Anti-leukemic effect of graft-versus-host disease on bone marrow and extramedullary relapses in acute leukemia

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Background and Objectives. Several reports have suggested that the graft-versus-leukemia effect at extramedullary (EM) sites might be less prominent than that in the bone marrow (BM). We analyzed the effect of graft-versus-host disease (GVHD) on BM and EM relapses in 194 consecutive patients with acute leukemia who underwent allogeneic hematopoietic cell transplantation at a single institute.

Design and Methods. We compared relapse-free survival (RFS), BM RFS, and EM RFS after allografting according to the occurrence of GVHD. We also investigated the clinical outcome of patients who relapsed after their allogeneic transplantation.

Results. Relapse occurred in 65 patients; in 41 (63%) relapse occurred in the BM only, in 9 (14%) it occurred in both BM and EM sites, and 15 (23%) in EM sites only. Patients who developed acute GVHD after transplantation had significantly higher relapse-free survival (69.2% vs. 52.4%; p=0.042) and BM RFS (80.7% vs. 59.1%; p=0.030) compared to those who did not. However, EM RFS was similar between patients with and without acute GVHD (76.7% vs. 78.2%; p=0.744). Among the 65 patients who relapsed, 32 patients attained complete remission with salvage treatments and 22 experienced a second relapse, which occurred in the BM (n=9), BM and EM sites (n=1), or EM sites (n=12).

Interpretations and Conclusions. Our study confirms that GVHD after allogeneic hematopoietic cell transplantation has an anti-leukemic effect, thus preventing relapse; however, it may be less effective in preventing EM relapse.

Key words: allogeneic HCT, AML, extramedullary relapse, GVHD, anti-leukemic effect.

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The curative potential of allogeneic hematopoietic cell transplantation for acute leukemia is, in part due to the graft-versus-leukemia effect produced by allogeneic immune cells. The existence of a graft-versus-leukemia effect has been demonstrated by numerous clinical observations as well as animal models.[1] In humans, the graft-versus-leukemia effect has been associated with the presence of graft-versus-host disease (GVHD)[2-4] although efforts to separate the two have been made.[3,5] Relapse rates after allogeneic hematopoietic cell transplantation are significantly decreased in patients with GVHD[2-4] and rare cases have been reported in which a second remission was concurrently induced with onset of clinically significant GVHD.[6-8] Relapse remains the major cause of treatment failure after allogeneic hematopoietic cell transplantation for acute leukemia.[9,10] It usually occurs in the bone marrow (BM), however, compared to non-transplant leukemic treatments, a high frequency of extramedullary (EM) relapse has been observed.[11,12] In several retrospective series, 7% to 46% of total relapses occurred in EM sites, with or without BM involvement.[13-18] EM relapses occurred in extremely diverse sites[12,19,20] and the median time from hematopoietic cell transplantation to relapse was significantly longer for EM relapse than for BM relapse only.[13,17,18,21] EM relapses, despite continued hematologic remission, have also been reported after donor-leukocyte infusion for the treatment of post-transplant relapses.[8,22-26] These observations suggest that the graft-versus-leukemia effect at EM sites may be less prominent than that in the BM.[22,23,27,28]

To prove this hypothesis, we investigated the anti-leukemic effect of GVHD on BM and EM relapses in 194 consecutive patients who underwent allogeneic hematopoietic cell transplantation for acute leukemia at a single institute.

Design and Methods

Patients

Between March 1995 and August 2004, 129 patients with acute non-lymphoblas-

tic leukemia and 65 patients with acute lymphoblastic leukemia underwent allogeneic hematopietic cell transplantation at the Asan Medical Center, College of Medicine University of Ulsan, Seoul, Korea. Table 1 shows the patients' characteristics. At the time of transplantation, 151 patients were in their first complete remission, 21 patients were in their second or third complete remission, and 22 patients had either relapsed (n=15), refractory (n=3), or untreated (n=4) leukemia. Cytogenetic risk groups were classified according to cytogenetic results at the time of diagnosis. The poor-risk group (n=62) was defined as having chromosomal abnormalities of an unfavorable risk category determined by SWOG criteria for patients with acute non-lymphoblastic leukemia,[29] or as having a t(9;22), t(4;11), or complex abnormalities (three or more unrelated abnormalities) for patients with acute lymphoblastic leukemia. Seven patients had a history of treatment for central nervous system leukemia prior to their transplantation. The hematopoietic cell donor was a sibling for 135 patients, an unrelated volunteer for 55, a haplo-identical family member for 3, and cord blood for 1 patient. All patients and donors gave informed consent to all procedures involved in the hematopietic cell transplantation. All research programs were approved by the institutional review board of the Asan Medical Center, Seoul, Korea.

