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ORIGINAL ARTICLE

Clinical significance of hemophagocytosis in BM clot sections during the peri-engraftment period following allogeneic hematopoietic SCT

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The effects of macrophage activation on the outcome of allogeneic hematopoietic SCT (allo-HSCT) have yet to be fully examined. A total of 70 adult patients who received a first allo-HSCT for hematological diseases were studied. We counted the number of hemophagocytic cells in BM clot sections on day +14±7, and analyzed its impact on subsequent outcome. In all, 23 patients were diagnosed as having increased numbers of hemophagocytic cells (HP group), whereas 47 were not (non-HP group). The HP group was not associated with an increased incidence of acute or chronic GVHD, but was associated with worse hematopoietic recovery than the non-HP group. The 2-year OS for the HP group and the non-HP group was 30 and 65% ($P<0.01$), respectively, and 2-year non-relapse mortality was 48% and 27% ($P<0.01$), respectively. Multivariate analysis confirmed that the HP group was associated with a lower OS (hazard ratio (HR) = 2.3; 95% confidence interval (CI), 1.0–5.4; $P=0.048$) and higher non-relapse mortality (HR = 4.0; 95% CI, 1.6–9.9; $P<0.01$). The HP group had higher incidences of death due to graft failure ($P<0.01$) and endothelial complications, such as sinusoidal obstruction syndrome and transplant-associated microangiopathy ($P=0.01$). Macrophage activation is a previously unrecognized complication with negative impact on outcome of allo-HSCT.

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Keywords: hemophagocytosis; macrophages; SCT; non-relapse mortality

Introduction

Macrophages have an indispensable role in both innate and acquired immunity and they have at least 3 major functions: antigen presentation, phagocytosis and immunomodulation.[1,2] Following allogeneic hematopoietic SCT (allo-HSCT), macrophages contribute to the development of acute GVHD by producing pro-inflammatory cytokines.[3] In addition to aGVHD, pro-inflammatory cytokine release is implicated in the pathogenesis of various early complications after allo-HSCT, such as sinusoidal obstruction syndrome, engraftment syndrome (ES) and capillary leakage syndrome.[4–7] Although the role of macrophages in these complications is undetermined, macrophages have an ability to secrete significant amounts of pro-inflammatory cytokines.[2] Furthermore, fatal outcomes of hemophagocytic syndrome after allo-HSCT have been described in case reports.[8] This evidence suggests that activation of macrophages has a significant impact on post-transplantation outcome. However, there are only a few clinical studies that have analyzed the effects of macrophage activation on outcome of allo-HSCT.[9]

Measuring the levels of cytokines or chemokines produced by activated macrophages, such as IL-1, IL-6, IL-12, TNF-α and macrophage inflammatory protein-1, may be a possible method to evaluate the activation of macrophages.[1,2,10] However, as these cytokines and chemokines are produced by many cell types, their elevated levels are not specific to macrophage activation.[1,10–12] An alternative method for evaluating the activation of macrophages is to assess the morphological change associated with macrophage activation, namely phagocytosis. Although phagocytosis reflects only a part of macrophage activation, the increased number of phagocytic cells provides direct evidence that macrophages are activated.[2] In addition, assessment of hemophagocytosis can be carried out easily using BM clot sections. Thus, hemophagocytosis serves as a specific and simple marker of macrophage activation. We assessed hemophagocytosis in BM clot sections during the early post-transplantation period, and analyzed its impact on subsequent outcome.

Patients and methods

Patients

We reviewed 96 consecutive adult patients who received their first allo-BM or PBSCT between December 2005 and December 2008 at the Japanese Red Cross Nagoya First Hospital. As our purpose was to examine the impact of

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hemophagocytosis in BM clot sections on day $+14\pm7$ on subsequent outcome, two patients who died within the first 21 days after transplantation were excluded. Although BM aspiration is routinely performed on day $+14\pm7$ in our institution, it was not performed in 17 patients. In addition, specimens were insufficient for evaluation in seven patients. As a result, 70 patients were included in the analysis, all of whom received T cell-replete grafts. Standard risk diseases were defined as AML in first or second CR, ALL in first CR, CML in first chronic phase, myelodysplastic syndrome as refractory anemia, malignant lymphoma in CR, chronic active EB-virus infection, aplastic anemia and paroxysmal nocturnal hemoglobinuria, whereas high-risk diseases were defined as the others. This study was approved by the institutional review board. All patients provided written informed consent.

