrome, *ML* malignant lymphoma, *MM* multiple myeloma, *SAA* severe aplastic anaemia, *R* related, *BMT* bone marrow transplantation, *PBSCT* peripheral blood stem cell transplantation, *UR* unrelated, *CBT* cord blood transplantation, *TBI* total body irradiation, *BU* busulfan, *CST* conventional stem cell transplantation, *RIST* reduced-intensity conditioning stem cell transplantation, *GVHD* graft-versus-host disease, *CsA* cyclosporine, *FK506* tacrolimus

analysis were included in the multivariate analysis. Factors that remained significant were retained in the final model. Factors that correlated with each other were not entered simultaneously into the model.

Patients were also analysed for overall survival (OS) and relapse. Semi-landmark plots were constructed to illustrate the effect of BO on survival. In patients who developed BO, the post-transplant day of BO development was defined as the landmark day; in those who did not develop BO, post-transplant day 335, which is the median day of BO occurrence as per the Kaplan–Meier estimate for the median time interval from transplantation to BO diagnosis, was defined as the landmark day. OS was calculated from the landmark day to death from any cause or the date of last

contact. Relapse was defined based on the respective malignancy, and its cumulative incidence was plotted as a function of time since the landmark day.

Survival analyses were performed by the Kaplan–Meier method [17] and the log-rank test was used for univariate comparisons. The cumulative BO incidence was calculated using the Gray method considering death without BO as the competing risk [18]. For most statistical analyses, the SPSS software version 11 (SPSS Inc., Chicago, IL, USA) was used. The cumulative incidence analyses were conducted using the R statistical software 2.1.0 (the R Foundation for Statistical Computing, Vienna, Austria; available at http://www.r-project.org) 'cmprsk' package. All p values were two-sided and differences were considered statistically significant when a p value was <0.05. Differences with p values >0.10 are reported as not significant, whereas those with p values between 0.05 and 0.1 are reported in detail.

3 Results

3.1 Cumulative incidence of BO

Fifty-seven patients met the diagnostic criteria for BO with a 5-year cumulative incidence of 2.8% post-transplantation (Fig. 1). The median time interval from transplantation to BO diagnosis was day 335 (range 83–907 days). The 5-year cumulative incidence of BO was 1.62% (12/653) for R-BMT, 3.83% (16/444) for R-PBSCT, 2.91% (24/793) for UR-BMT and 2.65% (5/197) for unrelated CBT (Fig. 2). The incidence of BO after R-PBSCT was significantly higher than that after any other type of allo-SCT ($p = 0.02$). As for conditioning intensity, there was no significant difference in the cumulative incidence of BO between CST and RIST recipients (2.80% in CST vs. 2.93% in RIST, $p = 0.996$). In 244 patients who underwent RIST, 6 patients developed BO (1 in RBMT, 3 in R-PBSCT, 1 in UR-BMT) without any significant difference. In R-PBSCT recipients, the cumulative incidence of BO in CST was higher than in RIST but there was no significant difference (4.39% in CST vs. 2.55% in RIST, $p = 0.407$).

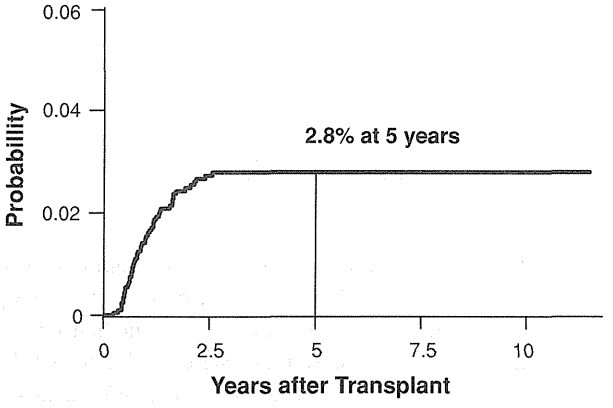

Fig. 1 Cumulative incidence of bronchiolitis obliterans (BO) in all patients

3.2 Bronchoscopy and PFTs

Bronchoscopy was performed in 15 patients (26.3%) and TBLB was performed in 14 (24.6%). However, no histological diagnosis of BO was made with TBLB. Only one patient (1.8%) had histological confirmation of BO with a lung biopsy by VATS. The remaining patients were diagnosed based on PFT results and a CT scan.

At the time of BO diagnosis, the mean FEV1/FVC value decreased from 82.2 ± 9.0% pre-transplantation to 52.1 ± 14% (Fig. 3). The mean FEV1/VC-predicted value decreased from 84.7 ± 12 to 37 ± 14%. The worst FEV1/FVC data and FEV1/VC predicted after BO diagnosis

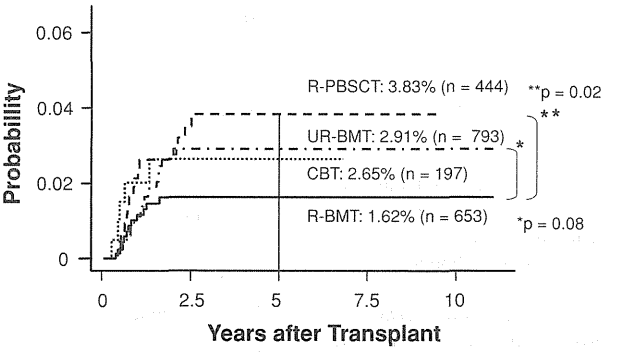

Fig. 2 Cumulative incidence of BO according to stem cell source

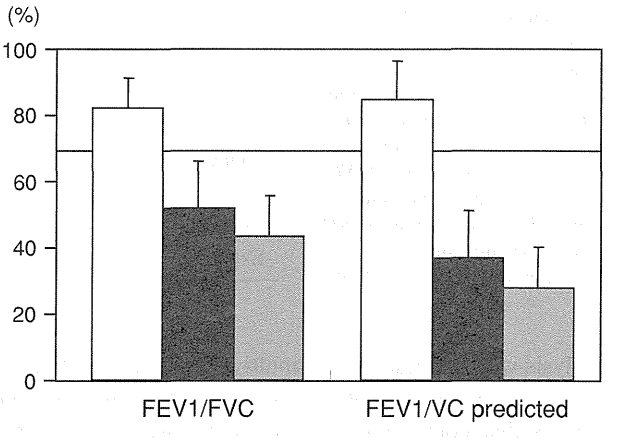

Fig. 3 Forced expiratory volume for 1 s (FEV1)/forced vital capacity (FVC) and FEV1/VC predicted by pulmonary function tests at pre-transplantation (*white bar*, $n = 52$), at diagnosis of BO (*black bar*, $n = 57$) and at the time worst results are obtained in the clinical course (*grey shaded bar*, $n = 34$)

during the clinical course were 43.5 ± 12 and 28.0 ± 12%, respectively.