Transplantation procedure

For conditioning therapy, 170 patients received busulfan plus cyclophosphamide and 24 received busulfan, fludarabine, plus antithymocyte globulin (Table 1). For the busulphan-cyclophosphamide regimen, before August 2002, busulfan was administered orally (4 mg/kg/day on days -7 to -4; n=116), and thereafter intravenously (3.2 mg/kg/day on days -7 to -4; n=54); cyclophosphamide was administered intravenously to all patients (60 mg/kg on days -3 and -2). For the busulphan-fludarabine-antithymocyte globulin regimen, busulfan (4 mg/kg/day orally or 3.2 mg/kg/day intravenously) was given for two days, except in one patient, who received busulfan for four days prior to cord blood transplantation. In addition to busulfan, fludarabine (30 mg/m^2) was given for six days and antithymocyte globulin (Atgam® 30 mg/kg/day or Thymoglobuline® 1.5-3 mg/kg/day) for three or four days.

In 183 patients, donor BM grafts were received on day 0, and in 10 patients, peripheral donor blood hematopoietic cells mobilized with granulocyte colony-stimulating factor (10 μg/kg/day subcutaneously for 4 days) were received on days 0 and 1. The hematopoietic cell grafts were not T-cell-depleted. All patients received prophylactic therapy for GVHD with cyclosporine only (n=47), or cyclosporine plus methotrexate (n=147). Cyclosporine (1.5 mg/kg) was given intravenously every 12 hours starting on day –1 and then orally once oral intake became feasible. Intravenous methotrexate was given at a dose of 15 mg/m^2 on day 1 and at 10 mg/m^2 on days 3, 6, and 11. The day 11 dose was omitted for patients conditioned with the busulphan-fludarabine-antithymocyte globulin regimen for matched sibling donor hematopietic cell transplantation. For prevention of hepatic veno-occlusive disease, patients conditioned with the busulphan-cyclophosphamide regimen were given heparin (100 units/kg/day) on days -7 to 30; they were also treated with mesna and hyperhydration with normal saline to prevent hemorrhagic cystitis. All patients received intravenous granulocyte colony-stimulating factor (450

Table 1. Characteristics of the patients and donors.

Age, year, median (range)	33 (15-57)
Sex, male vs. female	103 (53%)/91 (47%)
Diagnosis, ANLL vs. ALL	129 (67%)/65 (33%)
Disease status at HCT, CR1 vs. > CR1	151 (78%)/43 (22%)
Cytogenetic risk group*, other vs. poor	119 (66%)/62 (34%)
History of CNS treatment, no vs. yes	187 (96%)/7 (4%)
Pre-HCT transfusion, unit, median (range)	94 (0-1190)
Karnofsky performance score, ≤80 vs. ≥90	28 (14%)/166 (86%)
History of liver disease, no vs. yes	154 (79%)/40 (21%)
Hepatitis B surface antigen, negative vs. positive	181 (93%)/13 (7%)
Graft donor, sibling vs. unrelated volunteer vs. haplo-identical family vs. cord blood	135 (69%)/55 (28%) /3 (2%)/1(1%)
Donor-recipient ABO mismatch, no vs. yes	98 (51%)/96 (49%)
Donor-recipient sex pair, female to male vs. others	40 (21%)/154 (79%)
Conditioning regimen, Bu-Cy vs. Bu-Fludara-ATG#	170 (88%)/24 (12%)
GVHD prophylaxis, CSA/MTX vs. CSA only	147 (76%)/47 (24%)
Mononuclear cell dose, $\times10^8$/kg, median (range)	0.87 (0.25-13.67)
CD34+ cell dose, $\times10^6$/kg, median (range)	3.88 (0.01-73.50)

*ANLL: acute non-lymphoblastic leukemia; ALL: acute lymphoblastic leukemia; HCT: hematopoietic cell transplantation; CR1: first complete remission; CNS: central nervous system; Bu-Cy: busulfan plus cyclophosphamide; Bu-Fludara-ATG: busulfan, fludarabine, plus antithymocyte globulin; CSA: cyclosporine; MTX: methotrexate. *cytogenetic risk group was classified according to the cytogenetic results at the time of diagnosis of acute leukemia; #eight patients received matched sibling transplantations, 3 haplo-identical transplantations, 12 unrelated donor transplantations, and 1 cord blood transplantation.*

μg, once daily) starting on day 0 or 5, until the peripheral blood absolute neutrophil count was over 3000/μL.