Transplantation procedure

Conditioning included myeloablative and reduced-intensity regimens. The myeloablative regimens were mainly CY/TBI based, whereas the reduced-intensity conditioning regimens were mainly fludarabine 125 mg/m^2 plus melphalan 135–180 mg/m^2. Antithymocyte globulin was added in two patients who received HLA-mismatched transplants, and alemtuzumab was added in one patient with aplastic anemia. For GVHD prophylaxis, CYA and short-term MTX were used for allo-HSCT from a related donor, and tacrolimus and short-term MTX for allo-HSCT from an unrelated donor. All patients were cared for in laminar airflow units and received oral gut decontamination. Standard prophylaxis against *Pneumocystis carinii*, fungal infections and herpes simplex virus was given. G-CSF was administered after transplantation in all patients until engraftment was confirmed. Engraftment was defined as an ANC of more than 500/μL for 3 consecutive days. Primary graft failure was said to have occurred when engraftment was not seen in patients surviving more than 21 days after transplantation.[13] Secondary graft failure was defined as loss of neutrophil engraftment as determined by an ANC of less than 500/μL for 3 consecutive days after having achieved neutrophil engraftment, and no evidence of disease progression in the marrow.[14] ES was diagnosed, if patients presented with two or more of the following symptoms within 96 h of the start of neutrophil recovery (ANC >100/μL): (1) fever (temperature $>=38.5$°C) without an identifiable infectious cause; (2) weight gain $>=5\%$ over the pre-transplantation baseline weight; (3) erythematous rash not attributable to a medication; and (4) hypoxia, pulmonary infiltrates or both not attributable to infection or cardiac disease.[15,16] Acute GVHD was evaluated by established criteria.[17] Chronic GVHD was evaluated in patients who survived beyond day $+100$ without a relapse according to the traditional Seattle criteria.[18]

BM examination

BM aspiration was routinely performed on day $+14\pm7$. All specimens were fixed in formalin solution, embedded in paraffin and stained with hematoxylin-eosin. BM clot sections were reviewed retrospectively and the total number of hemophagocytic cells in three fields at a 200-fold magnification was counted (Figure 1).

Statistical considerations

Chi-square, Fisher's exact and Mann–Whitney tests were used to compare clinical and patient characteristics. The probability of survival was calculated using the Kaplan–Meier method, and the differences between groups were compared using log-rank statistics. Probabilities of non-relapse mortality (NRM) and relapse were calculated using the cumulative incidence function.[19] For NRM, relapse was the competing event, and for relapse, death in the absence of persistent or recurrent disease was the competing event. As our purpose was to examine the impact of hemophagocytosis in BM clot sections on day $+14\pm7$ on subsequent outcomes, all time-to-event comparisons were made from day $+21$ after transplantation. The Cox proportional hazards regression model was used to test the statistical significance of several potential prognostic factors for relapse, NRM and OS. Variables with a significance level less than 0.1 in univariate analysis

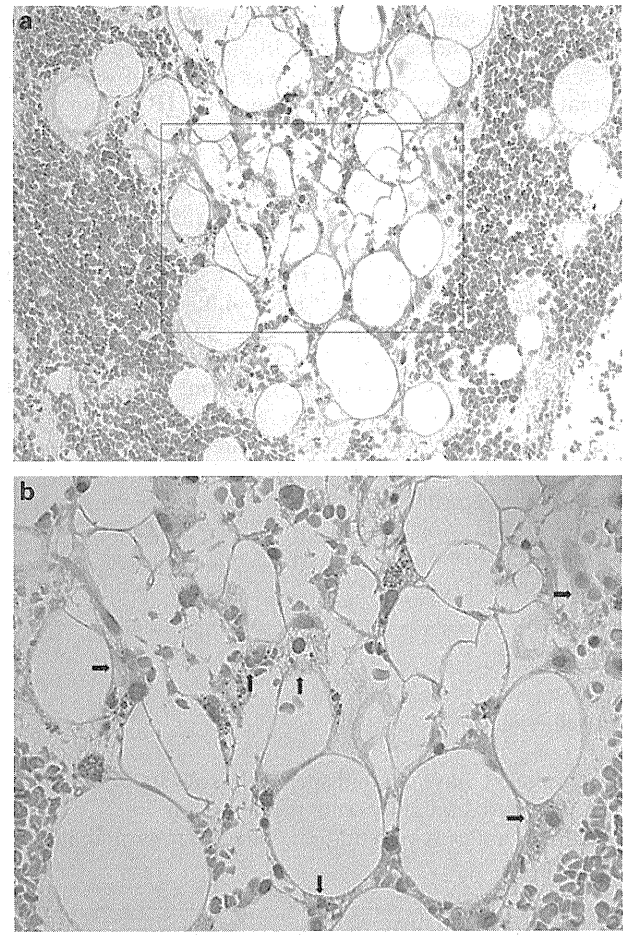

Figure 1 BM clot section stained with hematoxylin-eosin. (**a**) The specimen is from a representative patient who had an increased number of hemophagocytic cells. The indicated region is magnified in (**b**). Original magnification ×200. (**b**) Arrow indicates hemophagocytosis. Original magnification ×400.

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 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 ($P<0.01$).

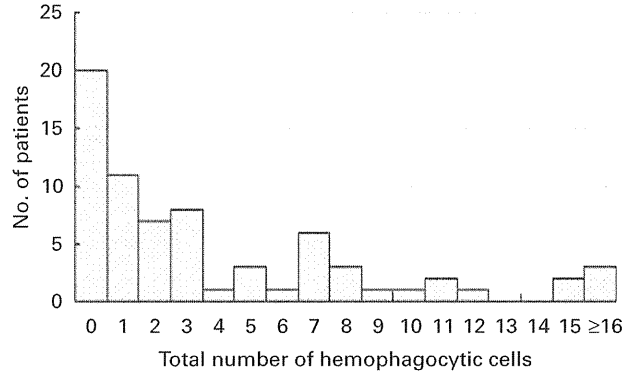

Figure 2 Distribution of numbers of hemophagocytic cells.