3.3 GVHD and risk factors for developing BO

The patients with BO had a higher incidence of acute GVHD (0: $n = 13$, I: $n = 15$, II: $n = 25$, III–IV: $n = 4$) (Table 1), but univariate analysis revealed that acute GVHD was not a significant risk factor for developing BO (0 vs. I–IV: $p = 0.099$ [HR = 1.76, 95% CI: 0.90–3.42] 0–I vs. II–IV: $p = 0.146$ [HR = 1.50, 95% CI: 0.87–2.63]; Table 2). Of 57 BO patients, 54 (94.7%) were already diagnosed with chronic GVHD (limited 18%, extensive 82%) before the onset of BO and their median onset of chronic GVHD post-transplantation was 144 days (range 55–471), which was 173 days earlier than the development of BO. One of the 3 patients without chronic GVHD at the diagnosis of BO developed chronic GVHD symptoms other than BO at 53 days after the diagnosis of BO. The onset of chronic GVHD was de novo type in 12 patients, quiescent type in 33 patients, and progressive type in 8 patients and there was no significant difference in the type of chronic GVHD onset.

Risk factors for BO were R-PBSCT (compared to R-BMT, $p = 0.019$) and preceding chronic GVHD (time-dependent covariate, $p < 0.001$) (Table 2). These two factors were also significant in the multivariate analysis. Patient age, sex, TBI, full-dose BU and GVHD prophylaxis were not significant risk factors for developing BO. Also, CST was not a significant risk factor compared to RIST.

3.4 Treatment, outcome, cause of death and relationship with relapse

All patients received immunosuppressive therapy but only eight patients showed improvement in BO, whereas 60.9% had progressive respiratory failure (Table 3). Home oxygen therapy was introduced to 15 patients. Twenty-eight patients died after developing BO of whom 17 died of respiratory failure. Three patients died of disseminated aspergillosis. Among 22 patients whose FEV1/FVC was less than 50% at the diagnosis of BO, 14 patients died after developing BO. However, 13 out of 29 patients whose FEV1/FVC more than 50% died and there was no significant difference in survival between the two groups (2-year survival rate after developing BO; 52.0% in patients with

Table 2 Univariate analysis of risk factors for developing BO

Factor	*n*	*p*	HR (95% CI)
Age			
<20 versus ≥20	3/189 versus 54/1965	0.331	1.78 (0.56–5.71)
<30 versus ≥30	14/691 versus 43/1463	0.149	1.59 (0.85–2.96)
Sex			
Male versus female	28/1271 versus 29/816	0.168	1.46 (0.85–2.50)
Primary disease			
Non-malignancy versus malignancy	0/88 versus 57/1999	0.261	1.68 (0.63–4.28)
Conditioning			
Non-TBI versus TBI	19/673 versus 38/1430	0.498	1.23 (0.68–2.24)
Full dose BU versus non-full dose BU	14/441 versus 43/1637	0.193	0.89 (0.75–1.06)
Stem cell source			
R-BMT	13/653	–	1.0
R-PBSCT	16/444	0.019	2.54 (1.16–5.51)
UR-BMT	23/793	0.083	1.90 (0.92–3.92)
CBT	5/197	0.258	1.84 (0.64–5.30)
GVHD prophylaxis			
CsA versus FK506	48/1595 versus 9/401	0.501	0.77 (0.36–1.65)
Conditioning			
CST versus RIST	51/1882 versus 6/220	0.594	1.26 (0.54–2.95)
Acute GVHD			
0 versus I–IV	13/581 versus 44/1309	0.085	1.80 (0.92–3.50)
0–I versus II–IV	28/1082 versus 29/807	0.148	1.50 (0.87–2.60)
Preceding chronic GVHD (time-dependent covariate)			
Present versus absent	54/666 versus 3/930	<0.001	17.1 (4.15–70.4)

FEV1/FVC ≥50 vs. 50.3% in patients with FEV1/FVC <50%, $p = 0.85$). However, there was a significant difference in survival between patients with FEV1/VC predicted ≥40 and <40% (69.7 vs. 41.3%, $p = 0.046$).

Primary disease relapsed in eight patients, of which it relapsed in six before developing BO. Only two patients had relapsed disease after developing BO, with the cumulative relapse rate after developing BO of 4.6%. Among the 8 patients, 6 patients died but only one patient died of relapsed disease and the remaining 5 patients died of respiratory failure or infections. Non-relapse mortality in patients with BO was 49.8%. The 5-year OS of patients with BO post-transplantation was lower than that without BO, but the difference was not significant (45.2 vs. 59.5%, $p = 0.34$) (Fig. 4a). However, the 5-year OS of patients with BO from the time of diagnosis was 45.4%, which was significantly less than that of patients without BO (77.6% from day 335, $p < 0.001$) by semi-landmark analysis (Fig. 4b).

Table 3 Outcomes of BO

	n (%)
Outcomes of BO	
Improved	8 (16.7%)
No change	10 (21.7%)
Progressed	28 (60.9%)
Relapse of primary disease	
(−)	49 (86.0%)
(+)	8 (14.0%)
Before developing BO	6 (10.5%)
After developing BO	2 (3.6%)
Cause of death	$n = 28$
Respiratory failure	17 (60.7%)
Aspergillosis	3 (10.7%)
Sepsis	2 (7.2%)
Relapse	1 (3.6%)
Interstitial pneumonia	1 (3.6%)
Other causes	4 (14.3%)

4 Discussion

In this study, the 5-year cumulative incidence of BO was 2.8%, and the incidence of BO in patients with R-PBSCT was significantly higher than that in UR-BMT, CBT or R-BMT patients. The International Bone Marrow Transplantation Registry (IBMTR) reported the incidence and risk factors for BO in 6275 adults leukaemia patients who underwent BMT or PBSCT from HLA-identical sibling donors [8]. In the report, the 2-year cumulative incidence of BO was 1.7% and the median time to onset of BO was 431 days. In a multivariate analysis, the factors associated with an increased risk for BO included: R-PBSCT, a busulfan-based conditioning regimen, ≥14-month interval from diagnosis to transplantation, female donor to male recipient sex match, prior interstitial pneumonia and an episode of moderate-to-severe acute GVHD. The hazard ratio for PBSC was 3.35 compared to that for BMT. Our data showed the same result that R-PBSCT recipients have a significantly higher risk for BO than UR-BMT recipients.

Dudek et al. [19] reported that 58 patients underwent CBT and none of them developed BO; however, most patients were children who had undergone a recent transplantation, and there is no data on the incidence of BO in adult CBT recipients. In our case series, the incidence of BO in CBT recipients was higher than that in R-BMT recipients (2.65 vs. 1.62%, Dudek = 0.381), but the