Monitoring of patients

The first day with an absolute neutrophil count of 500/μL or more for two consecutive days was recorded as the day of bone marrow engraftment. The first day of unsupported platelet counts of 20,000/μL or more for 7 consecutive days was also recorded. All patients were prospectively monitored for post-transplant toxicities, including GVHD, hepatic veno-occlusive disease, infections, and other transplantation-related toxicities. Acute and chronic GVHD was diagnosed on the basis of clinical symptoms, laboratory tests, and whenever possible, histopathological findings of the skin, oral mucosa, liver, or gastrointestinal tract,[30,31] and was classified according to clinical criteria.[32,33]

Hepatic veno-occlusive disease was diagnosed according to clinical criteria[34] and its severity was classified as mild, moderate or severe.[35] Cytomegalovirus infection was monitored weekly by shell vial culture[36] until July 1997, thereafter, both shell vial culture and the cytomegalovirus antigenemia assay were used.[37,38] Ganciclovir (5 mg/kg every 12 hours) was initiated when cytomegalovirus infection or disease was documented.

Statistical analysis

Categorical values were compared by the χ^2 test. Median times to BM relapse, BM relapse with simultaneous EM relapse (BM/EM relapse), and EM relapse were compared by the Kruskal-Wallis test. The probabilities of overall survival and relapse-free survival (RFS) were calculated by the Kaplan-Meier method[39] and compared by a log-rank test.[40] For the calculation of RFS, patients who died without relapse were censored at the time of death. Patients who relapsed at EM sites only or in the BM only were censored at the time of their relapse for calculation of BM RFS and EM RFS, respectively. Relapse was defined as at least 5% leukemic blasts in a BM aspirate (BM relapse) or new extramedullary leukemia (EM relapse).

For variables that change with time after hematopoieic cell transplantation such as GVHD, the effects of the variables on survival were analyzed in a time-dependent fashion in Cox regression models.[41] For multivariate analysis of independent prognostic factors for survival, the Cox proportional hazards regression model was used.[42]

A multiple logistic regression analysis was used for multivariate analysis of prognostic factors for complete remission rate after salvage treatment for relapsed patients.[43] For the analysis of cumulative incidence of relapse, patients alive without relapse were censored, whereas those who died without relapse were counted as a competing cause of failure.[44] For the analysis of cumulative incidence of non-relapse mortality, patients who relapsed were counted as a competing cause of failure. The cumulative incidence was calculated and compared by Gray's method.[45]

Results

Post-transplant outcomes

All patients, except three who died early after transplantation, achieved an absolute neutrophil count over 500/μL at a median of day 15. Transfusion-independent platelet counts over 20×10^3/μL were achieved in 173 of 194 patients at a median of day 26. Acute GVHD occurred in 53 (28%) of 192 evaluable patients at a median of day 27; it was grade I in 21, grade II in 15, grade III in 10, and grade IV in 7. Chronic GVHD occurred in 75 (45%) of 166 evaluable patients at a median of day 133; it was limited in 27 patients and extensive in 48. Seventy-five patients (39%) experienced cytomegalovirus infection, however only 7 (4%) developed cytomegalovirus disease. Interstitial pneumonia occurred in 9 patients (5%) and hepatic veno-occlusive disease developed in 71 patients (37%) at a median of day 10. Ten of 71 patients with veno-occlusive disease had severe disease and 8 died of this complication. After a median follow-up of 2.8 years (range, 0.3 to 9.6 years) among surviving patients, 65 patients had relapsed and 76 had died. Of these deaths, 30 were not related to leukemia relapse. The causes of 30 non-relapse deaths were engraftment failure (n=2; one with interstitial pneumonia), acute GVHD (n= 6; one with veno-occlusive disease), chronic GVHD (n= 4; one with interstitial pneumonia), interstitial pneumonia (n=7; one with engraftment failure and one with chronic GVHD), veno-occlusive disease (n=8; one with acute GVHD), bleeding (n=4) and others (n=2). The 5-year probabilities of overall and relapse-free survival were 58.3% and 56.0%, respectively, and the 5-year cumulative incidences of relapse and non-relapse mortality were 38.0% and 18.5%, respectively. Of 65 relapses, 41 (63%) occurred in BM only, 9 (14%) in both BM and EM sites, and 15 (23%) in EM sites only. The involved EM sites were bone/peri-osseous soft tissue (n=2), brain (n=3), breast (n=4), breast/subcutaneous tissue (n=1), leptomeninges (n=4), spinal cord (n=1), head and neck soft tissue (n=2), intestine (n=1), intra-abdomen (n=1), pelvis (n=1), mediastinum/pleura (n=1), and skin/subcutaneous tissue (n=3). The 5-year cumulative incidence of BM relapse was 28.0% and that of EM relapse was 15.7%.