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.

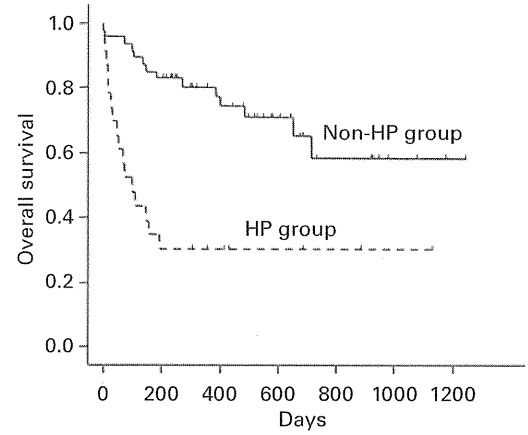

Figure 3 Probabilities of OS were significantly lower in the HP group than in the non-HP group (HP group: 52% at day +100 and 30% at 2 years; non-HP group: 94% at day +100 and 65% at 2 years; $P<0.01$).

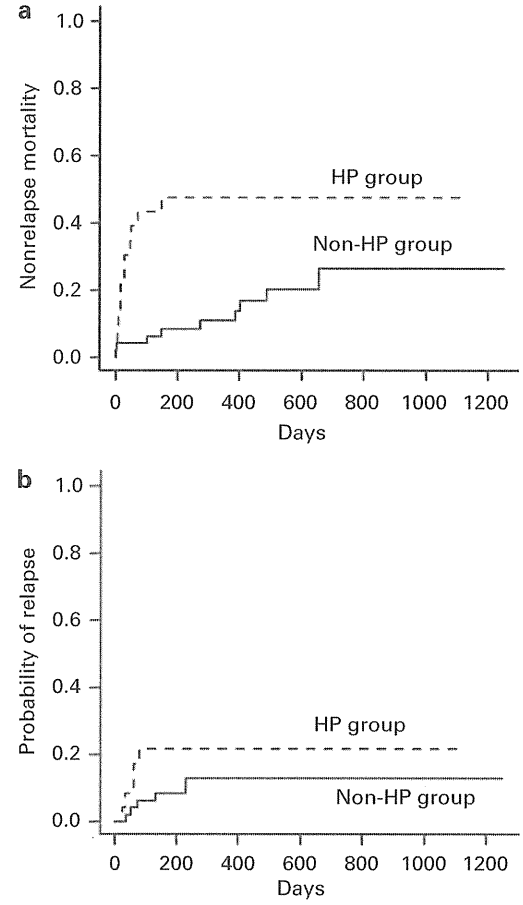

Figure 4 (**a**) Cumulative incidences of NRM were significantly higher in the HP group than in the non-HP group (HP group: 43% at day +100 and 48% at 2 years; non-HP group: 4% at day +100 and 27% at 2 years; $P<0.01$). (**b**) Cumulative incidences of relapse did not differ significantly between the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

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 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.

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Minimal Residual Disease following Allogeneic Hematopoietic Stem Cell Transplantation

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Minimal residual disease (MRD), both before and after transplantation, is a clinically important yet relatively poorly defined aspect of allogeneic hematopoietic stem cell transplantation (alloHSCT). The clinical relevance of MRD in the context of alloHSCT has been demonstrated by its association with the development of clinical relapse. However, with the possible exception of chronic myeloid leukemia (CML), the specific techniques, timing, frequency, and clinical utility, relative to improvement in patient outcomes, for monitoring MRD in the setting of alloHSCT has yet to be clearly defined. A concise overview of monitoring techniques for detecting MRD, as well as treatment strategies and biological and clinical research initiatives for MRD suggested by the National Cancer Institute First International Workshop on the Biology, Prevention, and Treatment of Relapse after Allogeneic Hematopoietic Stem Cell Transplantation, is covered in this article.

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KEY WORDS: Minimal residual disease, Allogeneic, Relapse, Graft-versus-tumor, DLI

INTRODUCTION

Minimal residual disease (MRD), in the setting of allogeneic hematopoietic stem cell transplantation (alloHSCT), poses several interesting questions and complex challenges. The relevance of these questions and challenges is personified by the relationship between MRD and the risk of relapse, which is primary cause of treatment failure and death after alloHSCT [1]. The clinical relation of posttransplant MRD with relapse, particularly in relationship to chronic myeloid leukemia (CML), was recognized early with development of cytogenetic and molecular techniques of detection [2]. The clinical relevance of MRD has been further recognized with the increased use of nonmyeloablative and reduced-intensity conditioning (RIC) regimens, with which relapse is even a greater clinical problem [3,4].