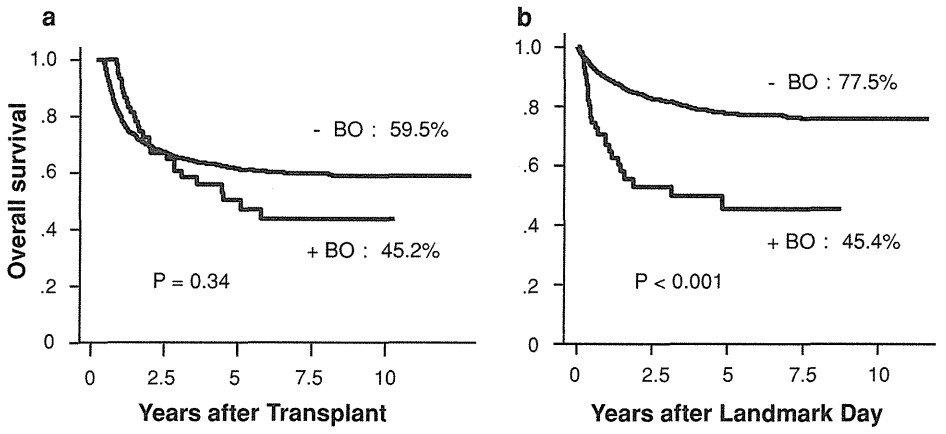

Fig. 4 Overall survival (OS) post-transplantation (**a**) and OS from landmark day (**b**). In patients who developed BO, the post-transplant day of BO development is defined as the landmark day; in those who did not develop BO, post-transplant day is day 335, which is the median day of occurrence of BO, is defined as the landmark day. The 5-year overall survival of patients with BO from the time of diagnosis was significantly less than that of patients without BO from day 335 (45.4 vs. 77.6%, $p < 0.001$)

difference was not significant. These data suggest that the incidence of BO after CBT may not be lower than that after transplantation from other stem cell sources. In our series, UR-PBSCT was not included, but UR-PBSCT patients might have a higher risk for developing BO than R-PBSCT recipients. Further study is necessary for this issue.

Regarding the incidence of BO in relation to the intensity of conditioning regimen, there was no significant difference between the patients with CST and RIST. Yoshihara et al. [20] reported that the incidence of BO following non-myeloablative HSCT was 2.3% compared with that of 17% following conventional myeloablative HSCT. A reason for this difference is that the incidence of BO in CST recipients was much higher in the report by Yoshihara than that reported by us. In their report, there was no difference in the incidence of BO between BMT and PBSCT recipients, although most patients with RIST received PBSCT (PBSC in RIST: 46/48). Tomas et al. [8] reported that the busulfan-based conditioning regimen was a significant risk factor for BO, but neither TBI nor non-TBI regimens were significant risk factors for BO in our study. Therefore, the type or intensity of conditioning might not be a definite risk factor for BO.

In the present study, 94% of the patients already suffered from chronic GVHD before developing BO and most patients suffered from extensive-type chronic GVHD. Time-dependent analysis revealed that preceding chronic GVHD was the strongest risk factor for developing BO. Although acute GVHD was a significant risk factor for BO but chronic GVHD was not in the IBMTR study, they did not correctly estimate the effect of preceding chronic GVHD for BO because the registry questionnaire incorporated BO as a manifestation of chronic GVHD [8].

The main cause of death was respiratory failure and the second cause was disseminated aspergillosis. Sakaida et al. reported that the relapse rate because of haematological malignancy was significantly low in LONIPC patients, and LONIPC is strongly associated with the GVL effect [5]. In our study, primary disease relapsed in six patients before developing BO and two patients after developing BO. Rapid reduction of immunosuppressive drugs might influence the development of BO in patients who relapsed before developing BO but only one died of relapse from primary disease. The low relapse rate and low relapse mortality suggest that patients with BO might have strong GVL effect, although non-relapse mortality is high.

There was no significant difference in survival from the time of transplantation between patients with and without BO. However, considering that the median time for developing BO was day 335 post-transplantation, we performed semi-landmark analysis by defining the median day of developing BO as the landmark day for patients without BO and showed that there was significant difference in survival. The reason for this discrepancy is that in total 503 patients died within 335 days post-transplant for various reasons and the 5-year OS of the long-term survivors beyond the day 335 was 77.6%, while only 8 patients with BO died within 335 days post-transplant and non-relapse mortality in patients with BO was 49.8%. These data suggest that BO is an important late-onset complication which significantly affects the survival of long-term survivors after allo-SCT.

In this study, we used an FEV1 of the predicted value <75% and an FEV1/FVC <70% as BO criteria. However, FEV1 and FEV1/FVC decreased significantly from pre-transplant values, and BO prognosis was poor. Chien et al. [9] recently proposed a new criterion for airway obstruction after allo-SCT in which patients were said to be suffering from airway obstruction if the annualized rate of the predicted FEV1 decline was >5% per year, and the lowest documented FEV1/FVC ratio was <80%. Soubani et al. [7] also proposed an FEV1/FVC ratio of <70% and a reduction in FEV1 of >20% from the pre-transplant values as criteria for BO after allo-SCT. Inhaled corticosteroids and macrolides have been recently reported to be effective for the treatment and management of BO following allo-SCT [21, 22]. Therefore, repeated PFTs after allo-SCT should be necessary to diagnose BO at the early stage, and starting early interventions with these agents may improve the prognosis of patients with BO.

In summary, we described the incidence and risk factors for BO in allo-SCT recipients. The incidence of BO was significantly higher in patients who underwent R-PBSCT than in those who underwent transplantation from other stem cell sources. R-PBSCT recipients who have already developed chronic GVHD have a high risk for developing BO and need extensive care and repeated PFTs.

Conflict of interest The authors declare no conflict of interest.

References

1. Yoshihara S, Yanik G, Cooke KR, Mineishi S. Bronchiolitis obliterans syndrome (BOS), bronchiolitis obliterans organizing pneumonia (BOOP), and other late-onset noninfectious pulmonary complications following allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2007;13:749–59.
2. Afessa B, Litzow MR, Tefferi A. Bronchiolitis obliterans and other late onset non-infectious pulmonary complications in hematopoietic stem cell transplantation. Bone Marrow Transplant. 2001;28:425–34.
3. Palmas A, Tefferi A, Myers JL, Scott JP, Swensen SJ, Chen MG, et al. Late-onset noninfectious pulmonary complications after allogeneic bone marrow transplantation. Br J Haematol. 1998; 100:680–7.
4. Patriarca F, Skert C, Sperotto A, Damiani D, Cerno M, Geromin A, et al. Incidence, outcome, and risk factors of late-onset noninfectious pulmonary complications after unrelated donor stem cell transplantation. Bone Marrow Transplant. 2004;33:751–8.

