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Different Kinetics and Risk Factors for Isolated Extramedullary Relapse after Allogeneic Hematopoietic Stem Cell Transplantation in Children with Acute Leukemia

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Relapse after allogeneic hematopoietic stem cell transplantation (allo-HSCT) remains the most frequent cause of post-transplantation mortality. Isolated extramedullary (EM) relapse (iEMR) after HSCT is relatively rare and not well characterized, particularly in pediatric patients. We retrospectively analyzed 1527 consecutive pediatric patients with acute leukemia after allo-HSCT to study the incidence, risk factors, and outcome of iEMR compared with systemic relapse. The 5-year cumulative incidence of systemic relapse (either bone marrow [BM] only or BM combined with EMR) was 24.8%, and that of iEMR was 5.5%. The onset of relapse after allo-HSCT was significantly longer in EM sites than in BM sites (7.19 and 5.58 months, respectively; $P = .013$). Complete response (CR) 2 +/active disease at transplantation (hazard ratio [HR], 3.1; $P < .001$) and prior EM disease (HR, 2.3; $P = .007$) were

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independent risk factors for iEMR. Chronic graft-versus-host disease reduced the risk of systemic relapse (HR, 0.5; P = .043) but did not protect against iEMR. The prognosis of patients who developed iEMR remained poor but was slightly better than that of patients who developed systemic relapse (3-year overall survival, 16.5% versus 15.3%; P = .089). Patients experiencing their first systemic relapse continued to have further systemic relapse, but only a minority progressed to iEMR, whereas those experiencing their iEMR at first relapse developed further systemic relapse and iEMR at approximately similar frequencies. A second iEMR was more common after a first iEMR than after a first systemic relapse (58.8% versus 13.0%; P = .001) and was associated with poor outcome. iEMR has a poor prognosis, particularly after a second relapse, and effective strategies are needed to improve outcomes.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a treatment option for patients with acute leukemia who have relapsed or refractory disease or other unfavorable risk factors. Relapse after transplantation is a devastating complication and the main reason for treatment failure. The incidence of relapse after allo-HSCT ranges from 10% to 60%, depending on patient type and disease stage at the time of transplantation and the transplantation characteristics, such as donor type and conditioning regimen [1]. Leukemia relapse usually occurs in the bone marrow (BM), but extramedullary (EM) relapse (EMR) also accounts for a significant proportion. Isolated EMR (iEMR), in which leukemia cells are observed in extremely diverse sites while full donor chimerism and morphologic remission in the marrow are retained [2,3], is relatively rare and not well characterized. Although BM relapse (BMR) has been well studied, there are limited systematic data on iEMR in the literature, most of which come from studies including only adult patients [3-6] or adult and pediatric patients together [7-9]. However, the biology of leukemia and indications for allo-HSCT in pediatric patients differ from those in adult patients. In an effort to better understand post-HSCT iEMR in the pediatric population, we evaluated iEMR and its features in a large pediatric cohort of allo-HSCT recipients, including patients with acute lymphoblastic leukemia (ALL) and acute myelogenous leukemia (AML), in a multicenter registry-based study.

METHODS

Study Design and Patient Population

A retrospective chart review of 1553 consecutive patients who underwent allo-HSCT for acute leukemia between January 1998 and December 2019 and were registered in the Turkish Pediatric Bone Marrow Transplantation Registry was performed for this study. The study cohort comprised 1527 patients with ALL or AML. Patients who developed graft rejection were excluded. We retrospectively extracted patient data, including details of the transplantation procedure, disease status, response rate, toxicity, survival time, and time to progression, from our prospectively acquired database. All patients were treated based on standard institutional protocols. Written informed consent for allo-HSCT was obtained from all patients or their legal guardians, and the study was approved by each center's Ethics Committee and conducted in accordance with the Declaration of Helsinki.

Disease status at transplantation was defined according to the Center for International Blood and Marrow Transplant Research criteria. Patients with early disease were defined as patients achieving first complete remission (CR); those with intermediate disease, as those achieving second CR or beyond; and those with advanced disease, as patients who did not achieve CR [10].