Effects of acute and chronic GVHD on BM and EM relapses after hematopoietic cell transplantation

Table 2 shows the RFS and cumulative incidence of relapse according to the occurrence of acute and chron-

Table 2. Relapse-free survival and cumulative incidence of relapse according to the occurrence of acute and chronic GVHD.

	5-year probabilities					
	RFS	*BM RFS*	*EM RFS*	*CI of relapse*	*CI of BM relapse*	*CI of EM relapse*
Acute GVHD						
No	52.4	59.1	78.2	43.7	34.0	15.0
Yes	69.2	80.7	76.7	22.8	11.4	18.6
p value	0.042*	0.030*	0.744*	0.014#	0.005#	0.475#
Chronic GVHD						
No	55.5	63.6	79.7	42.3	31.4	15.7
Yes	60.1	66.5	76.0	37.1	26.9	19.4
p value	0.776*	0.789*	0.672*	0.231#	0.233#	0.666#

*RFS, relapse-free survival; BM, bone marrow; EM, extramedullary; CI: cumulative incidence. *For GVHD that changed with time after HCT, effects of GVHD on survival were analyzed in a time-dependent fashion in the Cox regression models. #Cumulative incidence was compared by Gray's method.*

ic GVHD. Patients who developed acute GVHD after allografting had significantly higher RFS (69.2% vs. 52.4%; p=0.042) and BM RFS (80.7% vs. 59.1%; p=0.030) compared to those who did not, whereas EM RFS (76.7% vs. 78.2%; p=0.744) was similar between patients with and without acute GVHD (Figure 1). The cumulative incidences of relapse (22.8% vs. 43.7%; p=0.014) and BM relapse (11.4% vs. 34.0%; p=0.005) were lower in patients with acute GVHD than in those without acute GVHD, whereas the cumulative incidence of EM relapse (18.6% vs. 15.0%; p=0.475) was similar in patients with and without acute GVHD. In contrast to acute GVHD, chronic GVHD had no significant influence on relapse, BM relapse, or EM relapse (Table 2 and Figure 2). A separate analysis of acute non-lymphoblastic and acute lymphoblastic leukemia showed that the anti-leukemic effects of GVHD were much more prominent in acute non-lymphoblastic leukemia than in acute lymphoblastic leukemia. In patients with acute non-lymphoblastic leukemia, relapse and BM relapse occurred less frequently in patient with acute GVHD (RFS, 86.9% vs. 66.2%, p=0.035; BM RFS, 78.8% vs. 58.0%, p=0.056; EM RFS, 85.0% vs. 80.2%, p=0.420) as well as those with chronic GVHD (RFS, 75.2% vs. 56.9%, p=0.096; BM RFS, 81.7% vs. 66.1%, p=0.335; 83.9% vs. 79.5%, p=0.443). In contrast, we did not find anti-leukemic effects of GVHD in acute lymphoblastic leukemia.

Prognostic factors for survival

Table 3 shows the results of multivariate analyses to define independent prognostic factors for overall survival, RFS, BM RFS, and EM RFS. Poor-risk cytogenetics was an independent poor prognostic factor for overall survival (odds ratio, 1.899; p=0.012), RFS (odds ratio, 1.961; p=0.010), BM RFS (odds ratio, 1.941; p=0.026), and EM RFS (odds ratio, 2.316; p=0.064). Disease status at time of transplantation was an independent prognostic factor for RFS (odds ratio, 3.141; p<0.001), BM RFS (odds ratio, 2.538; p=0.003), and EM RFS (odds ratio, 4.242; p=0.001). A history of central nervous system treatment (odds ratio, 3.303; p=0.005) and the occur-