5. Sakaida E, Nakaseko C, Harima A, Yokota A, Cho R, Saito Y, et al. Late-onset noninfectious pulmonary complications after allogeneic stem cell transplantation are significantly associated with chronic graft-versus-host disease and with the graft-versus-leukemia effect. Blood. 2003;102:4236–42.
6. Roca J, Granena A, Rodriguez-Roisin R, Alvarez P, Agusti-Vidal A, Rozman C. Fatal airway disease in an adult with chronic graft-versus-host disease. Thorax. 1982;37:77–8.
7. Soubani AO, Uberti JP. Bronchiolitis obliterans following haematopoietic stem cell transplantation. Eur Respir J. 2007; 29:1007–19.
8. Santo Tomas LH, Loberiza FR Jr, Klein JP, Layde PM, Lipchik RJ, Rizzo JD, et al. Risk factors for bronchiolitis obliterans in allogeneic hematopoietic stem-cell transplantation for leukemia. Chest. 2005;128:153–61.
9. Chien JW, Martin PJ, Gooley TA, Flowers ME, Heckbert SR, Nichols WG, et al. Airflow obstruction after myeloablative allogeneic hematopoietic stem cell transplantation. Am J Respir Crit Care Med. 2003;168:208–14.
10. Clark JG, Crawford SW, Madtes DK, Sullivan KM. Obstructive lung disease after allogeneic marrow transplantation. Clinical presentation and course. Ann Intern Med. 1989;111:368–76.
11. Clark JG, Schwartz DA, Flournoy N, Sullivan KM, Crawford SW, Thomas ED. Risk factors for airflow obstruction in recipients of bone marrow transplants. Ann Intern Med. 1987; 107:648–56.
12. Sanders JE. Chronic graft-versus-host disease and late effects after hematopoietic stem cell transplantation. Int J Hematol. 2002;76(Suppl 2):15–28.
13. Filipovich AH, Weisdorf D, Pavletic S, Socie G, Wingard JR, Lee SJ, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. Biol Blood Marrow Transplant. 2005;11:945–56.
14. Lentz D, Bergin CJ, Berry GJ, Stoehr C, Theodore J. Diagnosis of bronchiolitis obliterans in heart-lung transplantation patients: importance of bronchial dilatation on CT. AJR Am J Roentgenol. 1992;159:463–7.
15. Sargent MA, Cairns RA, Murdoch MJ, Nadel HR, Wensley D, Schultz KR. Obstructive lung disease in children after allogeneic bone marrow transplantation: evaluation with high-resolution CT. Am J Roentgenol. 1995;164:693–6.
16. Cox DR. Regression models and life-tables. J Roy Stat Soc Ser B. 1972;34:187–220.
17. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc. 1958;53:457–81.
18. Gray RJ. A class of K-sample tests for comparing the cumulative incidence of a competing risk. Ann Stat. 1988;16:1141–54.
19. Dudek AZ, Mahaseth H, DeFor TE, Weisdorf DJ. Bronchiolitis obliterans in chronic graft-versus-host disease: analysis of risk factors and treatment outcomes. Biol Blood Marrow Transplant. 2003;9:657–66.
20. Yoshihara S, Tateishi U, Ando T, Kunitoh H, Suyama H, Onishi Y, et al. Lower incidence of Bronchiolitis obliterans in allogeneic hematopoietic stem cell transplantation with reduced-intensity conditioning compared with myeloablative conditioning. Bone Marrow Transplant. 2005;35:1195–200.
21. Bashoura L, Gupta S, Jain A, Couriel DR, Komanduri KV, Eapen GA, et al. Inhaled corticosteroids stabilize constrictive bronchiolitis after hematopoietic stem cell transplantation. Bone Marrow Transplant. 2008;41:63–7.
22. Maimon N, Lipton JH, Chan CK, Marras TK. Macrolides in the treatment of bronchiolitis obliterans in allograft recipients. Bone Marrow Transplant. 2009;44:69–73.

Bone Marrow Transplantation (2012), 95–100

www.nature.com/bmt

ORIGINAL ARTICLE

Secondary solid tumors after allogeneic hematopoietic SCT in Japan

A Yokota[1], S Ozawa[2], T Masanori[3], H Akiyama[3], K Ohshima[4], Y Kanda[4], S Takahashi[5], T Mori[6], C Nakaseko[2], M Onoda[1], K Kishi[7], N Doki[8], N Aotsuka[9], H Kanamori[10], A Maruta[10], H Sakamaki[3] and S Okamoto[6], for the Kanto Study Group for Cell Therapy (KSGCT)

[1]*Department of Internal Medicine, Chiba Aoba Municipal Hospital, Chiba, Japan;* [2]*Department of Hematology, Chiba University Graduate School of Medicine, Chiba, Japan;* [3]*Department of Hematology, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan;* [4]*Division of Hematology, Saitama Medical Center, Jichi Medical University, Saitama, Japan;* [5]*Department of Hematology and Oncology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan;* [6]*Division of Hematology, Department of Medicine, Keio University School of Medicine, Tokyo, Japan;* [7]*Division of Hematology and Oncology, Department of Medicine, Tokai University School of Medicine, Kanagawa, Japan;* [8]*Division of Hematology, Saiseikai Maebashi Hospital, Gunma, Japan;* [9]*Division of Hematology and Oncology, Narita Red Cross Hospital, Chiba, Japan and* [10]*Department of Hematology, Kanagawa Cancer Center, Kanagawa, Japan*

To evaluate the incidence and risk factors for secondary solid tumors in Japan after allogeneic hematopoietic SCT (allo-HSCT), 2062 patients who had received allo-HSCT between 1984 and 2005 were retrospectively analyzed. Twenty-eight patients who developed 30 solid tumors were identified a median of 5.6 years after transplantation. The risk for developing tumors was 2.16-fold higher than that of the age- and sex-adjusted general population. The cumulative incidence of solid tumors at 10 years after allo-HSCT was 2.4%. The risk was significantly higher for tumors of the skin, oral cavity and esophagus (standard incidental ratio 40.23, 35.25 and 10.73, respectively). No increase in gastric, colon or lung cancer, despite being the most prevalent neoplasm in the Japanese, was observed. In multivariate analysis, occurrence of chronic GVHD and malignant lymphoma as a primary disease was associated with a higher risk for developing solid tumors. Eighteen patients are still alive, and their 5-year probability of survival since diagnosis of solid tumors is 59.7%. Our data suggest that the incidence and risk factors of secondary solid tumors in Japanese allo-HSCT recipients are comparable to those reported in Western countries and emphasize that the early detection of solid tumors has a crucial role in improving OS.
Bone Marrow Transplantation (2012) **47**, 95–100; doi:10.1038/bmt.2011.23; published online 28 February 2011
Keywords: allo-SCT; secondary solid tumors; late effects of transplantation; chronic GVHD

Introduction

Allogeneic hematopoietic SCT (allo-HSCT) offers curative therapy for malignant and non-malignant hematological diseases. Improvements in outcomes after transplantation have led to a continuous increase of long-term survivors among allo-HSCT recipients. The development of secondary neoplasms is one of the serious late complications after allo-HSCT, and is associated with considerable morbidity and mortality.[1,2] Several studies reported that the incidence of secondary solid tumors in recipients aged 10 years or older ranged from 2.2 to 6.1%, and that TBI, T-cell depletion, GVHD and immunosuppressive therapy may be important risk factors.[3,4] Although these studies demonstrated that the risk of developing secondary solid tumors is significantly higher in allo-HSCT recipients in Western countries, only a few data documenting the incidence for secondary solid tumors in the Japanese population are available.[5] Thus, we retrospectively analyzed 2062 Japanese allo-HSCT recipients registered to the Kanto Study Group for Cell Therapy database, in order to determine the incidence and risk factors of secondary solid tumors following allo-HSCT.

Patients and methods

Patient characteristics

Between July 1984 and June 2005, 2062 patients underwent allo-HSCT in 14 facilities of the Kanto Study Group for Cell Therapy, and were retrospectively evaluated. The occurrence of each non-hematological secondary solid tumor was identified by using the Kanto Study Group for Cell Therapy database, and detailed clinical information, including histological type of malignancy, site of occurrence, date of diagnosis, treatment and its outcome, were obtained for each secondary tumor by chart review. The tissue diagnoses of secondary solid tumors were given in each institution.