All patients underwent transplantation according to the standard European Society for Blood and Marrow Transplantation indications following receipt of a myeloablative conditioning regimen. For patients with ALL, if total body irradiation (TBI) was used in the conditioning regimen, then a 6-Gy radiotherapy boost was administered for central nervous system (CNS) and testicular relapses. For patients with EM disease (EMD) who were not administered TBI, 18 Gy of radiotherapy was delivered for local control. In patients with EMD other than CNS or testicular relapse, if EM involvement disappeared after reinduction therapy, then no additional treatment was planned. For patients with AML, local radiotherapy to control EMD was not routinely recommended. Patients received graft-versus-host disease (GVHD) prophylaxis with a calcineurin inhibitor (mostly cyclosporin A) and a short course of methotrexate (on days +1, +3, +6, ± +10) or mycophenolate mofetil according to institutional protocols. Rabbit antithymocyte globulin was used only in umbilical cord blood transplantations. Post-transplantation cyclophosphamide or TCR-$\alpha\beta$/CD19 depletion was used to provide T cell depletion for haploidentical transplantations.

Grafts were obtained from related donors in 1024 cases (67%), including matched sibling donors (MSDs) (HLA 6/6; n = 715; 47%), matched nonsibling related donors (HLA 10/10; n = 152; 10%), mismatched related donors (HLA ≤8/10, n = 141; 9%), and related umbilical cord blood (UCB; HLA 6/6; n = 16; 1%), and from unrelated donors in 503 cases (33%), including matched unrelated donors (HLA 10/10 or 9/10; n = 481; 31%), mismatched unrelated donors (HLA ≤8/10; n = 12; 1%), and unrelated UCB transplants (HLA 5/6 or 6/6; n = 10; 1%). Stem cell sources were BM (n = 776; 51%), peripheral blood (PB; n = 664; 43%), UCB (n = 10; 1%), PB + BM (n = 61; 4%), and UCB + BM (n = 16; 1%).

Definitions, Diagnosis, and Treatment of Relapse

Patients who experienced relapse after allo-HSCT were subdivided into systemic relapse (including only BMR or BMR + EMR) and iEMR groups. Patients who presented with EMR but progressed to BMR within 30 days after diagnosis of relapse were included in the systemic relapse group. For iEMR, the evaluation of BM status was required to determine complete remission (CR), and a chimerism study was required to reveal >95% donor chimerism. We were then able to diagnose EMR by physical examination, imaging studies, and biopsy analysis of the involved tissue when possible. CNS relapse was diagnosed when leukemic cells were identified in the cerebrospinal fluid.

For iEMR, CR was defined as the disappearance of all clinical signs of EM leukemia, confirmed by physical examination, imaging studies, and/or cerebrospinal fluid examination. For isolated BMR, CR was defined as <5% BM blasts and cytogenetic remission if any cytogenetic alteration was noted at the time of relapse. As there was no standardization of minimal residual disease (MRD) assessment in Turkey during the study period, MRD status was not taken into consideration as a criterion for the assessment of CR in this study. For patients who had EMR with concurrent BMR, CR was defined as described above for both iEMR and BMR.

Poor genetic alterations were defined as t(9;22), t(4;11), t(17;19) iAMP21, hypodiploidy ≤44 chromosomes for ALL and isolated monosomy 7, der12p, t(12;12), t(4;11), t(5;11), t(6;11), t(10;11), t(6;9), t(7;12), t(9;22), complex karyotype (5 or more abnormalities), WT1 mut/FLT-ITD for AML.

Regarding treatment of post-transplantation relapse, early withdrawal of immune suppression based on chimerism (if available, based on MRD) was performed at the first stage. When an overt morphologic relapse developed, either palliative therapy or therapy with curative intent was used, namely palliative therapy consisting of low-dose chemotherapy and/or supportive therapy alone or therapy with curative intent consisting of chemotherapy and/or targeted therapy, surgery (eg, orchiectomy, granulocytic sarcoma excision), radiotherapy, and donor lymphocyte infusion (DLI) either alone or in combination.