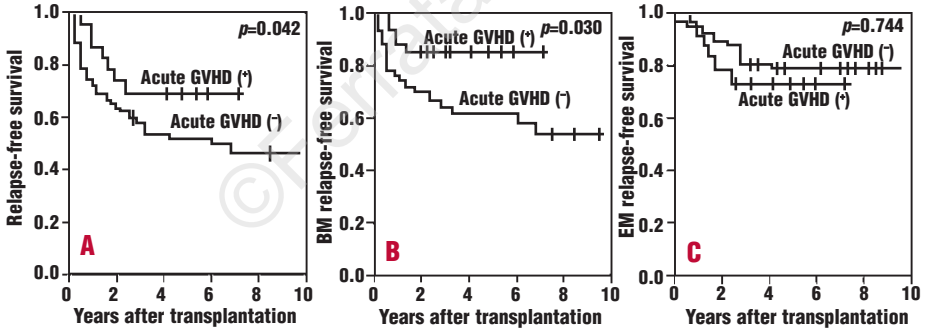

Figure 1. Relapse-free survival (RFS) according to the occurrence of acute GVHD. Patients who developed acute GVHD after hematopoietic cell transplantation had significantly higher RFS (A) and BM RFS (B) compared to those who did not, whereas EM RFS (C) was similar in patients with and without acute GVHD.

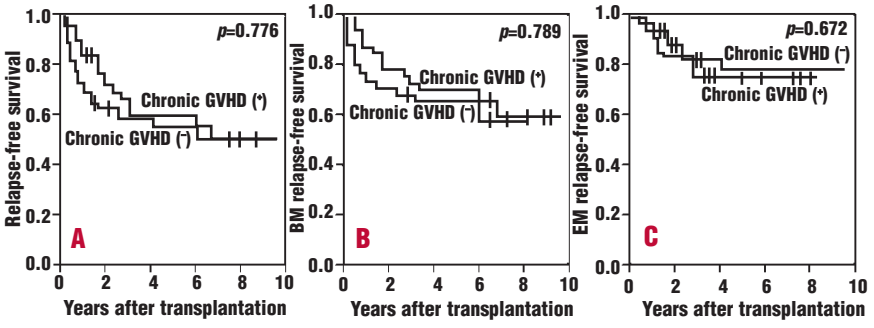

Figure 2. Relapse-free survival (RFS) according to the occurrence of chronic GVHD. Patients who developed chronic GVHD after hematopoietic cell transplantation did not show any significant differences in RFS (A), BM RFS (B), or EM RFS (C) compared to those who did not.

Table 3. Multivariate analysis of prognostic factors for overall survival, relapse-free survival, bone marrow relapse-free survival, and extramedullary relapse-free survival.

Variable	OR	95% CI	p value
Overall survival			
History of CNS treatment, no vs. yes	3.303	1.439-7.581	0.005
Interstitial pneumonitis, no vs. yes	4.683	1.988-11.034	<0.001
Hepatic VOD, no vs. yes	1.800	1.129-2.870	0.013
Cytogenetic risk group*, other vs. poor	1.899	1.155-3.124	0.012
Relapse-free survival			
Acute GVHD, no vs. yes	0.421	0.191-0.928	0.032
Disease status at HCT, CR1 vs. > CR1	3.141	1.855-5.318	<0.001
Cytogenetic risk group*, other vs. poor	1.961	1.179-3.263	0.010
Bone marrow relapse-free survival			
Acute GVHD, no vs. yes	0.302	0.108-0.845	0.022
Disease status at HCT, CR1 vs. > CR1	2.538	1.366-4.713	0.003
Cytogenetic risk group*, other vs. poor	1.941	1.084-3.475	0.026
Extramedullary relapse-free survival			
History of CNS treatment, no vs. yes	4.807	1.268-18.226	0.021
Disease status at HCT, CR1 vs. > CR1	4.242	1.788-10.064	0.001
Cytogenetic risk group*, other vs. poor	2.316	0.952-5.634	0.064

*OR: odds ratio; CI: confidence interval; HCT: hematopoietic cell transplantation; CR1: first complete remission; CNS: central nervous system; VOD: veno-occlusive disease; GVHD: graft-versus-host disease. *Cytogenetic risk group was classified according to the cytogenetic results at the time of diagnosis of acute leukemia.*

rence of interstitial pneumonia (odds ratio, 4.683; p<0.001) and hepatic veno-occlusive (odds ratio, 1.800; p=0.013) had poor prognostic impacts on overall survival. The occurrence of acute GVHD had a favorable influence on RFS (odds ratio, 0.421; p=0.032) and BM RFS (odds ratio, 0.302; p=0.022), but not on EM RFS.