Patient characteristics are summarized in Table 1. The median duration of follow-up for surviving patients after

Correspondence: Dr A Yokota, Department of Internal Medicine, Chiba Aoba Municipal Hospital, 1273-2 Aoba-cho, Chuo-ku, Chiba 260-0852, Japan.
E-mail: taroyok@xb4.so-net.ne.jp
Received 2 August 2010; revised 13 December 2010; accepted 17 December 2010; published online 28 February 2011

allo-HSCT was 5.7 years, and 1290 (62.6%) patients were followed through to the end of study date. Majority of the patients were adults, with only 97 patients (4.7%) aged <18 years at the time of allo-HSCT. The median age at transplant was 36 years (range, 7–68 years). Most of the patients underwent allo-HSCT for a primary diagnosis of hematological malignancies including ALL (20.9%), AML (32.3%), CML (21.2%), myelodysplastic syndrome (10.5%) or malignant lymphoma (5.4%); the median time from diagnosis to allo-HSCT was 10.6 months (range, 0.3–503.5 months). Donor and hematopoietic stem cell sources included BM from related donors ($n=852$, 41.3%), peripheral blood from related donors (R-PBSCT; $n=329$, 16.0%), BM from unrelated donors ($n=706$, 34.2%) and cord blood from unrelated donors ($n=160$, 7.8%). TBI-based regimen was used for 68% and non-TBI regimen for 32% of the patients as the conditioning regimen. Prophylaxis for GVHD was attempted mainly with calcineurin inhibitor (CYA or tacrolimus) plus MTX in 2041 patients (99.0%). Acute GVHD (aGVHD, grade I–IV) developed in 1390 (67.4%) and chronic GVHD (cGVHD) in 1140 patients (55.3%). The median duration of follow-up was 3.3 years (range, 0.2–21.9 years) for all patients and 5.6 years (range, 0.3–21.9 years) for survivors.

Statistical analysis

The number of person-years at risk was calculated for each patient from the date of transplantation till the date of last contact, death, diagnosis of a new neoplasm or completion of the study (18 January 2006) in the order of their first occurrence. Age (by 5-year bands)-, sex- and year-specific incidence rates for all cancers at specific anatomical sites were applied to the appropriate person-years at risk to compute the expected numbers of cancers. Incidence of all cancers in the general population was obtained from the database of the population-based cancer registration in Japan.[6] Standardized incidence ratios (SIRs) were then calculated by obtaining the ratio of the observed to the expected number of cases, accompanied by the 95% confidence intervals (CIs).[7,8] The cumulative incidence of developing a secondary malignancy was estimated after adjusting for the competing risk of patient death using Gray's method.[9,10] We estimated the survival rate among patients who developed secondary tumors by the Kaplan–Meier method. Univariate Cox proportional hazards regression analysis was used to determine statistically significant predictor variables for the development of a secondary malignancy. A multivariate analysis was performed using variables significant at univariate levels, which were potentially associated with the development of a secondary malignancy. All tests were two-sided, and a P value of $\leqslant 0.05$ was considered statistically significant.

Results

Diagnosis and outcome of secondary solid tumors

In total, 28 patients developed 30 solid malignancies at a median of 5.6 years (range, 0.3–17.6 years) after allo-HSCT. These secondary tumors involved the skin (6), oral cavity or pharynx (11), esophagus (3), stomach (3), colon (3), lung (2), thyroid gland (1) and prostate (1). Characteristics of patients who developed a secondary solid malignancy are shown in Tables 1 and 2. The median age of these patients at the time of allo-HSCT was 35 years (range, 10–57 years), and the median age at the time of diagnosis with a secondary malignancy was 42 years (range, 17–61 years).

Table 1 Characteristics of the patient population

	Total		*Solid tumor number*
	Number	*%*	
Number of patients	2062		28
Median age at HSCT (range), years	36 (7–68)		35 (10–57)
Sex			
Male	1225	59.4	18
Female	837	40.6	10
Primary diagnosis			
AA	104	5.0	1
ALL	431	20.9	5
AML	666	32.3	8
ATL	12	0.6	
CML	438	21.2	8
MDS	216	10.5	2
ML	112	5.4	4
MM	30	1.5	
MPDs	23	1.1	
Others	30	1.5	
Median number of months from diagnosis to HSCT (range)	10.6 (0.3–503.5)		8.1 (2.1–82.3)
Stem cell source			
R-BMT	852	41.3	18
R-PBSCT	329	16.0	5
R-PB and BMT	15	0.7	
U-BMT	706	34.2	5
U-CBT	160	7.8	
TBI			
Yes	1402	68.0	20
No	660	32.0	8
GVHD prophylaxis			
CsA	1689	81.9	27
FK	352	17.1	1
Others	21	1.0	
AGVHD			
0	672	32.6	6
I	582	28.2	15
II	592	28.7	6
III	153	7.4	1
IV	63	3.1	
CGVHD			
None	923	44.7	7
Limited	377	18.3	7
Extensive	727	35.3	14
Grade unknown	36	1.7	

Abbreviations: AA = aplastic anemia; ATL = adult T-cell leukemia/lymphoma; CBT = cord blood transplantation; FK = tacrolimus; MM = multiple myeloma; MDS = myelodysplastic syndrome; ML = malignant lymphoma; MPD = myeloproliferative disease; PB = peripheral blood; R = related; U = unrelated.

Table 2 Characteristics of patients who developed a secondary cancer

Age (years)[a]	*Sex*	*Secondary tumor*	*Site*	*Primary disease*	*Donor*	*Radiation dose (Gy)*	*aGVHD*	*cGVHD*	*Latency (years)*[b]	*Therapy*	*Outcome*	*Cause of death*
40	M	Adenocarcinoma	Colon	ALL	R-BM	12	I	None	3.7	ST+RT	Dead	Tumor
41	M	Adenocarcinoma	Colon	CML	U-BM	7	I	None	4.7	ST+CT	Alive	
53	M	Adenocarcinoma	Colon	AML	R-BM	None	I	Extensive	3.1	ST+CT	Alive	
18	F	Adenocarcinoma	Stomach	AML	U-BM	12	I	Extensive	2.0	EMR	Dead	cGVHD
10	F	SCC	Tongue	CML	R-BM	12	III	Extensive	7.2	RT	Dead	Tumor
37	M	Mucoepidermoid ca	Lung	AA	R-BM	7.5	None	Limited	10.2	ST	Alive	
50	F	BCC	Skin	AML	R-BM	12	I	Limited	8.4	ST	Alive	
37	M		Stomach	AML	U-BM	12	II	Extensive	6.0	ST	Alive	
23	F	SCC	Gingiva	MDS	R-BM	12	II	Limited	4.8	ST	Alive	
33	M	SCC	Tongue	CML	R-BM	12	I	Extensive	9.0	ST	Alive	
26	F	Adenocarcinoma	Stomach	NHL	R-PB	12	None	None	0.3	None	Dead	Primary disease
46	F	SCC	Skin	CML	R-PB	12	II	Extensive	1.6	ST+RT	Dead	cGVHD
44	M	SCC	Lung	CML	R-PB	None	I	Extensive	7.4	None	Dead	Tumor
36	M	SCC	Esophagus	CML	R-PB	None	None	Extensive	9.5	ST	Alive	
47	M	Adenocarcinoma	Prostate	ALL	R-PB	12	I	Limited	6.6	ST	Alive	
51	M	Melanoma	Skin	NHL	R-PB	12	None	Limited	2.1	ST+CT	Dead	Tumor
57	F	SCC	Esophagus	MDS	U-BM	12	I	Extensive	4.7	RT+CT	Dead	Tumor
26	M	SCC	Tongue	CML	R-BM	12	I	Extensive	12.3	CT	Dead	Tumor
25	F	Mucoepidermoid ca	Oral mucosa	ALL	R-BM	12	I	None	12.6	ST	Alive	
31	M	SCC	Gingiva	NHL	R-BM	12	I	Extensive	5.6	ST	Alive	
		SCC	Oral mucosa						6.7	ST	Alive	
		SCC	Esophagus						6.7	ST+RT+CT	Alive	
19	M	Myxofibrosarcoma	Skin	CML	R-BM	12	I	Limited	2.4	ST	Alive	
38	M	SCC	Pharynx	ALL	R-BM	12	II	Extensive	6.7	RT+CT	Alive	
32	F	SCC	Gingiva	NHL	R-BM	None	None	Extensive	4.2	ST	Alive	
42	M	SCC	Tongue	AML	R-BM	None	None	Extensive	9.9	ST	Alive	
40	F	BCC	Skin	AML	R-BM	12.5	I	Extensive	7.1	ST	Alive	
17	F	BCC	Skin	ALL	R-BM	12.5	I	None	17.6	ST	Alive	
32	M	SCC	Tongue	AML	R-BM	None	II	None	4.4	ST+RT	Alive	
27	M	Adenocarcinoma	Thyroid	AML	U-BM	None	II	None	2.3	ST	Dead	Tumor

