

# Extramedullary relapse of leukemia after allogeneic hematopoietic stem cell transplantation

## A retrospective study

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### Abstract

Extramedullary relapse (EMR) rarely occurs after allogeneic hematopoietic stem cell transplantation (HSCT) in leukemia. This study was to investigate the clinical characteristics of EMR.

We retrospectively investigated 316 consecutive patients undergoing HSCT for acute leukemia or chronic myeloid leukemia (CML) at 2 institutions between January 2012 and February 2017. Furthermore, we analyzed and compared the risk factors and outcomes between EMR and bone marrow relapse (BMR).

The 5-year cumulative incidence of EMR was 14.1%. The EMR incidence in acute myeloid leukemia, lymphoblastic leukemia, and CML was 17.5%, 18.9%, and 5.3%, respectively. CML had a lower EMR incidence rate. Compared to the BMR group, the EMR group had a longer median relapse-free time (10.5 months vs 5 months,  $P = .02$ ), and the EMR group had a higher incidence rate of chronic graft-versus-host disease (50.0% vs 20.9%,  $P = .009$ ). EMR had better estimated 3-year survival rates post-HSCT, and post-relapse, than did BMR (39.5% vs 9.5%,  $P < .001$ , and 21.9% vs 10.8%,  $P = .001$ ). Multivariate analysis identified that adverse cytogenetics (hazard ratio [HR] = 9.034,  $P < .001$ ) and extramedullary leukemia before HSCT (HR = 2.685,  $P = .027$ ) were the independent risk factors for EMR after HSCT. In the EMR group, patients who achieved complete remission (CR) had a significantly better, estimated 3-year survival than did patients who did not achieve CR (38.4% vs 14.3%,  $P = .014$ ).

EMR is a significant contributor to mortality after HSCT, which appears to be resistant to most of the current therapies. Establishing effective strategies for EMR is important in improving outcomes after HSCT.

**Abbreviations:** Ag = antigen, aGVHD = acute GVHD, AL = acute leukemia, ALL = acute lymphocytic leukemia, AML = acute myeloid leukemia, BM = bone marrow, BMR = BM relapse BU/CY2 = busulfan and cyclophosphamide, cGVHD = chronic GVHD, CML = chronic myeloid leukemia, CSA = cyclosporine, DLI = donor leukocyte infusion, EBMT = European group for blood and marrow transplantation, EMR = extramedullary relapse, GVHD = graft-versus-host disease, GVL = graft-versus-leukemia, HLA = human leukocyte antigen, HSCT = hematopoietic stem cell transplantation, MAL = mixed-lineage acute leukemia, MTX = methotrexate, TBI = total body irradiation.

**Keywords:** allogeneic hematopoietic stem cell transplantation, bone marrow relapse, extramedullary relapse, leukemia

## 1. Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is now considered an effective treatment modality for patients with acute leukemia (AL) and chronic myeloid leukemia (CML). The

graft-versus-leukemia (GVL) effect contributes to sustained remission and long-term survival in some patients. Although allogeneic HSCT reduces the risk of relapse, leukemia relapse has emerged as a frequent cause of treatment failure and mortality. Leukemia relapse usually occurs in the bone marrow (BM) while increasing reports suggest that extramedullary (EM) relapse (EMR) also accounts for a significant proportion. The incidence of EMR remains uncertain and varied widely in previous studies.<sup>[1–4]</sup> EMR occurs in diverse sites such as the brain, breast, urogenital tract, bone, and skin, where the GVL effect is less active in comparison to the BM.<sup>[2]</sup> The reduced effectiveness of the GVL reaction in EM sites has been suggested as one of the mechanisms for the increased frequency and wide distribution of EMR after allogeneic HSCT.<sup>[3,4]</sup> While the prognosis of EMR is generally unfavorable, there is dispute on whether EMR, as opposed to BM relapse, is associated with improved survival.<sup>[5,6]</sup> In addition, knowledge regarding clinical characteristics and prognostic factors of EMR, as well as consensus on treatment, is limited.

In order to better understand EMR of leukemia after allogeneic HSCT, we retrospectively investigated 316 consecutive patients with AL and CML who underwent successful allogeneic HSCT between January 2012 and February 2017 in the Affiliated Cancer Hospital of Zheng Zhou University and the First Affiliated Hospital of Zheng Zhou University.

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## 2. Methods

### 2.1. Patients

Written informed consent for hematopoietic cell collection and transplantation was obtained from all donors and patients. Overall, 316 patients were enrolled in this retrospective study, including 130 (41.1%) with acute myeloid leukemia (AML), 97 (30.7%) with acute lymphocytic leukemia (ALL), 82 (25.9%) with CML, and 7 (2.2%) with mixed-lineage acute leukemia (MAL). These patients included 190 males and 126 females, with a median age of 26 years (range, 3–59 years). Patients had 202 sibling donors, 48 unrelated donors, and 66 haploidentical donors who had a 1 to 3 antigen (Ag) mismatch serologically in the human leukocyte antigen (HLA) A, B, or DR loci with recipient. Overall, 313 patients received peripheral blood stem cells and 3 received BM cells. We collected clinical information of all patients and computed their risk score according to the European group for blood and marrow transplantation (EBMT). Table 1 shows the clinical characteristics of 316 patients.