Clinical features and outcome of patients who relapsed after hematopoietic cell transplantation

Table 4 shows the patients' characteristics according to the site of relapse. All of the patients who relapsed and had a history of central nervous system treatment prior to hematopoietic cell transplantation (n=3), had isolated EM relapses. Patients with BM/EM or EM relapse were more likely to have had acute GVHD before relapse than those with BM relapse only; 33% and 27% vs. 5%, respectively (p=0.021). The median time to BM relapse, BM/EM relapse, and EM relapse was 193 days (range, 24-2508 days), 369 days (range, 147-1035 days), and 508 days (range, 38-1498 days), respectively (p=0.030 by the Kruskal-Wallis test). All but one patient received at least one kind of salvage treatment for relapse of leukemia. Nine patients, who were under prophylactic GVHD treatment at the time of relapse, abruptly withdrew their immunosuppressive treatment. Salvage chemotherapy was given to 30 patients with donor lymphocyte infusions and to 20 patients without such infusions; one patient received donor lymphocyte infusion without chemotherapy. Of patients with BM/EM or EM relapse, five received

Table 4. Patients' characteristics according to relapse pattern.

Characteristic	Relapse site BM	BM/EM	EM	p value
Age, year, median	35	21	31	0.126#
Sex, male/female	19/22	6/3	5/10	0.284$
Diagnosis, ANLL vs. ALL	25/16	5/4	9/ 6	0.956$
Disease status at HCT, CR1 vs. > CR1	27/14	7/2	8 / 7	0.523$
Cytogenetic risk group*, other vs. poor	23 / 16	3 / 5	9 / 6	0.510$
History of CNS treatment, no vs. yes	41 / 0	9 / 0	12 / 3	0.005$
Graft donor, sibling vs. unrelated volunteer vs. haplo-identical family vs. cord blood	27/11 /2/1	7/2/ 0/0	13/2/ 0/0	0.767$
Donor-recipient ABO mismatch, no vs. yes	23/18	4/5	12/3	0.160$
Donor-recipient sex pair, female to male vs. others	5/36	2/7	2/13	0.731$
Conditioning regimen, Bu-Cy vs. Bu-Fludara-ATG	34/7	9/0	15/0	0.101$
GVHD prophylaxis, CSA/MTX vs. CSA only	7/34	3/6	4/11	0.482$
Acute GVHD, no vs. yes	39/2	6/3	11/4	0.021$
Chronic GVHD, no vs. yes	24/12	4/5	8/6	0.447$
Time from HCT to relapse, day, median	193	369	508	0.030#

*ANLL: acute non-lymphoblastic leukemia; ALL: acute lymphoblastic leukemia; HCT: hematopoietic cell transplantation; CR1: first complete remission; Bu-Cy, busulfan plus cyclophosphamide; Bu-Fludara-ATG, busulfan, fludarabine, plus antithymocyte globulin; CSA, cyclosporine; MTX, methotrexate. *Cytogenetic risk group was classified according to the cytogenetic results at the time of diagnosis of acute leukemia. #Kruskal-Wallis test; χ^2 test.*

radiotherapy in addition to salvage chemotherapy, and nine with isolated EM relapse received radiation therapy only without systemic chemotherapy. The four patients with leptomeningeal relapse received intrathecal chemotherapy. After salvage treatment, 21 patients experienced newly developed or flared-up GVHD. Among 61 evaluable patients, 32 attained complete remission after salvage treatment., with a median complete remission duration of 156 days (range, 25-2672). A second relapse occurred in 22 patients; the sites were in BM (n=9), BM/EM (n=1), or EM (n=12). BM/EM or EM relapse occurred in 8 of 11 patients with development or flare-up of GVHD after salvage treatment, whereas it occurred in only 5 of 11 patients without GVHD (p=0.193). After a median follow-up of 264 days (range, 3-2713), 12 patients were alive without

Table 5. Multivariate analysis of prognostic factors for complete remission rate after salvage treatment, complete remission duration, and survival after relapse.

Variable	OR	95% CI	p value
Complete remission rate after salvage chemotherapy			
GVHD after salvage treatment, no vs. yes	4.487	1.272-15.830	0.020#
Cytogenetic risk group*, other vs. poor	0.303	0.091-1.007	0.051#
Complete remission duration			
GVHD after salvage treatment, no vs. yes	0.384	0.152-0.968	0.042$
Time to relapse after HCT, year, ≤1 vs. > 1	0.352	0.137-0.903	0.030$
Survival after relapse			
Time to relapse after HCT, year, ≤1 vs. > 1	0.412	0.212-0.801	0.009$
Induction of complete remission, no vs. yes	0.116	0.057-0.234	<0.001$

*HCT, hematopoietic cell transplantation; *cytogenetic risk group was classified according to the cytogenetic results at the time of diagnosis of acute leukemia. #A multiple logistic regression analysis; $Cox proportional hazards regression model.*

Table 6. Published data regarding patterns of relapse after allogeneic HCT for hematologic malignancies.