Statistical Analysis

Data were analyzed using SPSS version 18.0 (SPSS, Chicago, IL) and XLSTAT version 2017.2 statistical package (Addinsoft, Paris, France). The incidences of systemic relapse and iEMR w calculated using cumulative incidence analysis, with each factor and treatment-related mortality considered a competing risk. Overall survival (OS) was calculated from the time of relapse after transplantation to the date of mortality due to any reason or the last follow-up and was estimated using the Kaplan-Meier method. Patient characteristics were compared using the chi-square test for qualitative variables and the Mann-Whitney U test for continuous variables. To identify the risk factors according to relapse type, univariate and multivariate analyses were performed with such variables as sex, diagnosis, disease status at transplantation, poor cytogenetics, prior EMD in the course of therapy before transplantation, donor type, conditioning regimen, prior grade II-IV acute GVHD (aGVHD) and prior chronic GVHD (cGVHD). The log-rank test for OS

Table 1
Essential Clinical Characteristics of the Patients According to Relapse Type

Characteristic	All Patients (N = 1527)	All Relapses (N = 432)	Systemic Relapse (N = 357)	iEMR (N = 75)	*P* Value (Systemic vs iEMR)
Age at HSCT, yr, median (range)	9.09 (0.04- 22.39)	9.31 (0.57-22.39)	8.52 (0.40- 22.08)	8.72 (1.52-19.50)	.667
Sex, n (%)					.041
Male	998 (65)	290 (67)	232 (65)	58 (77)	
Female	529 (35)	142 (33)	125 (35)	17 (23)	
Diagnosis, n (%)					.103
ALL	983 (64)	287 (67)	231 (65)	56 (75)	
AML	544 (36)	145 (33)	126 (35)	19 (25)	
Transplantation status, n (%)					.010
Early	877 (57)	166 (38)	144 (40)	22 (29)	
Intermediate	573 (38)	223 (52)	173 (49)	50 (67)	
Advanced	77 (5)	43 (10)	40 (11)	3 (4)	
Poor cytogenetic alterations, n (%)	283 (18)	74 (17)	67 (19)	7 (9)	.051
Prior EMD, n (%)	149 (10)	45 (10)	29 (8)	16 (21)	.001
Matched sibling donor, n (%)	715 (47)	234 (54)	192 (53)	42 (47)	.691
Conditioning regimen, n (%)					.872
TBI-based, n (%)	419 (27)	112 (26)	92 (26)	20 (27)	
Chemotherapy -based, n (%)	1108 (73)	320 (74)	265 (74)	55 (73)	
Prior aGVHD grade II-IV, n (%)	425 (28)	99 (23)	72 (20)	27 (36)	.003
Prior cGVHD, n (%)*	252 (16)	49 (11)	33 (10)	16 (22)	.003
Time to relapse, mo. median (range)	—	5.84 (0.43-136.54)	5.58 (0.43-136.54)	7.19 (1.15-48.79)	.013

* Data available in 1463 patients who survived >100 days post-transplantation.

and Gray's test for relapse were used for comparisons. Univariate comparisons with a *P*value <.2 were included in the multivariate analysis, which was performed using the Cox proportional hazards model. The results are expressed as hazard ratios (HRs) and their corresponding 95% confidence intervals (CIs). An HR>1 denotes an unfavorable effect on relapse and OS. A *P* value <.05 was considered to indicate statistical significance.

RESULTS

Patient Population, Relapse Characteristics, and Incidence

We included 1527 consecutive patients with ALL (n = 983) and AML (n = 544) who underwent transplantation at 1 of 22 pediatric transplantation centers. The patient characteristics are summarized in Table 1. Nine hundred ninety-eight patients (65%) in this study cohort were male, and the minority of the cohort (5%) had active disease at the time of transplantation. Poor cytogenetic alterations were found in 283 patients (18%). One hundred forty-nine patients (10%) had EMD at the time of diagnosis.