### 2.2. Transplantation procedure

A total of 27 patients received a busulfan and cyclophosphamide (BU/CY2) conditioning regimen consisting of BU (4 mg/kg/d p.o. or 3.2 mg/kg/d i.v.) on days –7 to –4, and CY (60 mg/kg) on days –3 to –2. Furthermore, 245 patients received a modified BU/CY regimen (methyl-chlorethyl-cyclohexyl-nitroso-urea 250 mg/m<sup>2</sup> on day –10, cytarabine 4 g/m<sup>2</sup>/d on days –9 to –8, BU 1 mg/kg/6 h on days –7 to –5, and CY 1.8 g/m<sup>2</sup>/d on days –4 to –3), 38 patients received a total body irradiation (TBI)/CY regimen (TBI 7–8 Gy on day –6, cytarabine 2 g/m<sup>2</sup>/d on days –5 to –4, and CY 1.8 g/m<sup>2</sup>/d on days –3 to –2), and 4 patients intolerant to a myeloablative regimen received a nonmyeloablative regimen. All patients received a daily dose of granulocyte-colony stimulating factor 5–7.5 ug/kg intravenously starting on day 5 of infusion of donor hematopoietic cells until peripheral blood absolute neutrophil count was over  $0.5 \times 10^9/L$ .

Regimens for graft-versus-host disease (GVHD) prophylaxis were comprised of cyclosporine (CSA) and short-term methotrexate (MTX) for patients with sibling identical donors, and CSA, MTX and mycophenolate mofetil for patients with unrelated or haploidentical donors. The haploidentical grafts were non-T cell depleted. Acute GVHD (aGVHD) and chronic GVHD (cGVHD) were classified according to the criteria of Przepiorka et al.<sup>[7]</sup> and Sullivan et al.<sup>[8]</sup> respectively. Mild (grade I–II), and severe (grade III–IV) aGVHD occurred in 180, and 29 cases, respectively, while 163 patients developed cGVHD.

Intrathecal chemotherapy consisting of MTX, cytarabine, and dexamethasone was administered to patients with ALL, high-risk AML, and high-risk CML, for the prophylaxis of central nervous system (CNS) leukemia before conditioning.

### 2.3. Diagnosis and definitions

EMR was at times isolated; however, it also occurred concurrently with BMR. BMR in AL was defined as BM blasts >5%, and in CML as cytogenetic relapse with or without hematological relapse. In isolated EMR patients, the evaluation of BM status was required to reveal complete remission (CR), and a chimerism study was required to reveal full-donor chimerism. We were then able to diagnose EMR by physical examination and

**Table 1**

**Clinical characteristics of 316 patients with leukemia.**

Characteristics	AML n=130	ALL n=97	CML n=82	MAL n=7
Age (yr), n				
<20	30	36	21	2
20–40	68	46	48	4
>40	32	15	13	1
Sex, n				
Male	71	63	51	5
Female	59	34	31	2
Hyperleukocytosis at diagnosis, n	20	34	66	0
Adverse cytogenetics, n	32	35	8	3
EM leukemia before HSCT, n	9	12	6	0
Disease stage, n				
Early	104	69	58	5
Intermediate	11	19	16	1
Late	15	9	8	1
Disease status at HSCT				
CR or CP	121	92	67	7
Others	9	5	15	0
Stem cell source, n				
BM	2	0	1	0
Peripheral blood	128	97	81	7
Donor type, n				
Sibling	92	50	57	3
Unrelated	22	15	11	0
haploidentical	16	32	14	4
Donor recipient gender combination, n				
Female-male	17	22	12	2
Others	113	75	39	5
Conditioning regiment, n				
BU/CY2	19	2	6	0
Modified BU/CY	103	63	74	5
TBI/CY	6	31	1	0
Nonmyeloablative	2	1	1	0
Time from diagnosis to HSCT (mo), n				
<12	93	70	52	3
≥12	37	27	30	4
EBMT score				
Low	86	66	57	4
Medium	39	27	20	2
High	5	4	5	1
Acute GVHD, n				
0	47	35	23	2
I–II	74	54	48	4
III–IV	9	8	11	1
cGVHD, n				
Yes	69	45	46	3
No	61	52	36	4

ALL = acute lymphocytic leukemia, AML = acute myeloid leukemia, BU/CY2 = busulfan and cyclophosphamide, cGVHD = chronic graft-versus-host disease, CML = chronic myeloid leukemia, EBMT = European group for blood and marrow transplantation, HSCT = hematopoietic stem cell transplantation, MAL = mixed-lineage acute leukemia, TBI = total body irradiation.

imaging studies only. CNS relapse was diagnosed when leukemic cells were identified in the cerebrospinal fluid.