Author	Year	Disease	No. of HCT	No. of relapse	Relapse sites		
					BM	BM+EM	EM
Mortimer[17]	1989	AL	821	225	162 (72%)	21 (9%)	42 (19%)
Simpson[18]	1998	AML	81	22	12 (55%)	4 (18%)	6 (27%)
Mehta[15]	1997	AL	–	114	106 (93%)	3 (3%)	5 (4%)
Michel[16]	1997	AML	202	44	33 (75%)	11 (25%)	
Chong[13]	2000	AL/CML /MM	183	51	36 (71%)	3 (6%)	12 (23%)
Lee*	–	AL	194	65	41 (63%)	9 (14%)	15 (23%)

*HCT: hematopoietic cell transplantation; Year: published year; BM: bone marrow; EM: extramedullary; AL: acute leukemia; AML: acute myelogenous leukemia; CML: chronic myeloid leukemia; MM: multiple myeloma. *This study.*

leukemia, 7 were alive with leukemia, 6 had died without leukemia, and 40 had died of leukemia. The estimated 5-year survival after relapse was 18.2%.

Table 5 shows the results of multivariate analyses of prognostic factors for complete remission rate after salvage treatment, duration of complete remission, and survival after relapse. Development or flare-up of GVHD after salvage treatment was an independent prognostic factor for induction of complete remission (odds ratio, 4.487; p=0.020) and duration of the complete remission (odds ratio, 0.384; p=0.042). Time to relapse after hematopoietic cell transplantation (≤1 year vs. > 1 year) was an independent prognostic factor for the duration of complete remission (odds ratio, 0.352; p=0.030) and survival after relapse (odds ratio, 0.412; p=0.009). Achievement of complete remission after salvage treatment was an independent prognostic factor for survival after relapse (odds ratio, 0.116; p<0.001).

Discussion

Patterns of relapse of acute leukemia seem to be different after allogeneic hematopoietic cell transplantation and after chemotherapy. EM relapse has been observed frequently after allogeneic transplantation (Table 6),[13,15-18] while it is uncommon after chemotherapy only.[11,46] In our study, 37% of all relapsed patients had leukemic disease at various EM sites at the time of their first relapse and 15% had isolated EM relapse despite continued BM remission. The time to relapse after hematopoietic cell transplantation was longest in patients with isolated EM relapse followed by those with BM/EM relapse, then those with BM relapse only; this finding is consistent with previous reports.[13,17,18,21]

The above clinical observations have led to the concept of immunologic sanctuaries. This means that graft-versus-leukemia effects at EM sites are not as efficient as those in the BM.[22,23,27,28] To test this hypothesis, we investigated anti-leukemic effects of GVHD in both the BM and in EM sites. GVHD is usually a parallel event to the graft-versus-leukemia effect, and the former's anti-leukemic effects on relapse have been well demonstrated.[2-4] Acute GVHD was associated with significantly longer RFS and lower cumulative incidence of relapse in our patients. In a further analysis of acute GVHD effects on BM versus EM relapse, acute GVHD had similar effects on BM relapse, but it did not have any significant effect on EM relapse. Our findings confirm that GVHD has anti-leukemic effects and strongly support the hypothesis that the graft-versus-leukemia effect is less at EM sites. The clinical features and course of relapsed patients provided additional evidence that the graft-versus-leukemia effect has a lower impact on EM relapse. In our study, only 2 of 41 patients with BM relapse had a history of acute GVHD before relapse, while 7 of 24 relapsed patients with an EM component had acute GVHD before relapse. Previous reports have shown that GVHD has favorable effects on maintaining remission following second transplants or immunotherapy.[15,47,48] Our study also demonstrated that development or flare-up of GVHD

after salvage treatment is significantly associated with reinduction of complete remission and its maintenance. However, GVHD did not seem to be effective in preventing EM relapse because many patients who attained a complete remission from salvage treatment had second relapses at EM sites. Other studies have also shown the ineffectiveness of donor lymphocyte infusion in preventing EM relapse.[8,13,22-26]