Abbreviations: AA = aplastic anemia; BCC = basal cell carcinoma; CT = chemotherapy; EMR = endoscopic mucosal resection; MDS = myelodysplastic syndrome; NHL = non-Hodgkin lymphoma; PB = peripheral blood; R = related; RT = radiotherapy; ST = surgical therapy.
Mucoepidermoid ca refers to mucoepidermoid carcinoma.
[a]At transplantation.
[b]From transplantation.

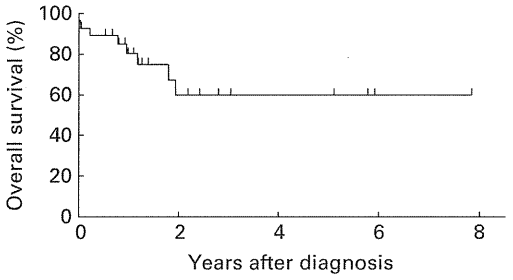

Figure 1 OS after diagnosis of secondary cancers.

Among the 30 patients with secondary tumors, 29 received various therapies, with the exception of 1 patient with an early gastric cancer, for whom the diagnosis was made by autopsy after death from primary disease. Surgery was performed in 16 patients, and endoscopic mucosal resection was performed in 1 patient with gastric cancer, of which 16 operations were curative. All patients who received a curative operation, with the exception of one patient who died from cGVHD, survived for 1 to 94 months without the recurrence of solid tumors after the diagnosis of secondary solid tumors. Seven patients were treated with surgery and chemotherapy with or without radiotherapy, and five of seven survived for 6 to 48 months. Only one of the five patients who were treated with chemotherapy and/or radiotherapy survived 6 months from diagnosis. Overall, of the 28 patients, 1 died from primary disease, 2 from cGVHD and 7 from their secondary neoplasm at a median of 10.4 months (range, 0–23.2 months) after the diagnosis. The probability of a 5-year survival since diagnosis of secondary tumors was 59.7% (Figure 1).

Probability and risk factors of developing secondary solid tumors

The cumulative incidence of developing any secondary solid tumors at 5, 10 and 15 years after transplantation was 0.9, 2.4 and 3.7%, respectively (Figure 2). The overall risk of developing solid tumors had significantly increased in the study population; 30 cancers were observed compared with 13.90 cases expected in an age- and sex-matched general population (SIR 2.16; 95% CI 1.46–3.08). The overall risk was especially elevated for neoplasms of the skin (SIR 40.23; 95% CI 14.77–87.57), oral cavity or pharynx (SIR 35.25; 95% CI 17.59–63.06) and esophagus (SIR 10.73; 95% CI 2.21–31.36). In contrast, the risk of gastric cancer, a common cancer in Japan, had not increased compared with that in the general population (SIR 1.33; 95% CI 0.27–3.89) (Table 3).

In univariate analysis, R-PBSCT as the stem cell source ($P=0.041$), malignant lymphoma as the primary disease ($P=0.014$) and presence of cGVHD ($P=0.05$) were significant risk factors for development of a secondary solid tumor. Presence of aGVHD was marginally significant ($P=0.062$). These four factors were added to the Cox regression model in multivariate analysis. Two independent risk factors, malignant lymphoma as the primary disease and occurrence of cGVHD, were identified ($P=0.005$, relative risk (RR) 4.7 and $P=0.043$, RR 2.4, respectively). In the oral cavity and esophageal squamous cell carcinoma, these two risk factors were also significant in multivariate analysis ($P=0.010$, RR 8.1 and $P=0.019$, RR 4.9, respectively). Gray's method was used to analyze these two risk factors to confirm this result. Malignant lymphoma as primary disease and occurrence of cGVHD remained significant ($P=0.006$ and 0.009, respectively). In contrast, no significant risk factor was identified for secondary solid tumors in other sites of the body.

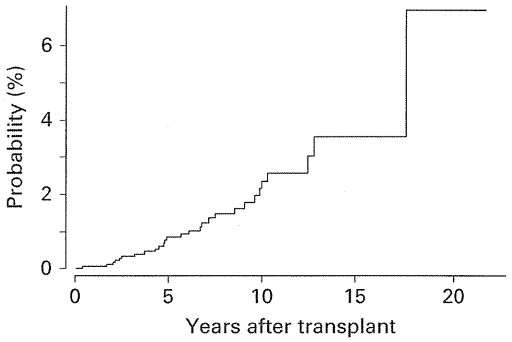

Figure 2 Cumulative incidences of secondary solid tumor post allo-HSCT.

Table 3 SIRs of secondary solid tumors

Site	*Observed*	*Expected*	*SIR*	*95% CI*
All solid tumor	30	13.90	2.16	1.46–3.08
Skin	6	0.15	40.23	14.77–87.57
Oral cavity or pharynx	11	0.31	35.25	17.59–63.06
Esophagus	3	0.28	10.73	2.21–31.36
Stomach	3	2.25	1.33	0.27–3.89
Colon	3	1.92	1.56	0.32–4.56
Lung	2	0.95	2.10	0.25–7.58
Thyroid	1	0.49	2.05	0.05–11.43
Prostate	1	0.15	6.76	0.17–37.64

Abbreviations: CI = confidence interval; SIR = standardized incidence ratio.

Discussion

Among the 2062 patients who received allo-HSCT, 30 secondary solid tumors occurred in 28 patients, resulting in an estimated cumulative risk of 0.9, 2.4 and 3.7% at 5, 10 and 15 years, respectively (Figure 2). The latency of secondary tumors was discovered at a median of 5.6 years (range, 0.3–17.6 years). The overall incidence was 2.16-fold higher compared with age- and sex-adjusted cancer rates (Table 3) for the general population.