At diagnosis, we defined hyperleukocytosis as a peripheral WBC  $>100 \times 10^9/L$  in AML,  $>30 \times 10^9/L$  in B lymphoblastic leukemia,  $>100 \times 10^9/L$  in T lymphoblastic leukemia, and  $>100 \times 10^9/L$  in CML. Adverse cytogenetics were defined as monosomy 5/5q-, monosomy 7/7q-, t (9; 22), complex karyotype, FLT-3-positive, MLL gene rearrangement, and CD56-positive in AML; t (9; 22) and complex karyotype in ALL; and additional chromosomal abnormalities in CML.<sup>[9–11]</sup> The EBMT

risk score was classified as low (EBMT risk score 0–2), medium (EBMT risk score 3–4), or high (EBMT risk score  $\geq 5$ ) according to the age at transplant, disease stage, time interval from diagnosis to transplant, donor-type, and donor-recipient gender combinations.<sup>[12]</sup>

For isolated EMR, CR was defined as the disappearance of all clinical signs of EM leukemia, confirmed by a physical examination, imaging studies, and/or examination of cerebrospinal fluid. For isolated BMR, CR was defined as BM blasts  $>5\%$  for AL, and as major cytogenetic remission, at least, for CML.<sup>[13]</sup> For patients that had EMR with concurrent BMR, CR was defined as meeting the CR criteria above, of both isolate EMR and isolate BMR.

#### 2.4. Statistical analysis

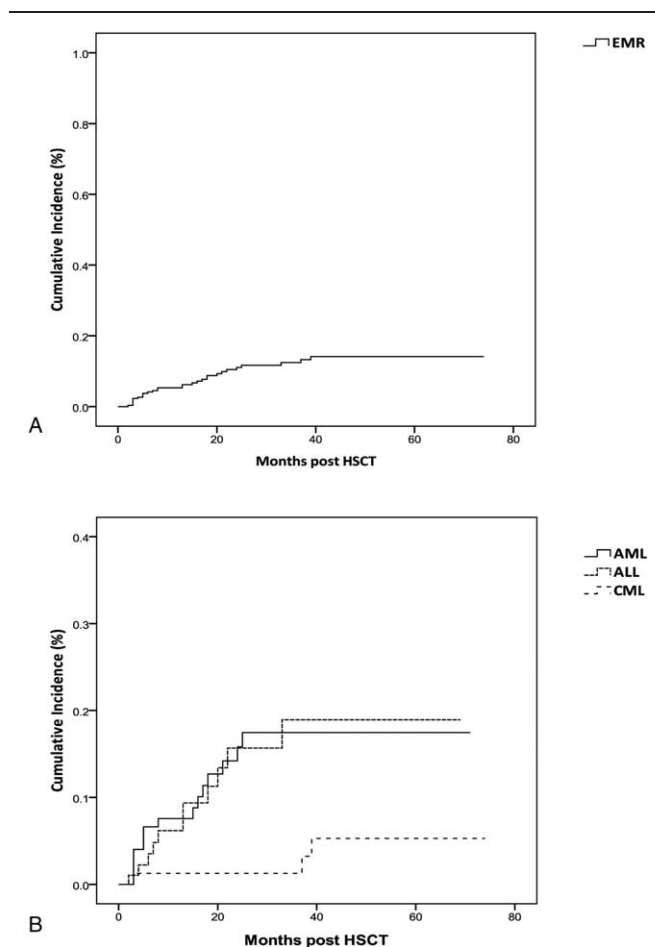
The relapse time was calculated from the HSCT date to the date of leukemia relapse, the date of last contact, or the date of death, whichever came first. Patients who died due to HSCT complications without relapse were excluded. The Kaplan–Meier method was used to plot cumulative incidence curves for relapse which were compared using a log-rank test. Categorical variables were compared using Chi-square and Fisher exact tests. Overall survival (OS) was calculated from the HSCT date to last follow-up or death date, using the Kaplan–Meier estimate method, and compared with a log-rank test. Hazard ratios (HRs) for EMR and BMR were estimated from univariate and multivariate Cox regression analyses.  $P < .05$  was considered to be statistically significant. All reported  $P$  values were based on 2-sided hypothesis tests. All analyses were conducted using SPSS ver. 17.0 (SPSS, Chicago, IL).

### 3. Results

#### 3.1. Incidence and characteristics of EMR

After a follow-up ranging from 1 to 74 months (median, 25 months), 73 patients relapsed within a median time of 6 months (range, 2–71 months). Of all 316 patients, 43 (13.6%) experienced BMR, and 30 (9.5%) experienced EMR, including 13 with isolated EMR, and 17 with EMR and concurrent BMR. The 5-year cumulative EMR incidence was 14.1%. The EMR incidence in CML was 5.3%, which was significantly lower than that in AML (17.5%;  $P = .009$ ) and ALL (18.9%;  $P = .010$ ). Although the 5-year cumulative EMR incidence in those with ALL was higher than that in those with AML, the difference was not statistically significant ( $P = .094$ ) (Fig. 1). There was no significant difference between the 5-year cumulative EMR incidence in patients who had haploidentical donors and patients who had HLA-identical donors (13.7% vs 12.3%,  $P = .654$ ).

Among the 30 EMR patients, there were 20 males and 10 females with a median age of 23.5 (range, 6–47) years. Of these, 16 had AML (FAB: AML-M2 (10); AML-M4 (2); AML-M5 (4)), 11 had ALL, and 3 had CML. At diagnosis, 21 patients had adverse cytogenetics including 43.8% (7/16) of AML patients with CD56 expression, 81.8% (9/11) of ALL patients with  $t(9; 22)$ , and 66.7% (2/3) of CML patients with additional chromosomal abnormalities. At the time of HSCT, 26 were at CR or chronic phase (CP) of CML, whereas 29 were at non-CR or more advanced stages. There were 19 patients at low risk, 9 at medium risk, and 2 at high risk according to the EBMT risk score. Before the onset of EMR, 19 patients developed mild aGVHD,



**Figure 1.** (A) Cumulative incidence of EMR after HSCT for all patients; (B) Cumulative incidence of EMR after HSCT for AML versus ALL versus CML (CML vs AML,  $P = .009$ ; CML vs ALL,  $P = .010$ ; ALL vs AML,  $P = .094$ ). ALL = acute lymphocytic leukemia, AML = acute myeloid leukemia, CML = chronic myeloid leukemia, EMR = extramedullary relapse, HSCT = hematopoietic stem cell transplantation.