By the end of the study period, 958 of 1527 patients (62.8%) were alive, with a median follow-up time of 40.80 months (range, 0.26 to 266.09 months). Three hundred and thirteen patients died of treatment-related causes (infections in 184, GVHD in 62, veno-occlusive disease in 17, bleeding in 11, and other reasons in 39; 5-year cumulative rate, 21.3%; 95% CI, 19.2 to 23.6), and 432 patients relapsed, 350 of whom died (including 256 who succumbed to progressive disease). The cumulative incidence of first relapse at any site at 5 years post-transplantation was 30.3% (95% CI, 27.9% to 32.8%); 357 patients experienced systemic relapse (303 with isolated BMR and 54 combined with EMR; 5-year cumulative incidence, 24.8%; 95% CI, 22.6 to 27.1), and 75 had iEMR (5-year cumulative incidence, 5.5%; 95% CI, 4.4 to 6.9) (Figure 1A). At the time of iEMR, MRD from BM was not available in 37 patients, was negative in 12 and positive in 4 by flow cytometry, and was negative in 21 and positive in 1 according to initial cytogenetic alterations.

Overall, 136 patients experienced EMR at some point during their disease course. The 5-year cumulative incidence of any EMR was 9.6% (95% CI, 8.1% to 11.3%). Seventy-five patients had isolated first EMR, 54 had combined first BMR and EMR, 6 had isolated second EMR, and 1 had combined second BMR and EMR. Seventeen patients had EMR, either isolated or combined with BMR, for both the first and second relapse events.

More patients with iEMR at relapse were male and had prior EMD and aGVHD and cGVHD, and fewer patients had CR2+/active disease at transplantation. iEMR developed significantly later than systemic relapse, at a median of 7.19 months (range, 1.15 to 48.79 months) versus 5.58 months (range, 0.43 to 136.11 months) after transplantation, respectively ($P = .013$) (Table 1). Sixteen of the patients with iEMR (21.3%) had EMD at the time of initial diagnosis. The sites of EMR are shown in Table 2. The CNS was the most common site, either isolated or combined with other EM sites, and the incidence of CNS involvement, either isolated or combined with other EM sites, was not significantly different between ALL and AML patients (44.2% versus 34.1%; $P = .259$).

Risk Factors for Systemic Relapse and iEMR after First HSCT

In an attempt to discriminate any impact of disease status at the time of transplantation, we planned to separately analyze and compare the cumulative incidence of relapse for patients who underwent transplantation in CR1 (early disease), in CR2 or beyond (intermediate disease), and not in CR (advanced disease). For patients with iEMR, we found a cumulative incidence of relapse of 2.7% (95% CI, 1.8% to 4.1%) in patients in CR1, of 10.0% (95% CI, 7.6% to 13.2%) in those in CR2 or beyond, and of 5.1% (95% CI, 2.6% to 11.7%) in those with no CR ($P < .001$). Although this is a large *P* value, it is not reflective of whether the rate of iEMR was higher in early versus advanced disease. Despite the significant difference, the percentage of risk was close (2.7% versus 5.1%); therefore, we examined CR1 versus CR2+/active disease instead of the definition mentioned above.