Despite the clear association of MRD with relapse, the clinical relevance of MRD in the alloHSCT setting remains to be determined. First and foremost, the definition of MRD needs to be defined for each disease, and needs to be distinguished from what we currently refer to as "remission" or "relapse." The detection of persistent disease posttransplant by immunophenotypic measures has significantly different implications for patients with acute lymphocytic leukemia (ALL) compared to someone with persistent chronic lymphocytic leukemia (CLL) [5,6]. Similarly, the molecular detection of a cytogenetic abnormality in the posttransplant is markedly different for a patient transplanted with chronic myeloid leukemia (CML) compared to a patient with acute myeloid leukemia (AML) [7]. Second, when and how often we should be using available techniques for a specific disease remains to be defined. This applies not only to the posttransplant setting, but also to the pretransplant setting, where multiple studies have demonstrated the prognostic significance of MRD prior to conditioning [8]. As the majority of relapses occur within the first 6 months after transplantation [1], it is important to determine the frequency of monitoring for recurrent disease within this posttransplant period. If we can determine when and how often, the next question is what tests should we be performing and are those tests adequately sensitive, specific, reproducible, practical, and economical. Finally, and most importantly, does monitoring for MRD make a clinical difference? There is sufficient evidence that detection of MRD provides prognostic information. However, does this information result in clinical decisions, relative to choice of conditioning regimen or stem cell product relative to detection of pretransplant MRD or intervention (eg, withdrawal of immune suppression

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or donor lymphocyte infusion) that result in improved outcomes? These remain essential questions for which there are relatively limited data and recommendations, with the possible exceptions of CML and ALL, and even with these diseases, there remains a need for further investigation.

This manuscript attempts to provide a concise overview of many of these issues. Specifically, it attempts to address methods for monitoring MRD and strategies to clinically manage patients once MRD is detected. In addition, a brief summary is provided on the National Cancer Institute First International Workshop on the Biology, Prevention, and Treatment of Relapse after Allogeneic Hematopoietic Stem Cell Transplantation, which attempted to address in a formal manner many of the issues described above.

MONITORING MRD AFTER ALLOSCT

Improved supportive care, the introduction of RIC regimen, and careful donor selection have substantially decreased the nonrelapse mortality (NRM) after alloHSCT in recent years, and therefore relapse has become the leading cause of death following alloHSCT. Furthermore, as inferred above, relapse remains the primary cause of death among patients surviving more than 2 years after alloHSCT [9]. Despite improved understanding of the biology that underlies the graft-versus-leukemia/tumor (GVL/GVT) effect, the relapse rate has not decreased over the past 20 years [10,11]. It is obvious that relapse after alloHSCT evolves from residual disease that escaped the preceding conditioning regimen as well as the graft-versus-malignancy effect.

New methodologic and technologic advances allow sensitive detection of MRD and early recognition of recurrence after alloHSCT. This is of clinical importance because intervention prior to florid relapse improves outcome for certain hematologic malignancies [12,13]. Standard diagnostic criteria that are widely employed in the definition of relapse for the different hematologic malignancies are based on morphologic bone marrow investigations, imaging, and/or specific laboratory findings. After alloHSCT, more sensitive methods, such as tumor-specific molecular primers, molecular genetics, fluorescence in situ hybridization (FISH), flow cytometry, and/or chimerism analysis, are commonly used to monitor patients with respect to relapse (Table 1).

Broadly, 2 different approaches are mainly used for the posttransplant surveillance of disease status: characterization of chimerism, and specific detection of MRD. The latter approach measures the malignant clone directly, whereas chimerism assessment characterizes the origin of posttransplant hematopoiesis. For chimerism as well as for specific detection of residual disease, a variety of techniques are available, although in general, there have been more studies looking directly at markers of residual tumor than of chimerism [14]. Despite the increasing sensitivity by the described methods of chimerism determination, because of its low specificity, this method is not a reliable means of detecting MRD. The specificity is higher in diseases that originate from a stem or progenitor cell (eg, AML, CML), whereas in B cell lymphoma or multiple myeloma, which originate from a late B cell stage of development, the specificity of chimerism to detect MRD or relapse is low. The lack of specificity might be overcome partly by performing lineage-specific chimerism in some diseases such as multiple myeloma [15].

A paradigm for the importance of minimal molecular disease and prediction of relapse after alloHSCT is CML. Here, it is now well established that the detection of the chimeric *BCR-ABL* mRNA transcript by reverse-transcriptase polymerase chain reaction (RT-PCR) is a powerful predictor of subsequent relapse [16]. The use of quantitative PCR has greatly increased the clinical value of monitoring MRD. It could be demonstrated that the kinetics of BCR-ABL level over time described impending relapse and response to donor lymphocyte infusion (DLI). Low or absence of residual BCR-ABL was associated with a very low risk of relapse (1%), compared to 75% relapse rate in CML patients with increasing or persistently high BCR-ABL levels [17]. The activating mutation V617F of the *JAK2* gene is an obvious target for monitoring MRD in patients with myeloproliferative disorders undergoing alloHSCT. There are emerging data suggesting that, similar to *BCR-ABL* in CML, PCR negativity for *JAK2-V617F* correlates with prolonged remission and that reappearance of a detectable *JAK2-V617F* clone is associated with relapse [18].

However, the utility of the available tools in the monitoring of disease status after alloHSCT has not yet been fully elucidated across all hematologic malignancies. In AML and myelodysplastic syndromes, several studies demonstrated the relevance of chimerism, and especially its kinetics, for the prediction of relapse. A variety of genetic markers are available for MRD in AML such as rearrangements t (15;17)/*PML-RARA*, inv(16)/*CBFB-MYH11*, and t(8;21)/*RUNX1-RUNX1T1*, *NPM1*, *FLT3*, or *MLL-PTD* but have not been studied in a larger cohort of patients.