1 experienced severe aGVHD, and 15 displayed cGVHD. In particular, 4 patients developed EMR after haploidentical-HSCT as a second HSCT.

The EMR site varied widely, including the CNS ( $n = 13$ ), skin or soft tissue ( $n = 8$ ), bone ( $n = 8$ ), testis ( $n = 4$ ), lymph nodes ( $n = 4$ ), breast ( $n = 1$ ), and gastrointestinal tract ( $n = 1$ ). Multiple sites of EMR ( $\geq 2$ ) were observed in 10 patients. Overall, 7 patients had EM leukemia before HSCT, 6 relapsed at the same sites as the previous leukemia, while 1 relapsed at an extra site. Characteristics of 30 EMR patients are presented in Tables 2 and 3.

#### 3.2. Comparison between EMR and BMR

The estimated 5-year cumulative incidence of BMR was 16.6%. The median relapse-free time of EMR was significantly longer than that for BMR (10.5 months vs 5 months,  $P = .020$ ). The proportion of BMR patients (29/43) who possessed medium or high EBMT risk scores was higher than that for EMR patients (11/30;  $P = .040$ ). The proportion of patients who developed cGVHD was higher in the EMR group when compared with the BMR group (50.0% vs 20.9%,  $P = .009$ ). The comparison of characteristics between EMR and BMR patients is detailed in Table 4.

**Table 2**  
**Characteristics of patients who developed isolate EMR.**

No.	Age/sex	DX/cytogenetics	EM leukemia before HSCT	Disease status at HSCT	EBMT risk score	GVHD	Relapse site	Results
1	41/F	M5, 46XX	No	CR1	3	cGVHD	Skin	Alive
2	32/M	M4, 46XY	No	NR	3	Mild aGVHD, cGVHD	CNS	Dead
3	19/M	M5, FLT-3 (+), MLL gene rearrangement	No	CR1	0	cGVHD	Bilateral tibia	Dead
4	16/M	ALL, t (9;22)	Yes	CR2	3	Mild aGVHD, cGVHD	Right femur	Alive
5	37/F	M2, t (8;21), CD56+	Yes	CR1	1	Mild aGVHD, cGVHD	CNS	Dead
6	42/F	M2, t (8;21), CD56+	No	CR2	4	Mild aGVHD, cGVHD	CNS, soft tissue	Dead
7	47/M	M2, 46XY	No	CR1	2	Mild aGVHD, cGVHD	CNS	Alive
8	25/M	ALL, t (9;22)	No	CR1	2	cGVHD	CNS	Dead
9	7/M	M2, t (8;21), CD56+	No	CR2	3	Mild aGVHD, cGVHD	Mandible	Dead
10	10/F	ALL, 46XX	Yes	CR1	1	Mild aGVHD, cGVHD	Lymph nodes	Dead
11	45/M	CML, t (9;22)	No	CR1	2	Severe aGVHD, cGVHD	Lymph nodes	Dead
12	26/M	ALL, t (9;22)	No	CR2	2	Mild aGVHD, cGVHD	CNS	Alive
13	45/F	ALL, t (9;22)	No	NR	5	cGVHD	CNS	Dead

aGVHD = acute graft-versus-host disease, ALL = acute lymphocytic leukemia, cGVHD = chronic graft-versus-host disease, CML = chronic myeloid leukemia, CNS = central nervous system, EBMT = European group for blood and marrow transplantation, GVHD = graft-versus-host disease, HSCT = hematopoietic stem cell transplantation.

The estimated 3-year OS of EMR patients was better than that for BMR patients (39.5% vs 9.5%,  $P=.000$ ). The median survival time post-HSCT for EMR, and BMR patients were 28, and 11 months, respectively. The estimated 3-year survival post-relapse of EMR was significantly higher than that for BMR (21.9% vs 10.8%,  $P=.001$ ). The median survival time post-relapse for EMR, and BMR patients were 18.5, and 3 months, respectively (Fig. 2).

### 3.3. Risk factors of EMR

We analyzed the clinical characteristics of interest using Cox proportional hazards modeling to identify risk factors for EMR and BMR, including sex, diagnosis, hyperleukocytosis at diagnosis, adverse cytogenetics, disease status at HSCT, EM leukemia before HSCT, EBMT risk score, aGVHD, and cGVHD.

The results of our univariate and multivariate analyses are summarized in Tables 5 and 6.

Univariate analysis suggested that CML patients were less likely to develop EMR than AML patients (HR=0.219; 95% CI, 0.064–0.756;  $P=.016$ ), whereas the risk for EMR in ALL or MAL patients was not significantly different from that of AML patients. The multivariate analysis showed that adverse cytogenetics (HR=9.034; 95% CI, 3.949–20.668;  $P<.001$ ) and EM leukemia before HSCT (HR=2.685; 95% CI, 1.122–6.426;  $P=.027$ ) were the independent risk factors for EMR. However, there was no significant impact on the incidence of EMR due to sex, hyperleukocytosis at diagnosis, disease status at HSCT, EBMT risk score, aGVHD, and cGVHD.