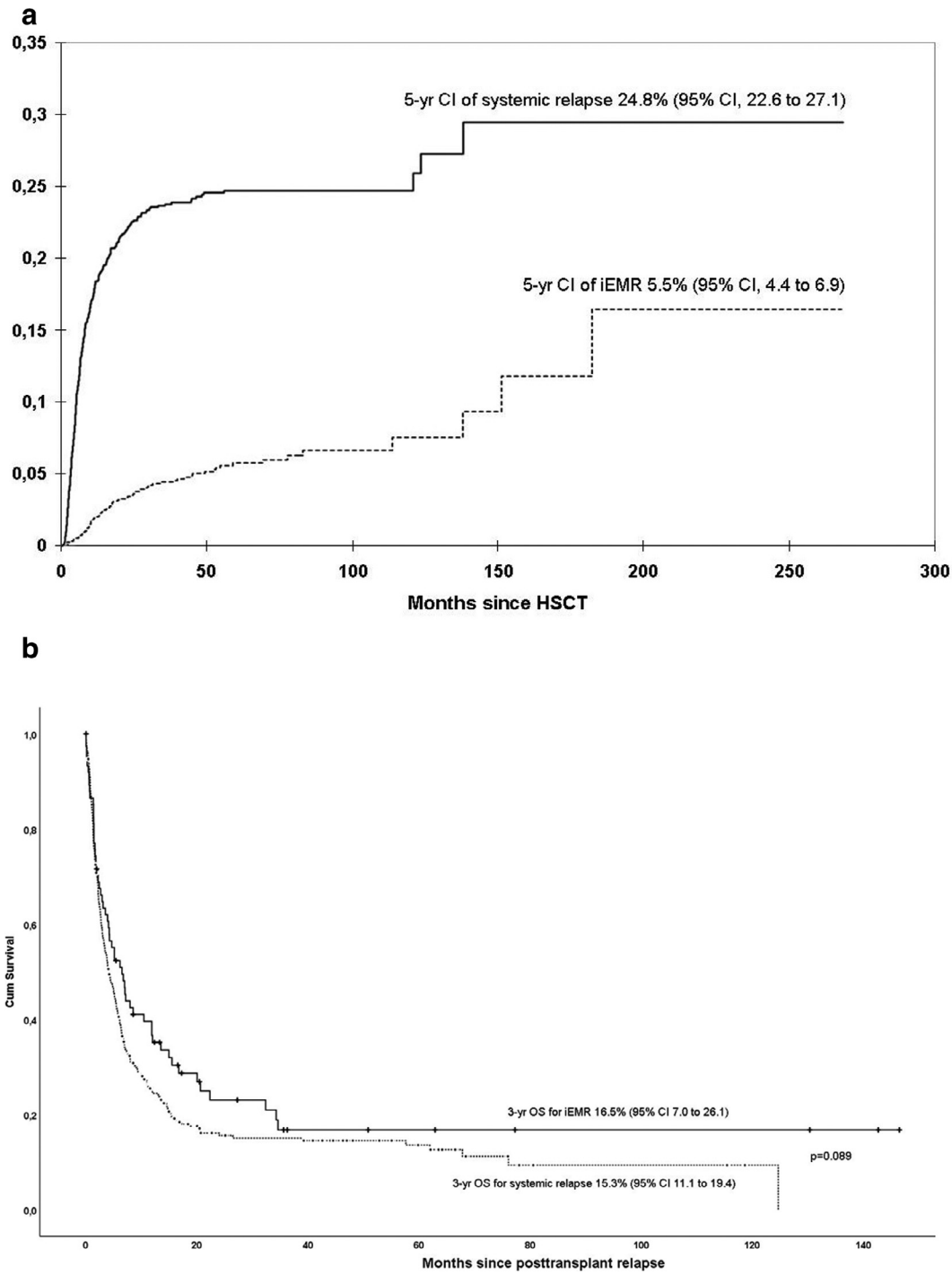

Figure 1. Cumulative incidence of first relapse (A) and survival after first relapse (B) by relapse type.

The risk factors for systemic relapse and iEMR differed. Disease status (17.6% for CR1 versus 34.4% for CR2+/active disease; $P < .001$), donor type (28.0% for matched sibling donors versus 21.7% for other donors; $P = .011$), prior grade II-IV aGVHD (no, 27.4% versus yes, 17.9%; $P < .001$), and prior cGVHD (no, 26.8% versus yes, 14.4%; $P < .001$) were associated with systemic relapse. The univariate analysis identified sex (6.5% for males versus 3.7% for females; $P = .026$), diagnosis (6.6% for ALL versus 3.7% for AML; $P = .043$), disease status (2.7% for CR1 versus 9.3% for CR2+/active disease; $P < .001$), poor cytogenetic alterations (yes, 6.2% versus yes, 2.6%; $P = .032$), prior EMD (yes, 11.5% versus no, 4.9%; $P < .001$), and local radiotherapy for EMD before transplantation (yes, 11.3% versus no, 5.0%; $P = .004$) as factors associated with iEMR. Donor type and prior aGVHD or cGVHD did not affect the incidence of iEMR (Table 3).