Methods for MRD monitoring in B- or T-lymphoid malignancies include PCR techniques aiming to quantitatively detect disease specific T cell receptor (TCR) or immunoglobulin (Ig) gene rearrangements. Multiple studies support the independent prognostic value of MRD measurements in pediatric and adult patients with B- and T-lineage ALL. Furthermore, the risk of relapse appears to be proportional to the level of MRD, which in some studies was found to be the most powerful prognostic factor for relapse in

Table 1. Diagnostic Methods to Monitor Residual Disease and Relapse after Allogeneic Stem Cell Transplantation

Detection of Residual Disease (MRD)	Karyotyping	FISH	Flow Cytometry	Antigen Receptor PCR	Translocation or Other mRNA PCR	Chimerisms: XY FISH	Chimerism: qPCR/VNTR-PCR
Utility	Subset of all types of neoplasms with chromosomal abnormalities	Subset of all types of neoplasms with know chromosomal abnormality	ALL; most AML; CLL; myeloma	ALL; lymphoma, myeloma	CML; subset of ALL; subset of AML; subset of lymphoma	All types of neoplasms (sex mismatched SCT) Disadvantage not specific for MRD	All neoplasms (precondition differences in donor/ recipient polymorphisms) Disadvantage: not specific for MRD
Sensitivity	10^{-1}	10^{-2}	10^{-3}-10^{-4}	10^{-4}-10^{-5}	10^{-3}-10^{-6}	10^{-2}	10^{-3}-10^{-6}

qPCR indicates quantitative real-time PCR (modified after [6]); CML, chronic myeloid leukemia; ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; VNTR, variable number tandem repeat.

multivariate analyses [13]. Similarly, detection of pretransplant MRD in pediatric and some adult studies is highly predictive of relapse following alloHSCT and, coupled with posttransplant MRD evaluation, may guide early posttransplant intervention such as early withdrawal of immunosuppression, administration of DLI, or addition of posttransplant maintenance therapy (eg, targeted tyrosine kinase inhibition for Ph+ ALL).

In CLL, 2 main approaches of MRD assessment have been followed: flow cytometry, taking advantage of the unique immunophenotype of CLL, and PCR-based strategies using the clonal rearrangement of the hypervariable region of the V_H part of the immunoglobulin heavy chain gene (CDR3 region). Several studies showed that MRD assessment after alloHSCT is predictive for durable freedom from CLL progression if: (1) MRD levels are below 1×10^{-4} at 1 year posttransplant, or (2) show decreasing or stable kinetics within the quantitative range. The clinical impact of MRD detection in different lymphomas is not identical.

Specific chromosomal translocations detectable by PCR amplification, particularly t(11;14) and t(14;18) translocation, are present in mantle cell lymphoma and follicular lymphoma, respectively, but t(14;18) translocation is also detectable by PCR at low levels in 10% to 25% of healthy individuals. For Hodgkin lymphoma, neither cytogenetics, flow cytometry, nor molecular testing is helpful for assessing residual disease [19].

In multiple myeloma, MRD can be detected by PCR using patient-specific primers derived from the rearrangement of immunoglobulin heavy-chain genes. It could be shown that durable PCR-negativity after allografting had a cumulative risk of relapse at 5 years of 0%, in comparison to 33% for PCR-mixed patients and 100% for patients who never achieved PCR-negativity [20]

Ongoing and further clinical trials investigate whether sensitive MRD detection will allow for earlier therapeutic intervention, and it is hoped that treatment prior to overt relapse may improve outcome of allogeneic stem cell transplantation for hematologic malignancies.

STRATEGIES AND OPTIONS FOR RECURRENT DISEASE FOLLOWING ALLOSCT

The clinical significance of MRD after alloHSCT is different among diseases. MRD has been extensively studied using the qualitative PCR method during the early 1990s. Detection of BCR-ABL by PCR in the first year after alloHSCT for CML patients disappears in the majority of patients, secondary to ongoing GVT

effects; however, detection of MRD after alloHSCT for Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ALL) is indicative of imminent hematologic relapse [21-24]. In the case with t(8;21) AML, MRD after chemotherapy does not always indicate eventual clinical relapse. In the last decade, quantitative PCR machines are widely available, and sequential and quantitative tests of leukemic genes have become available. With this technique, a rise in the amount of leukemic genes strongly suggests clinical relapse in the near future. Also, several investigators have tried to find thresholds for the amount of genes that are predictive of clinical relapse. However, because of a lack of standardization of this technique, hitherto universal threshold has not been clarified at any leukemia with the possible exception of CML.

Clinical Intervention

Because of the limitation of quantitative PCR as mentioned above, clinical intervention upon the emergence of MRD has not been well established. Clinical interventions for early relapse and MRD after alloHSCT are performed in 2 ways; 1 is adoptive immunotherapy including DLI and vaccination, and the other is administration of new agents, which are expected to preserve normal hematopoietic cells. Several questions are raised in this clinical setting. First, does early intervention have more clinical effects than the intervention performed at hematological relapse? Second, does clinical intervention affect the other parameters such as graft-versus-host disease (GVHD), related adverse events, and the subsequent alloHSCT. Third, which is the better way, prophylactic administration or intervention upon MRD, for patients with a high risk of relapse?