Several factors, including expression of certain integrins, contribute to the BM homing of hematopoietic cells.[49] A reduced expression of adhesion molecules on leukemic cells has been described in patients with leukemia.[50,51] These abnormalities may alter the homing of leukemic cells, and contribute to seeding outside of the BM. In one study, chimerism analysis was performed in three patients with EM relapse after allogeneic hematopoietic cell transplantation; no donor cells were detected at the relapsed EM sites.[28] Lymphocyte activation occurring in BM/blood or spleen may lead to the expression of lymphocyte surface molecules directing selective homing to only these tissues.[52,53] Immunologically active allogeneic T cells may not reach EM sites, and recruitment of allogeneic immune cells in these tissues may not be fully operative, or may be delayed. The lack of donor T cells may explain the high frequency of EM relapse at diverse sites after allogeneic hematopoietic cell transplantation. In addition, recruitment of accessory cells necessary to achieve efficient local anti-leukemic activity may be deficient in EM sites of leukemic relapse.[24] Furthermore, certain leukemic cells may be intrinsically resistant to graft-versus-leukemia effects through various immune escape mechanisms such as down-regulation of the Fas antigen,[54] induction of Fas ligand expression,[55] or deficient expression of co-stimulatory molecules.[56] Also, some EM sites of leukemic relapse may represent sanctuary sites for conditioning chemotherapy.[12] For instance, isolated central nervous system relapse is relatively common in acute lymphoblastic leukemia after chemotherapy as well as after allogeneic hematopoietic cell transplantation.[57] In a retrospective study, the busulphan-cyclophosphamide regimen resulted in a higher frequency of isolated EM relapse than did total body irradiation regimens.[18] This result may be due to the low plasma level of busulfan achieved in some patients;[58] however, the role of conditioning regimen in preventing EM relapse after hematopoietic cell transplantation has not been accurately evaluated.

In our study, chronic GVHD had no significant effect on relapse, while acute GVHD was associated with significantly longer RFS. These results are in contrast to those of other studies.[3,4] These results must, however, be interpreted cautiously. Statistical analysis for anti-leukemic effects of chronic GVHD is complicated by the presence of acute GVHD. In our study, chronic GVHD was evaluable in 166 patients. Of those, 20 developed acute GVHD only and they showed the best RFS (71.9%). The RFS of 24 patients with both acute and chronic GVHD was 64.7%, that of 51 patients with chronic GVHD only was 58.6%, and that of 71 patients without acute or chronic GVHD was 52.0%. Two recent reports from Japan showed similar results to ours in that relapse incidence was significantly lower in patients with acute GVHD, but not in those with chronic GVHD.[59,60] There might be some racial differences in the graft-versus-leukemia effects of acute or chronic GVHD. Anti-leukemic effects of acute GVHD might be superior to those of chronic GVHD in our patients and we focused on anti-leukemic effects of acute GVHD in the statistical analysis of correlation with BM versus EM relapse.

Our study has some limitations. EM relapse was the main event in our study, but there were only 24 EM relapses and this relatively small number may not provide enough power to prove that GVHD has no effect on EM relapse. The reduction in relapse attributed to the graft-versus-leukemia effect is known to be greater in acute myeloid leukemia than in acute lymphoblastic leukemia.[3] When we separately analyzed acute non-lymphoblastic leukemia and acute lymphoblastic leukemia , the anti-leukemic effects of GVHD in the former were much more prominent than those in the later. However, the subset analysis decreased the statistical power because of the small number of patients in each group.

In conclusion, our study confirmed the anti-leukemic effect of GVHD on relapse after allogeneic hematopoietic cell transplantation for acute leukemia, although it may be less effective in preventing EM relapse. Leukemic cells at the EM sites may escape the graft-versus-leukemia effects of allogeneic immune cells. Further efforts to elucidate these mechanisms are needed.

J-HL was primarily responsible for this work, from conception to submitted manuscript. K-HL, S-JC, J-HL participated in conception and design of this work; S-JC, J-HL, MS, Y-SL, S-GR, K-HL analyzed and interpreted the clinical data, which were prospectively collected in the Asan Medical Center HCT registry; H-SC and C-JP performed the pathologic studies and interpreted the results. M-SL, SCY analyzed the survival data. J-HL prepared the manuscript. J-SL, K-HL were involved in critically revising the content of the manuscript. Kyoo-Hyung Lee gave the final approval for its submission.

The authors are listed according to a criterion of decreasing individual contribution to the work, with the following exception: the last author had a major role as a senior author in designing the study, interpreting the data and preparing the article. The authors declare that thaey have no potential conflicts of interest.

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