Multivariate analysis found that CR2+/active disease (HR, 3.4; $P < .001$) and MSD (HR, 1.8; $P = .014$) were associated with an increased risk of systemic relapse, whereas cGVHD (HR, 0.5; $P < .001$) was an independent risk factor for lower risk. iEMR was independently higher in patients with CR2+/active disease (HR, 3.1; $P < .001$) and prior EMD (HR, 2.8; $P = .046$). Male sex

Table 2
Sites of EMR

Site	First Relapse after HSCT				
	ALL		AML		All
	iEMR	BMR + EMR	iEMR	BMR + EMR	
CNS	22	16	8	5	51
Testis	17	9	-	3	29
Soft tissue/lymph node	4	4	6	1	15
Bone	4	3	-	2	9
Visceral	3	2	1	2	8
Pleura/pericardium	2	-	1	-	3
Skin/subcutaneous	1	1	1	1	4
CNS + visceral	1	-	-	-	1
CNS + soft tissue	-	-	1	-	1
CNS + visceral + soft tissue	-	-	1	-	1
CNS + testis	-	1	-	-	1
CNS + bone	-	1	-	-	1
CNS + skin/subcutaneous	-	1	-	-	1
Visceral + testis	2	1	-	-	3
Bone + soft tissue	-	-	-	1	1
All sites	56	39	19	15	129

Table 4
Multivariate Analysis for 5-Year Cumulative Incidence of Relapse

Variable	Systemic Relapse		iEMR	
	HR (CI)	*P* Value	HR (CI)	*P* Value
Sex: Male	—	—	1.6 (0.9 to 2.8)	.092
Diagnosis: ALL	—	—	1.4 (0.8-2.4)	.255
Status at transplantation: CR2 +/active disease	3.4 (2.1-5.8)	<.001	3.1 (1.8-5.2)	<.001
Poor cytogenetics	—	—	1.9 (0.8-4.2)	.120
Prior EMD	—	—	2.8 (1.0-7.8)	.046
Prior local radiotherapy	—	—	1.3 (0.4-4.2)	.632
Donor: MSD	1.8 (1.1-3.0)	.014	1.7 (1.0-2.8)	.054
aGVHD grade II-IV	0.6 (0.4-1.1)	.100	1.5 (0.9-2.5)	.105
cGVHD	0.5 (0.3-0.7)	.043	—	—

and MSD had a marginal effect on increased risk of iEMR. Remarkably, whereas cGVHD was a low risk factor for systemic relapse in the multivariate analysis, neither the univariate analysis nor the multivariate analysis found that GVHD was