EM leukemia before HSCT (HR=2.777; 95% CI, 1.232–6.262;  $P=.014$ ) and non-CR or CP (CML) at HSCT

**Table 3**  
**Characteristics of patients who developed EMR concurrent BMR.**

No.	Age/sex	DX/cytogenetics	EM leukemia before HSCT	Disease status at HSCT	EBMT risk score	GVHD	Relapse site	Results
1	42/F	M2, t (8;21), CD56+	No	CR2	4	Mild aGVHD	Right humerus, soft tissue, BM	Alive
2	10/M	ALL, t (9;22)	No	CR1	1	No	Bilateral testical, BM	Dead
3	14/F	M5, 46XX	No	CR1	1	Mild aGVHD	Skin, BM	Dead
4	21/M	M5, complex karyotype	No	CR1	2	No	Left testis, BM	Alive
5	36/F	M2, FLT-3 (+), CD56+	No	CR1	2	Mild aGVHD	Lumbar, BM	Dead
6	20/M	ALL, t (9;22)	No	CR1	3	Mild aGVHD	Left testis, BM	Dead
7	12/M	CML, complex karyotype	No	CP2	2	Mild aGVHD	CNS, right tibia, BM	Dead
8	25/F	M4, FLT-3 (+)	Yes	NR	4	Mild aGVHD	Breast, soft tissue, BM	Dead
9	6/F	M2, t (8;21), CD56+	No	CR1	1	No	CNS, skull, soft tissue, BM	Dead
10	8/M	ALL, 46XY	Yes	CR2	2	Mild aGVHD, cGVHD	CNS, BM	Dead
11	22/M	ALL, t (9;22)	No	CR1	2	No	CNS, BM	Dead
12	6/M	M2, t (8;21), CD56+, MLL gene rearrangement	No	CR1	1	cGVHD	Soft tissue, right tibia, right testis, CNS, BM	Dead
13	17/M	CML, t (6;9;22)	No	CP1	0	Mild aGVHD	Lymph nodes, BM	Dead
14	41/M	M2, 46XY	No	CR1	2	No	Lymph nodes, soft tissue, gastrointestinal tract, BM	Dead
15	44/M	M2, 46XY	No	CR1	2	Mild aGVHD	Right femur, right anterior superior iliac spine, soft tissue, BM	Dead
16	44/M	ALL, t (9;22)	Yes	CR2	6	Mild aGVHD	CNS, BM	Dead
17	14/M	ALL, t (9;22)	Yes	NR	4	Mild aGVHD	CNS, BM	Alive

aGVHD = acute graft-versus-host disease, ALL = acute lymphocytic leukemia, BM = bone marrow, cGVHD = chronic graft-versus-host disease, CML = chronic myeloid leukemia, CNS = central nervous system, EBMT = European group for blood and marrow transplantation, GVHD = graft-versus-host disease, HSCT = hematopoietic stem cell transplantation.



**Table 4**  
**Comparison of characteristics between EMR and BMR.**

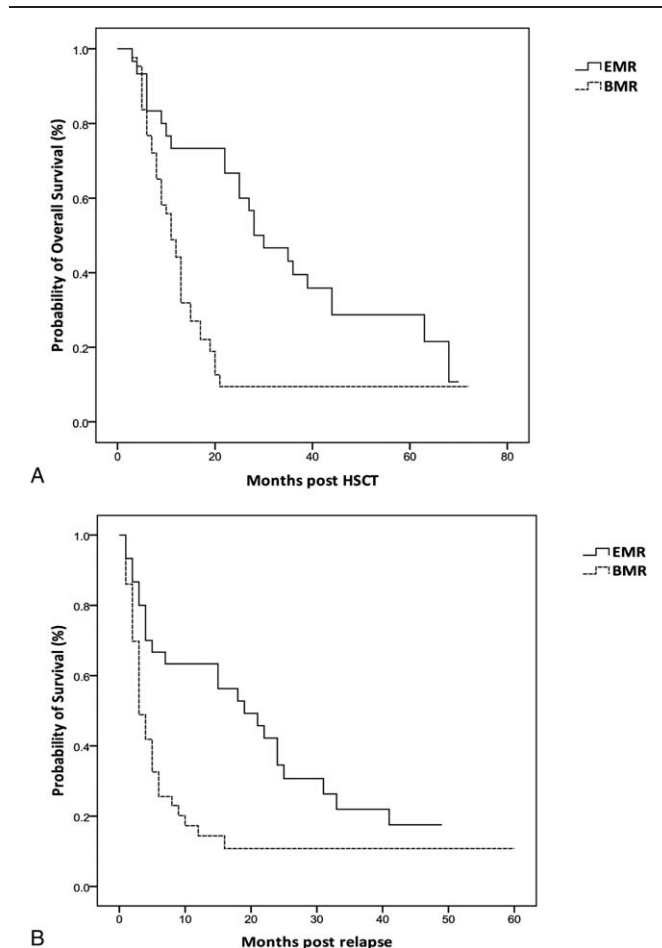
Characteristics	EMR (n=30)	BMR (n=43)	P-value
Sex			
Male	20	30	.803
Female	10	13	
Diagnosis			
AML	16	20	.779
ALL	11	15	
CML	3	6	
MAL	0	2	
Hyperleukocytosis			
Yes	13	22	.635
No	17	21	
Adverse cytogenetics			
Yes	21	23	.224
No	9	20	
EM leukemia before HSCT			
Yes	7	7	.550
No	23	36	
Disease status			
CR or CP	26	35	.750
Others	4	8	
EBMT risk score			
Low	19	14	.016
Medium or high	11	29	
aGVHD			
0	10	16	.793
I-II	19	24	
III-IV	1	3	
cGVHD			
Yes	15	9	.009
No	15	34	