Adoptive Immunotherapy

DLI was first developed for relapsed patients. Although they are dramatically effective for CML, DLI remains limited of limited utility for patients with other diseases because of inadequate responses and toxicity related to GVHD, which occurs in one-third of patients. As a strategy to reduce the incidence and severity of GVHD while preserving the GVL effect, tumor-specific DLI are proposed [25]. A protocol to generate hematopoietic cell–specific minor antigen (eg, HA-1, HA-2, ACC-1) specific T cell lines from mHag-negative donors was studied for adoptive immunotherapy. Warren et al. [26] conducted a phase I/II study to test the toxicity and effectiveness of CTL clones specific for minor H antigens. However, this strategy using cloned antigen-specific T cells has been shown to be ineffective mostly because these cells could not survive long enough to execute their cytotoxic ability in vivo. This problem could be overcome by: (1) infusion of a relatively young and small number of memory T cells without extensive expansion in vitro, and (2) infusion of autologous peripheral blood T cells transduced retrovirally with T cell receptor α and β cDNA cloned from tumor/minor antigen–specific T cell clones [27]. The latter approach has been shown to be promising in the setting of melanoma treatment in studies conducted by Rosenberg and colleagues at the National Cancer Institute [28]. Thus, T cells armed with TCR specific for WT-1, HA-1, HA-2, and ACC-1 would be great candidates for adoptive immunotherapy in the very near future. Another approach studied intensively in the clinical hematology field is a vaccination using epitope peptides such as WT-1, PR3, MUC-1, NY-ESO-1, and BCR-ABL fusion polypeptides. In particular, WT-1 is one of the most promising tumor antigens because WT1 vaccination-driven immunologic responses and clinical responses, including reduction of leukemic cells, and the reduction of the M-protein amount in myeloma, have been reported. Further enhancement of the efficacy of the WT1 peptide vaccine can be expected by coadministration of WT1-specific helper peptide, Th1-inducing adjuvant, or immunosuppressive chemotherapy prior to vaccinations to take advantage of inhibition of regulatory T cells and facilitation of homeostatic expansion of desired T cells. Adoptive immune therapies as prophylaxis or preemptive therapy would be performed in the near future.

New Agents

Chemotherapy for the patients with recurrent disease is hampered by the fact that these agents impel the normal hematopoietic cells, as well as the fact that tumor cells and tumor-specific agents have long been desired. Recently, a new molecular-specific targeting agent has been developed. The specific manner of these new agents prompts us to use them for earlier interventions. Nevertheless, most of these tumor-specific agents exert some effects on normal hematopoietic cells and interfere with immunologic functions after alloHSCT.

Tyrosine kinase inhibitors

Philadelphia chromosome-positive ALL is associated with highly aggressive disease. Although alloHSCT is at present the only curative treatment option, hematologic relapse still remains a major obstacle. Recently, there have been some reports of posttransplant imatinib administration, but its efficacy and administration methods are still controversial. Nishiwaki and colleagues [29] compared prophylactic administration of imatinib with intervention upon molecular relapse to evaluate the effect of posttransplant imatinib administration. MRD became positive in both groups, leading to hematologic relapse. It was therefore concluded that

posttransplant imatinib administration may not be an ideal prophylactic treatment for Ph+ALL patients. In contrast, Ottmann et al. [30] demonstrated that all Ph+ALL patients who received imatinib upon appearance of BCR-ABL and promptly achieved molecular response remained in remission for the duration of imatinib treatment.

Bortezomib

Recently, both conventional chemotherapy and autologous and alloHSCT combined with new agents, such as thalidomide, lenalidomide, and bortezomib, have improved the depth of response and survival of multiple myeloma patients. However, after transplantation, most patients still harbor residual disease. Ladetto et al. [31] reported the effect of posttransplant consolidation including bortezomib on MRD detected by PCR using tumor-clone–specific primers. Molecular remissions were achieved in 3% of patients after autologous HSCT and 18% after consolidation with bortezomib. It has been proposed that bortezomib increases the expression of Fas and DR5 and enhances GVT effects, and that this agent also suppresses the activity of NFkB, resulting in reduction of inflammatory cytokines related to graft-versus-host activity [32].

Lenalidomide

Lenalidomide is an immunomodulatory drug (IMiD) that has multiple effects on myeloma cells and their microenvironment. Administration of IMiDs for postautologous HSCT maintenance resulted in prolonged progression-free survival (PFS) even in patients who achieved very good partial response or complete response before lenalidomide administration. In the alloHSCT setting, lenalidomide plus low-dose dexamethasone combination therapy have shown significant disease and chronic GVHD (cGHVD) control for myeloma patients, who relapsed after transplantation [33]. GVHD control with IMiD is still controversial but a very attractive issue for investigation [34].

Hypomethylating agents

Low-dose 5-azacitidine (5-Aza) was used by investigators at the M.D. Anderson Cancer Center for patients with AML/MDS as a maintenance therapy or salvage therapy upon relapse after alloHSCT; an overall survival rate of 90% at 1 year was reported [35]. Additive effects of DLI to 5-Aza were also reported. The administration of 5-Aza was not associated with an increased incidence of GVHD. Sanchez-Abarca et al. [36] reported that 5-Aza inhibits T cell proliferation and activation, blocking the cell cycle in the G0 to G1 phase and decreasing the production of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ). They also reported that administration of 5-Aza after transplantation prevented the development of GVHD, leading to a significant increase in survival in a fully mismatched bone marrow transplantation mouse model. Recently, decitabine, another DNA hypomethylating agent, was reported to be used in patients experiencing cytogenetic relapse after alloHSCT [37].