This study is one of the largest surveys in Japan investigating secondary malignancies after allo-HSCT. These results are similar to those reported previously. Curtis *et al.*[4] reported the cumulative incidence of secondary solid cancer as 2.2 and 6.7% at 10 and 15 years, respectively (RR 2.7 compared with the general population) in 19 229 allografts; this was the largest study to evaluate the incidence of secondary malignancies after allograft to

date. Similarly, Baker *et al.*[11] studied 3372 allogeneic and autologous HSCT recipients, and reported a cumulative incidence of solid cancers of 3.8% at 20 years with a SIR of 2.8 in a retrospective analysis. Shimada *et al.*[5] reported that cumulative incidence at 5 and 10 years was 1.9 and 4.2%, respectively, in 809 Japanese HSCT recipients. But, these two studies included both allogeneic and autologous transplant recipients for analysis. Consistent with the previous reports, the current study confirmed the increasing incidence of secondary solid tumors, even after a period of observation of more than 15 years, thus emphasizing again the importance of longer follow-up in this population.

Various risk factors that may contribute to the development of secondary solid cancers after allo-HSCT have been identified. These include TBI as a part of the conditioning regimen, previous radiotherapy, immunodeficiency from incomplete recovery after allo-HSCT, the use of immunosuppressive agents, occurrence of aGVHD or cGVHD, and advanced age.[12] Curtis *et al.*[13] revealed that cGVHD and its therapy were strongly correlated with the risk for developing squamous cell carcinomas of the oral cavity and skin in a case–control study. The possible mechanism behind this correlation is that cGVHD leads to persistent inflammation in the involved organs, and may stimulate regeneration of the epithelium and subsequent emergence of neoplastic cells. Furthermore, instances of solid organ transplantation indicate that prolonged immunosuppressive therapy leading to impairment in immune surveillance is associated with an increased risk of both solid malignancies and non-Hodgkin's lymphoma.[14,15] This may be caused by co-carcinogenic effects of pretransplant chemo-radiation resulting in genetic damage. Most instances of microsatellite instability of the colon or buccal non-neoplastic epithelium after allo-HSCT were often caused by aGVHD or cGVHD. These genomic alternations may also be implicated in the evolution of post transplant complications such as secondary malignancies.[16] In our study, the occurrence of cGVHD was also identified as the risk factor for secondary tumors, particularly in the oral cavity and esophageal squamous cell carcinoma, where occurrence of secondary tumors was found to be 35- and 11-fold higher, respectively, than that expected in the general population (Table 3).

Only one case of squamous cell carcinoma of the skin was diagnosed in this study as compared with five non-squamous cell cutaneous malignancies. This is similar to the findings of previous reports for the Japanese population.[5] However, this contrasts with the finding for patients in Western countries.[3,11] This disparity may be explained by the difference in cGVHD manifestation or in genetic susceptibilities between ethnic groups. The most common tumors observed in our study were those of the skin and oral cavity. The predominance of cutaneous and oral cancers is similar to that reported previously.[17,18] Although gastric, colon and lung cancers are prevalent in the Japanese population,[6] the incidence of these neoplasms is not statistically different from that in the general population in this study.

Despite the well-known association between radiation and secondary solid malignancies, only two of the largest studies of secondary solid malignancies occurring after allo-HSCT describe a significant relationship with TBI. Curtis *et al.*[4] reported that a significant dose related to a 2.7- to 4.4-fold higher risk with TBI. Socie *et al.*[19] found a 3.1-fold risk with high-dose TBI. In contrast, other studies failed to demonstrate a significant association between secondary malignancies and TBI. Witherspoon *et al.*[20] reported a 3.9-fold increased risk of all secondary malignancies after TBI in 2246 auto- and allografts, but this was not significant when assessing solid malignancies alone. Bhatia *et al.*[21] showed a sixfold-increased risk for secondary malignancies in patients treated with TBI, which approached statistical significance ($P=0.08$). However, a more recent report with 6 more years of follow-up gave less conclusive results (RR 1.5, $P=0.27$).[11]

A TBI-containing conditioning regimen was not a statistically significant risk factor in this study. There may be several explanations for this finding. First, the follow-up period is still relatively short in comparison with the generally long latent period of radiogenic cancers. Several reports suggest that the risk of secondary solid malignancy after radiation exposure remains elevated for multiple decades;[22–24] several radiogenic malignancies take a long period to develop, and hence may require much longer follow-up. Second, the risk of such cancers is frequently high among patients undergoing irradiation at a young age.[19,25] Third, certain tumor types, such as those in the brain, thyroid, salivary gland and bone connective tissue, occur in association with radiation exposure.[26–29] Two large-cohort studies in children indicated that brain and thyroid cancers accounted for the increased risk of TBI.[2,4] In this study, which mainly included adult patients, only one case of thyroid cancer was observed. These results indicate that a distinctive mechanism may participate in the evolution of different post transplant solid tumors.

An unexpected finding was that of the malignant lymphoma as a primary disease conferring significant risks of developing into a secondary solid tumor. Among all secondary tumors, malignant lymphoma as the primary disease had an RR of 4.7, which was significantly higher compared with acute leukemia. When the cohort was limited to secondary tumors in the oral cavity and esophagus, much high risk (RR 8.1) was demonstrated. This observation has not been reported previously in the literature, and its explanation remains to be elucidated. Because allo-HSCT is not routinely recommended for treating malignant lymphoma during the first CR in most cases, lymphoma patients who undergo allo-HSCT may be heavily treated to start off with. Pre-transplant treatment before the conditioning therapies, such as salvage therapy, radiotherapy and autologous HSCT, might have already predisposed those patients to develop secondary solid malignancy after allo-HSCT. Indeed, it has been reported that the incidence of lung cancer increases with increases in radiation dose and cycles of alkylating agents in Hodgkin's lymphoma therapy.[30] Unfortunately, we did not have comprehensive records of pre-transplant chemotherapy and/or radiotherapy, and hence could not assess the influence of pre-transplant therapy on the risk of developing a secondary solid tumor after allo-HSCT. It is necessary to estimate this finding carefully, because patients of malignant lymphoma are only 5.4% of all patients.

Few data exist about treatment of secondary solid tumors; and the prognosis of secondary malignancies is

generally considered to be poor, depending on an early diagnosis at a potentially curable stage. Our results are consistent with those reported by Favre-Schmuziger *et al.*[31] They reported five patients developing secondary solid tumors after allo-HSCT, who were treated as *de novo* tumor, and four out of five are alive without tumor recurrence. All patients in our study who received curative care overcame their secondary tumors. Because the risk of developing secondary malignancies after allo-HSCT continues to increase with time, all transplant recipients should be followed for the long term to detect cancers at an early stage.

Conflict of interest

The authors declare no conflict of interest.