aGVHD = acute graft-versus-host disease, ALL = acute lymphocytic leukemia, AML = acute myeloid leukemia, BMR = bone marrow relapse, cGVHD = chronic graft-versus-host disease, CML = chronic myeloid leukemia, CP = chronic phase, CR = complete remission, EBMT = European group for blood and marrow transplantation, EMR = extramedullary relapse, HSCT = hematopoietic stem cell transplantation, MAL = mixed-lineage acute leukemia.

(HR = 2.908; 95% CI, 1.343–6.301; *P* = .007) correlated with an increased risk of BMR in the univariate analysis. The multivariate analysis revealed a higher frequency of BMR in patients with adverse cytogenetics (HR = 3.099; 95% CI, 1.609–5.971; *P* = .001), a medium or high EBMT risk score (HR = 3.900; 95% CI, 1.900–8.007; *P* = .000 vs HR = 6.716; 95% CI, 2.360–19.112; *P* = .000), and without cGVHD (HR = 4.072; 95% CI, 1.936–8.563; *P* = .000).

### 3.4. Treatments and outcomes of EMR

Four EMR patients who refused treatment died of relapse. In addition, 24 patients received chemotherapy while 10 received irradiation, 7 received donor leukocyte infusion (DLI), 3 received surgery, and 2 received repeated transplant, 16 patients receiving combination treatments (≥2 treatments). After treatment, 13 patients achieved CR whereas 7 experienced recurrence. The estimated 3-year survival post-relapse of CR patients was significantly better than that for non-CR patients (38.4% vs 14.3%, *P* = .014). Although 60% (6/10) of patients receiving irradiation ± chemotherapy achieved CR, the estimated 3-year survival post-relapse was not significantly better than for patients receiving other therapies (30.5% vs 25.0%, *P* = .453). CR was achieved in 42.9% (3/7) of patients receiving DLI/chemotherapy,



**Figure 2.** (A) Kaplan–Meier estimates overall survival after HSCT (EMR vs BMR, *P* = .000); (B) Kaplan–Meier estimates overall survival after relapse (EMR vs BMR, *P* = .001). BMR = bone marrow relapse, EMR = extramedullary relapse, HSCT = hematopoietic stem cell transplantation.

with only 1 survivor. One of the 2 patients who underwent repeated transplant developed EMR again after treatment and died of recurrence, and the other died of an infection.

Of the 30 EMR patients, 23 died within 15 (range, 1–44) months after relapse, with 15 dying of recurrence, 6 dying of infection, and 2 dying of GVHD. For EMR patients, the estimated 3-year survival post-relapse in CML patients was better than that in AML and ALL patients, but the difference was not statistically significant (33.3% vs 22.5% vs 16.4%, *P* = .785). The estimated 3-year survival post-relapse of patients receiving combination therapy was not significantly better than that in patients receiving a single therapy (32.7% vs 10.0%, *P* = .418).

## 4. Discussion

EMR after HSCT remains poorly understood compared with BMR. Most previous EMR studies focused on patients with AML or ALL; there is limited data on EMR in CML patients. We found a significant difference between CML and AL patients in terms of EMR. In addition, the EBMT risk score served as a risk factor for EMR which has not been reported previously to our knowledge.

The incidence of EMR in AL after HSCT ranges from 6% to over 20% as reported previously.<sup>[14]</sup> In an EBMT study, only 0.7% of 3071 AML patients experienced EMR post-HSCT,<sup>[11]</sup>

**Table 5**  
Univariate and multivariate analysis of risk factors for EMR.

Factor	Univariate HR 95% CI)	P-value	Multivariate HR 95% CI)	P-value
Sex				
Male	1.000	.427		
Female	0.735 (0.344–1.571)			
Diagnosis				
AML	1.000		1.000	
ALL	0.965 (0.448–2.079)	.927	0.753 (0.347–1.636)	.474
CML	0.219 (0.064–0.756)	.016	0.323 (0.092–1.142)	.080
MAL	0.000 (0.000–)	.974	0.000 (0.000–)	.978
Hyperleukocytosis				
No	1.000	.533		
Yes	1.258 (0.611–2.591)			
Adverse cytogenetics				
No	1.000	.000	1.000	.000
Yes	11.364 (5.115–25.245)		9.034 (3.949–20.668)	
EM leukemia before HSCT				
Yes	1.000	.000	1.000	.027
No	4.586 (1.961–10.725)		2.685 (1.122–6.426)	
Disease status				
CR or CP	1.000	.103		
Others	2.424 (0.837–7.016)			
EBMT risk score				
Low	1.000			
Medium	1.869 (0.834–4.187)	.129		
High	4.160 (0.923–18.754)	.064		
aGVHD				
0	1.000			
I-II	1.139 (0.529–2.449)	.739		
III-IV	0.523 (0.067–4.093)	.537		
cGVHD				
No	1.000	.600		
Yes	1.211 (0.592–2.479)			

aGVHD = acute graft-versus-host disease, ALL = acute lymphocytic leukemia, AML = acute myeloid leukemia, cGVHD = chronic graft-versus-host disease, CI = confidence interval, CML = chronic myeloid leukemia, CP = chronic phase, CR = complete remission, EBMT = European group for blood and marrow transplantation, EMR = extramedullary relapse, HR = hazard ratio, HSCT = hematopoietic stem cell transplantation, MAL = mixed-lineage acute leukemia.