Humanized monoclonal antibodies

Rituximab (anti-CD20 monoclonal antibody) was used for 9 chronic lymphocytic leukemia patients who had persistent disease after alloHSCT and underwent immuno-manipulation to augment GVT effects including immunosuppression withdrawal and DLI with rituximab treatment, and 8 patients had a complete response [38]. Alemtuzumab (anti-CD52 monoclonal antibody), as well as antithymocyte globulin (ATG), has been used as a T cell depletion method in alloHSCT. Because it is reported that the majority of precursor B-ALL blasts express CD52, and CD52 is expressed on other ALL cells, alemtuzumab is considered to potentially contribute to the eradication of MRD [39].

Summary on the Treatment of MRD

For decades, interventions for relapsed patients have been performed using DLI and chemotherapies; however, they are a 2-edged sword, hampering normal hematopoietic cells as well as tumor cells. Recently, the emergence of new strategies using tumor-specific DLI and tumor-specific new agents has prompted us to use these methods before clinical relapse. Some of them are used as prophylaxis, and some of them are used upon tumor emergence at molecular level. Trials confirming these strategies are just beginning, and there is a need for the definition of MRD. Thus, it is becoming more and more important that the measurement of MRD becomes standard practice; otherwise, clinical studies will be somewhat meaningless.

NATIONAL CANCER INSTITUTE FIRST INTERNATIONAL WORKSHOP ON THE BIOLOGY, PREVENTION, AND TREATMENT OF RELAPSE AFTER ALLOHSCT

As stated above, there is a strong association of MRD with relapse following alloHSCT. The growing recognition of relapse as one of the most significant posttransplant problems led to the organization and convening of the National Cancer Institute First International Workshop on the Biology, Prevention, and Treatment of Relapse after AlloHSCT [40]. The primary objectives of the Workshop were to review the current "state-of-the-science" relative to the biology, natural history, prevention, and treatment, and identify the most important biological and clinical questions that need to be addressed relative to relapse following alloHSCT.

The Workshop, which took place on November 2 and 3, 2009, in Bethesda, Maryland, USA, brought together an international group of more than 200 basic and clinical researchers. Over 50 formal presentations were made by the Workshop committee members that addressed both GVT and non-GVT biology, relapse epidemiology, and natural history, strategies, and therapies for prevention, disease-specific methods, and strategies for monitoring, and disease-specific treatment of relapse following alloHSCT. These presentations are available for viewing at https://ccrod.cancer.gov/confluence/display/NCIRelapse/Presentations+from+Workshop. Each of the 6 workshop committees subsequently prepared a "state-of-the-science" manuscript, which contained their commended research priorities; these manuscripts were published sequentially during 2010 in the *Biology of Blood and Marrow Transplantation* [1,14,19,41-44].

The central Workshop theme was that in its most simplistic form, relapse occurs because tumor cells are first able to resist the cytotoxic effects of the conditioning regimen. These surviving cells either never respond to initial GVT or they subsequently escape from GVT effects after initial control.

Central and recurrent research themes included the necessity to establish biorepositories to collect and store tumor samples before transplant when possible, and after transplant, store samples from allografts for analysis, and collect blood and serum samples at set posttransplant time points and at the time of relapse for study of immunology related to relapse. Second, there is a need for more careful study of the natural history of relapse for specific diseases, particularly in regard to MRD. To perform such studies, there needs to be international acceptance of standard definitions and techniques; it is hoped that the definitions and techniques proposed by the Workshop will be considered for this purpose. Finally, there needs to be multi-institutional collaboration in regard to prevention and treatment of relapse after alloHSCT. A formal summary of the workshop recommendations will be presented during the 2011 Tandem Transplant Meetings Educational Sessions.

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Reduced-intensity versus conventional myeloablative conditioning for patients with Philadelphia chromosome –negative acute lymphoblastic leukemia in complete remission

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To the editor:

Reduced-intensity versus conventional myeloablative conditioning for patients with Philadelphia chromosome–negative acute lymphoblastic leukemia in complete remission

We read with interest the results of the comparison between reduced-intensity conditioning (RIC) and conventional myeloablative conditioning (MAC) allogeneic stem cell transplantation (allo-SCT) for patients with acute lymphoblastic leukemia (ALL) in complete remission (CR), reported by Mohty et al.[1] They concluded that RIC allo-SCT was a potential therapeutic option for ALL.