References

1 Gallagher G, Forrest DL. Second solid cancers after allogeneic hematopoietic stem cell transplantation. *Cancer* 2007; **109**: 84–92.
2 Bhatia S, Louie AD, Bhatia R, O'Donnell MR, Fung H, Kashyap A *et al.* Solid cancers after bone marrow transplantation. *J Clin Oncol* 2001; **19**: 464–471.
3 Kolb HJ, Socié G, Duell T, Van Lint MT, Tichelli A, Apperley JF *et al.* Malignant neoplasms in long-term survivors of bone marrow transplantation: late effects working party of the European cooperative group for blood and marrow transplantation and the European late effect project group. *Ann Intern Med* 1999; **131**: 738–744.
4 Curtis RE, Rowlings PA, Deeg HJ, Shriner DA, Socie G, Travis LB *et al.* Solid cancers after bone marrow transplantation. *N Engl J Med* 1997; **336**: 897–904.
5 Shimada K, Yokozawa T, Atsuta Y, Kohno A, Maruyama F, Yano K *et al.* Solid tumors after hematopoietic stem cell transplantation in Japan: incidence, risk factors and prognosis. *Bone Marrow Transplant* 2005; **36**: 115–121.
6 Matsuda T, Marugame T, Kamo K, Katanoda K, Ajiki W, Sobue T. Cancer incidence and incidence rates in Japan in 2002: based on data from 11 population-based cancer registries. *Jpn J Clin Oncol* 2008; **38**: 641–648.
7 Vandenbroucke J. A shortcut method for calculating the 95% percent confidence interval of the standardized mortality ratio. *Am J Epidemiol* 1982; **115**: 303–304.
8 Esteve J, Benhamou E, Raymond L. Statistical methods in cancer research. Volume IV. Descriptive epidemiology. *IARC Sci Publ* 1994; **128**: 1–302.
9 Gray RJ. A class of K-sample tests for comparing the cumulative incidence of a competing risk. *Ann Stat* 1988; **16**: 1140–1154.
10 Lin DY. Non-parametric inference for cumulative incidence functions in competing risks studies. *Stat Med* 1997; **16**: 901–910.
11 Baker KS, DeFor TE, Burns JK, Ramsay NK, Neglia JP, Robison LL. New malignancies after blood or marrow stem-cell transplantation in children and adults: incidence and risk factors. *J Clin Oncol* 2003; **21**: 1352–1358.
12 Deeg HJ, Socie G. Malignancies after hematopoietic stem cell transplantation: many questions, some answers. *Blood* 1998; **91**: 1833–1844.
13 Curtis RE, Metayer C, Rizzo JD, Socié G, Sobocinski KA, Flowers ME *et al.* Impact of chronic GvHD therapy on the development of squamous cell cancers after hematopoietic stem cell transplantation: an international case–control study. *Blood* 2005; **105**: 3802–3811.
14 Jonas S, Rayes N, Neumann U, Neuhaus R, Bechstein WO, Guckelberger O *et al. De novo* malignancies after liver transplantation using tacrolimus-based protocols or cyclosporine-based quadruple immunosuppression with an interleukin-2 receptor antibody or antithymocyte globulin. *Cancer* 1997; **80**: 1141–1150.
15 Winter P, Schoeneich G, Miersch WD, Klehr HU. Tumour induction as a consequence of immunosuppression after renal transplantation. *Int Urol Nephrol* 1997; **29**: 701–709.
16 Faber P, Fisch P, Waterhouse M, Schmitt-Gräff A, Bertz H, Finke J *et al.* Frequent genomic alterations in epithelium measured by microsatellite instability following allogeneic hematopoietic cell transplantation in humans. *Blood* 2006; **107**: 3389–3396.
17 Hasegawa W, Pond GR, Rifkind JT, Messner HA, Lau A, Daly AS *et al.* Long-term follow-up of secondary malignancies in adults after allogeneic bone marrow transplantation. *Bone Marrow Transplant* 2005; **35**: 51–55.
18 Leisenring W, Friedman DL, Flowers MED, Schwartz JL, Deeg HJ. Nonmelanoma skin and mucosal cancers after hematopoietic cell transplantation. *J Clin Oncol* 2006; **24**: 1119–1126.
19 Socie G, Curtis RE, Deeg HJ, Sobocinski KA, Filipovich AH, Travis LB *et al.* New malignant diseases after allogeneic marrow transplantation for childhood acute leukemia. *J Clin Oncol* 2000; **18**: 348–357.
20 Witherspoon RP, Fisher LD, Schoch G, Martin P, Sullivan KM, Sanders J *et al.* Secondary cancers after bone marrow transplantation for leukemia or aplastic anemia. *N Engl J Med* 1989; **321**: 784–789.
21 Bhatia S, Ramsay NK, Steinbuch M, Dusenbery KE, Shapiro RS, Weisdorf DJ *et al.* Malignant neoplasms, following bone marrow transplantation. *Blood* 1996; **87**: 3633–3639.
22 Preston DL, Shimizu Y, Pierce DA, Suyama A, Mabuchi K. Studies of mortality of atomic bomb survivors. Report 13. Solid cancer and noncancer disease mortality. 1950-1997. *Radiat Res* 2003; **160**: 381–407.
23 Bhatia S, Robison LL, Oberlin O, Greenberg M, Bunin G, Fossati-Bellani F *et al.* Breast cancer and other second neoplasms after childhood Hodgkin's disease. *N Engl J Med* 1996; **334**: 745–751.
24 van Leeuwen FE, Klokman WJ, Veer MB, Hagenbeek A, Krol AD, Vetter UA *et al.* Long-term risk of second malignancy in survivors of Hodgkin's disease treated during adolescence or young adulthood. *J Clin Oncol* 2000; **18**: 487–497.
25 Charles M. UNSCEAR report 2000: sources and effects of ionizing radiation. United Nations Scientific Comittee on the effects of atomic radiation. *J Radiol Prot* 2001; **21**: 83–86.
26 Neglia JP, Meadows AT, Robison LL, Kim TH, Newton WA, Ruymann FB *et al.* Second neoplasms after acute lymphoblastic leukemia in childhood. *N Engl J Med* 1991; **325**: 1330–1336.
27 Cohen A, Rovelli A, van Lint MT, Merlo F, Gaiero A, Mulas R *et al.* Secondary thyroid carcinoma after allogeneic bone marrow transplantation during childhood. *Bone Marrow Transplant* 2001; **28**: 1125–1128.
28 Hawkins MM, Wilson LM, Burton HS, Potok MH, Winter DL, Marsden HB *et al.* Radiotherapy, alkylating agents, and risk of bone cancer after childhood cancer. *J Natl Cancer Inst* 1996; **88**: 270–278.
29 Tucker MA, D'Angio GJ, Boice Jr JD, Strong LC, Li FP, Stovall M *et al.* Bone sarcomas linked to radiotherapy and chemotherapy in children. *N Engl J Med* 1987; **317**: 588–593.
30 Mauch PM, Kalish LA, Marcus KC, Coleman CN, Shulman LN, Krill E *et al.* Second malignancies after treatment for laparotomy staged IA-IIIB Hodgkin's disease: long-term analysis of risk factors and outcome. *Blood* 1996; **87**: 3625–3632.
31 Favre-Schmuziger G, Hofer S, Passweg J, Tichelli A, Hoffmann T, Speck B *et al.* Treatment of solid tumors following allogeneic bone marrow transplantation. *Bone Marrow Transplant* 2000; **25**: 895–898.