but the incidence in this study might have been underreported. Among long-term survivors, the incidence has been reported to be over 20%.<sup>[2,11]</sup> In our study, 30 patients (9.5%) experienced EMR after HSCT, which is consistent with previously reported incidences of EMR post-HSCT in AL patients. The EMR incidence is reported to be higher in ALL compared with AML.<sup>[11,14]</sup> However, the 5-year cumulative EMR incidence in ALL was not significantly higher than in AML in our study. EMR in CML patients has been reported mostly in the form of case reports.<sup>[13]</sup> In the EBMT study, EMR occurred in only 0.45% CML patients, and the EMR incidence in CML was lower compared with that in AML.<sup>[1]</sup> A similar phenomenon was observed in our study, as CML patients were less likely to develop EMR than AL patients. In contrast, in a single-institution study involving 26 CML patients, the EMR incidence post-HSCT was as high as 21%, although the incidence might have been overestimated as a result of selecting high-risk patients.<sup>[16]</sup> Thus, the incidence of EMR in CML may be influenced by the proportion of high-risk patients.

Recently, EMR in haploidentical-HSCT has been reported in several studies, whereas few studies compare the EMR incidence in haploidentical-HSCT. Our study suggests that the 5-year cumulative EMR incidence in haploidentical-HSCT is not

**Table 6**  
Univariate and multivariate analysis of risk factors for BMR.

Factor	Univariate HR (95% CI)	P-value	Multivariate HR (95% CI)	P-value
Sex				
Male	1.000	.174		
Female	0.637 (0.332–1.221)			
Diagnosis				
AML	1.000			
ALL	1.045 (0.535–2.041)	.898		
CML	0.415 (0.166–1.037)	.060		
MAL	1.970 (0.460–8.440)	.361		
Hyperleukocytosis				
No	1.000	.068		
Yes	1.744 (0.959–3.172)			
Adverse cytogenetics				
No	1.000	.000	1.000	.001
Yes	4.468 (2.442–8.176)		3.099 (1.609–5.971)	
EM leukemia before HSCT				
No	1.000	.014	1.000	.693
Yes	2.777 (1.232–6.262)		1.196 (0.492–2.904)	
Disease status				
CR or CP	1.000	.007	1.000	.468
Others	2.908 (1.343–6.301)		0.734 (0.318–1.695)	
EBMT risk score				
Low	1.000		1.000	
Medium	5.183 (2.651–10.136)	.000	3.900 (1.900–8.007)	.000
High	10.076 (3.816–22.609)	.000	6.716 (2.360–19.112)	.000
aGVHD				
0	1.000			
I-II	0.876 (0.465–1.648)	.680		
III-IV	0.928 (0.270–3.190)	.906		
cGVHD				
Yes	1.000	.000	1.000	.000
No	4.451 (2.134–9.282)		4.072 (1.936–8.563)	

aGVHD = acute graft-versus-host disease, ALL = acute lymphocytic leukemia, AML = acute myeloid leukemia, cGVHD = chronic graft-versus-host disease, CI = confidence interval, CML = chronic myeloid leukemia, CP = chronic phase, CR = complete remission, EBMT = European group for blood and marrow transplantation, BMR = bone marrow relapse, HR = hazard ratio, HSCT = hematopoietic stem cell transplantation, MAL = mixed-lineage acute leukemia.

significantly higher than that in HLA-identical HSCT. The degree of GVHD is generally thought to be more severe in haploidentical-HSCT. The heavier GVHD associated with an occurrence of GVL may act as a protective factor for EMR to some extent. On the other hand, 2 patients in our study experienced EMR after haploidentical-HSCT performed as a second HSCT. Furthermore, Yoshihara et al<sup>[17]</sup> found that the incidence of EMR after haploidentical-HSCT performed as a second SCT was remarkably high. These findings strongly suggest that a potent GVL effect elicited by HLA disparity occurs preferentially in the BM.

It has long been thought that the GVL effect would protect patients from BMR and EMR by immune surveillance. However, the precise mechanism for the difference in the GVL effect between BM and EM tissues remains to be clarified. Moreover, there is a controversy whether cGVHD, with targets largely the same as those of GVL, is associated with increased risk of EMR. Some authors indicated that patients with cGVHD were more likely to develop EMR compared to those without cGVHD,<sup>[15,18]</sup> but this interpretation does not overlap with the results of other authors,<sup>[6,9,14]</sup> some of whom consider that the high cGVHD incidence in EMR may contribute to longer-term survival in EMR than in BMR. In our study, we also found the cGVHD incidence

to be higher in EMR patients than those with BMR, whereas cGVHD was not a risk factor for EMR. What is generally acknowledged is that the GVL process is less effective in preventing EMR than BMR. The GVL effect probably preferentially maintains remission in the BM while allowing leukemic cells in peripheral tissues to evade immune surveillance. The high concentration of cytotoxic CD8<sup>+</sup> T cells, the main mediators of the GVL effect,<sup>[5]</sup> in the BM, as well as deficient recruitment of the accessory cells necessary for antileukemic activity at the sites of EMR,<sup>[19]</sup> may provide evidence for the hypothesis above.