Although we agree in general with their conclusion, our major concern is that the cytogenetic background between MAC and RIC might differ. Adjustment for cytogenetic risk groups was not performed in a multivariate analysis, because there was no difference in the cytogenetic distribution between MAC and RIC when analyzed among 3 risk groups: t(9;22), t(4;11), or other ($P = .10$). However, when analyzed among 4 groups, including NA/failed as one group, a significant difference was noted between MAC and RIC ($P = .02$). In fact, the number of Philadelphia chromosome–positive [Ph^+] ALL was smaller in MAC than in RIC [104/449 (23%) vs 41/127 (32%), $P = .049$]. Since the relapse incidence (RI) was higher among Ph^+ ALL patients than in the whole study population (40% ± 5% vs 31% ± 2% in MAC, and 49% ± 9% vs 47% ± 5% in RIC), lower RI in MAC might be associated with a smaller number of Ph^+ ALL. Allo-SCT has been recognized as the only curative therapy for Ph^+ ALL,[2] and there are already several reports of RIC allo-SCT for Ph^+ ALL.[3,4] It may be better to treat Ph^+ ALL and Philadelphia chromosome-negative (Ph^-) ALL as different diseases because their treatment would differ in an era of tyrosine kinase inhibitors. Therefore, it would be more practical to present data only from patients with Ph^- ALL.[5]

The results of our 121 HLA-matched allo-SCT for adult Ph^- ALL in first (81 MAC, 21 RIC) or second (14 MAC, 5 RIC) CR for patients aged ≥ 45 years (between 1998 and 2007 using the Japan Society for Hematopoietic Cell Transplantation and the Japan Marrow Donor Program database) were comparable between MAC and RIC (Figure 1A-D). In a multivariate analysis, RIC was not a significant risk factor for relapse (Hazard ratio [HR] 1.66, 95% confidence interval [CI] 0.63-4.37, $P = .30$). The variables considered in our multivariate analyses were conditioning (MAC vs RIC), age (> 50 years vs ≤50 years), sex, white blood cell counts (< 30 000/μL vs ≥30 000/μL), lineage (T vs B), disease status (first vs second CR), donor source (sibling vs unrelated), and graft-versus-host disease prophylaxis (cyclosporine-based vs tacrolimus-based). Similarly, RIC posed no significant risk factor for leukemia-free survival (HR 1.00, 95% CI 0.51-1.96, $P = .99$), nonrelapse mortality (HR 1.05, 95% CI 0.53-2.05, $P = .89$), and overall survival (HR 1.06, 95% CI 0.64-2.07, $P = .87$).

There are several ways to deal with missing data.[6] Given that the cytogenetics of 54% (244/449) for MAC and 43% (55/127) for RIC were missing in the study by Mohty et al, differences in how to handle missing data may produce different results. Because there was a difference in the cytogenetic distribution between MAC and RIC when analyzing missing data as one category, data adjusted for cytogenetic risk groups, or those of Ph^- ALL, are of considerable interest.

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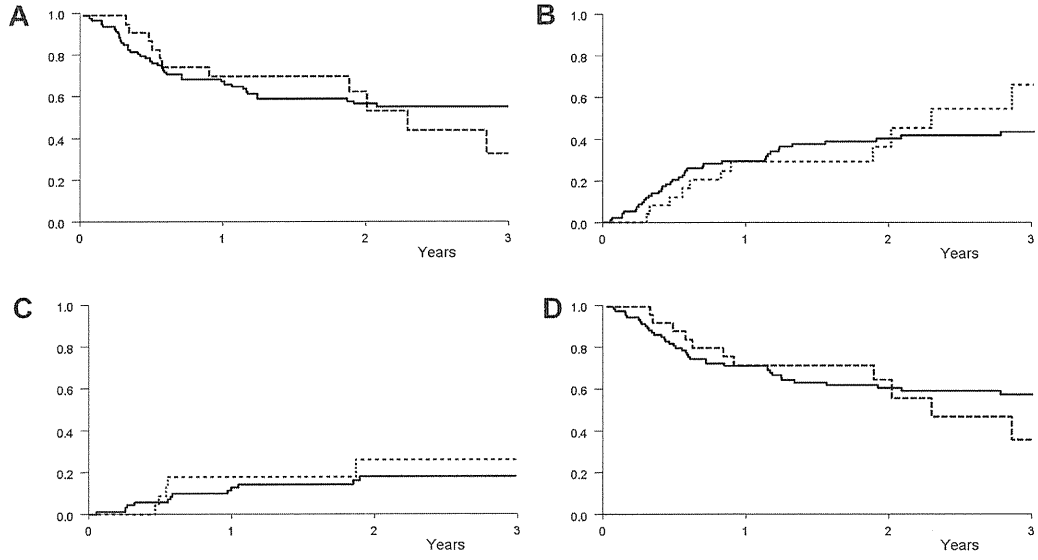

Figure 1. Survival probabilities. (A) Leukemia-free survival according to conditioning regimen: 58% ± 5% in myeloablative conditioning (MAC) vs 63% ± 11% in reduced-intensity conditioning (RIC) at 2 years ($P = .90$). (B) Nonrelapse mortality according to conditioning regimen: 40% ± 5% in MAC vs 36% ± 11% in RIC at 2 years ($P = .79$). (C) Relapse incidence according to conditioning regimen: 18% ± 5% in MAC vs 26% ± 11% in RIC at 2 years ($P = .27$). (D) Overall survival according to conditioning regimen: 59% ± 5% in MAC vs 63% ± 11% in RIC at 2 years ($P = .82$). Solid curve indicates MAC; dashed curve, RIC; x-axis, years after transplantation; and y-axis, probability.