A series of risk factors have been reported to contribute to the EMR occurrence post-HSCT, including male gender, AML subtype M2/M4/M5, adverse cytogenetics, hyperleukocytosis at diagnosis, EM leukemia before HSCT, non-CR status at HSCT, conditioning regimens containing TBI, and cGVHD.<sup>[9–11,20,21]</sup> In the present study, identified risk factors were consistent with previous studies including adverse cytogenetics and EM leukemia before HSCT; however, male gender, hyperleukocytosis at diagnosis, non-CR or CP (CML) at HSCT, and cGVHD were not risk factors for EMR. The presence of t (9; 22) was common in ALL EMR patients and the frequency of CD56 expression in AML EMR patients was considerably high. CD56/neural cell adhesion molecule has been reported to be highly expressed in various tissues, including neural tissues, gut, pancreas, testis, ovary, and visceral smooth muscle, mediating cell-to-cell interactions via homophilic adhesion.<sup>[22,23]</sup> Thus, EM involvement at these sites may result from the homing of leukemic cells to these sites via homophilic adhesion of CD56 Ag. Apart from factors above, the TBI/CY conditioning regimen which was applied mostly to ALL patients with advanced stage in our study failed to perform in the risk factors analysis for excluding the imbalance of factors in patients with different diagnosis. The number of patients who received stem cells from BM was too small to include in the risk analysis. The EBMT risk score, aimed at providing a tool to assess HSCT risks, has been reported to influence survival, non-relapse mortality, and the relapse risk after HSCT.<sup>[12]</sup> Our study implied that the EBMT risk score, with a combination of age, disease stage, time interval, donor type, and donor-recipient gender, was associated with the BMR risk; in contrast, it was not a risk factor for EMR. EMR patients with higher EBMT risk scores did not follow with shorter survival, which was possibly affected by treatment.

Differences were observed between EMR and BMR, aside from a divergence in risk factors in the study. Patients developed EMR later than BMR, while better survival post-HSCT and post-relapse were both observed in EMR patients compared with BMR patients, consistent with previous reports.<sup>[9,18,24]</sup> However, results of other authors suggested that EMR post-HSCT had similar outcomes as BMR treated in a similar way.<sup>[6]</sup> In other words, therapies after relapse are the main factors affecting the survival post-relapse. In addition, CML patients with EMR were reported to have a better survival than did AML patients, which might be partly explained by the administration of the second generation of tyrosine kinase inhibitors.<sup>[16,25]</sup> In our study, the estimated 3-year survival post-relapse in CML was better than that AL patients, but the difference was not significant. Further studies involving more EMR patients are needed.

In general, EMR patients have poor outcomes, and only a few patients exhibited long-term survival. There have been no established guidelines for clinical decision making in the

treatment of EMR after HSCT. Not only systemic chemotherapy, but also DLI and repeated transplants have been reported to have limited effect in improving the survival of EMR patients.<sup>[26,27]</sup> Although local radiotherapy has been observed to offer some patients long-term survival, most patients developed systemic relapse.<sup>[25]</sup> We also found a high CR rate in patients receiving irradiation; however, this did not contribute to the survival post-relapse. In contrast with views of some authors,<sup>[24]</sup> combination therapy could not improve survival of patients with EMR post-relapse compared with single therapy in the present study. Recently, gemtuzumab ozogamicin has been reported to present excellent efficacy in salvage therapy of multiple relapse in EM,<sup>[28,29]</sup> while the efficacy should be confirmed by further studies. In addition, the monoclonal Ab targeting CD56 Ag or WT1-derived peptides in peripheral blood, which were observed to play a predictive role in EMR,<sup>[30,31]</sup> may be promising candidates for future studies of EMR treatments.

In the present study, we investigated the characteristics of EMR after HSCT in patients with leukemia and analyzed the relevant risk factors, to help better predict whether patients with leukemia after HSCT have a tendency to develop EMR. However, our research has limitations, and therefore the results of this should be applied with caution. First, the number of subjects we included was not large enough. Because of the low incidence of EMR, the number of patients with EMR who underwent HSCT for 5 years was small, which will affect the comprehensiveness of the results. In addition, the correlational analysis will also have some biased due to the small sample size. There was also potential heterogeneity in the clinical parameters of each EMR patient, such as the exact time of recurrence, which can lead to bias in the final outcome. Given these limitations, the results of this study will need to be verified by a larger sample size and more accurate parametric analysis for further validation.

In summary, our study analyzed the prevalence of EMR after HSCT, and found that adverse cytogenetics and EM leukemia before HSCT are independent risk factors for EMR. These conclusions are useful for assessing the prognosis of patients with EMR and for planning effective treatment improving the poor outcomes of patients with EMR.

## Author contributions

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