Table 3
Univariate Analysis of 5-Year Cumulative Incidence of Relapse

Variable	All Patients, n	Systemic Relapse			iEMR		
		n	Cumulative Incidence, %, median (range)	*P* Value	n	Cumulative Incidence, %, median (range)	*P* Value
All	1527	357	24.8 (22.6- 27.1)		75	5.5 (4.4-6.9)	
Sex				.853			.026
Male	998	232	24.6 (22.0-27.6)		58	6.5 (5.0-8.4)	
Female	529	125	25.0 (21.7-29.3)		17	3.7 (2.3-6.0)	
Diagnosis				.697			.043
ALL	983	231	25.2 (22.5-28.2)		56	6.6 (5.0 to 8.5)	
AML	544	126	24.0 (20.5-28.0)		19	3.7 (2.4 to 5.9)	
Status at HSCT*				<.001			<.001
CR1	877	144	17.6 (15.1-20.5)		22	2.7 (1.8-4.1)	
CR2+/active disease	650	213	34.4 (30.8-38.5)		53	9.3 (7.1-12.9)	
Poor cytogenetics				.895			.032
Yes	283	67	25.0 (20.2-30.8)		7	6.2 (4.9-7.9)	
No	1244	290	24.7 (22.3-27.4)		68	2.6 (1.3-5.4)	
Prior EMD				.243			<.001
Yes	148	28	20.2 (14.4-28.2)		16	11.5 (7.2-18.2)	
No	1379	329	25.2 (22.9-27.8)		59	4.9 (3.8-6.3)	
Prior local radiotherapy				.301			.004
Yes	114	22	20.7 (14.2-30.2)		12	11.3 (6.6-19.4)	
No	1413	335	25.1 (22.8-27.6)		63	5.0 (3.9-6.5)	
Donor				.011			.162
MSD	715	192	28.0 (24.8-31.7)		42	6.6 (4.9-8.9)	
Other	812	165	21.7 (18.9-25.0)		33	4.3 (3.1-6.1)	
Conditioning regimen				.415			.829
TBI-based	419	92	23.4 (19.5-28.1)		20	5.6 (3.6-8.7)	
Chemotherapy-based	1108	265	25.3 (22.7-28.1)		55	5.4 (4.2-7.1)	
Prior aGVHD grade II-IV				<.001			.105
Yes	425	72	17.9 (14.5-22.2)		27	6.9 (4.8-10.0)	
No	1102	285	27.4 (24.7-30.3)		48	4.9 (3.7-6.6)	
Prior cGVHD*				<.001			.359
Yes	252	33	14.4 (10.4-19.8)		16	7.0 (4.3-11.3)	
No	1211	307	26.8 (24.3-29.6)		57	5.3 (4.1-7.0)	

* Data available in 1463 patients who survived >100 days post-transplantation.

Table 5
Three-Year OS Following First Relapse, Univariate and Multivariate Analyses

Variable	Number Alive	Univariable		Multivariable	
		3-Yr OS, % (95% CI)	*P* Value	HR (95% CI)	*P* Value
All Patients	82	15.2 (11.4-19.1)	–	–	–
Sex			.949	–	–
Male	54	13.2 (8.6-17.9)			
Female	28	18.7 (11.8-25.5)			
Diagnosis			.114		.122
ALL	65	17.5 (12.4-22.6)		1	
AML	17	11.1 (5.7-16.6)		1.2 (9.0-1.5)	
Status at transplantation			.252	–	–
CR1	34	18.0 (11.7-24.4)			
CR2+/active disease	48	13.2 (8.4-18.0)			
Poor cytogenetics			.426	–	–
No	65	14.4 (10.3-18.6)			
Yes	17	19.5 (9.4-29.6)			
Donor type			.380	–	–
MSD	42	16.3 (11.2-22.2)			
Other	40	13.7 (7.8-19.6)			
Conditioning regimen			.751	–	–
TBI-based	21	16.4 (8.8-23.9)			
Chemotherapy-based	61	14.8 (10.4-19.3)			
Relapse type			.089		.286
Systemic	65	15.3 (11.1-19.4)		1	
iEMR	17	16.5 (7.0-26.1)		0.8 (0.6-1.1)	
Time to relapse			<.001		<.001
≤6 mo	21	8.2 (4.4-12.0)		1	
>6 mo	61	23.1 (16.3-29.9)		0.5 (0.4-0.6)	
Prior aGVHD grade II-IV			.635	–	–
No	62	15.3 (11.0-19.6)			
Yes	20	15.3 (7.0-23.6)			
Prior cGVHD			.138		.507
No	68	14.8 (10.6-19.0)		1	
Yes	14	24.5 (10.9-38.1)		0.9 (0.6-1.3)	

associated with a lower risk for iEMR. Local radiotherapy for local control before or during the conditioning regimen did not appear to protect the patients with EMD from post-transplantation iEMR (Table 4).

We performed a subgroup analysis according to diagnosis. CR2+/active disease was a high risk factor for systemic relapse in both the ALL and AML groups (HR, 3.0 [$P < .001$] and HR, 3.4 [$P = .016$], respectively), and prior EMD was associated with a lower risk for systemic relapse only in the ALL group (HR, 0.4; $P = .005$). Although TBI had a marginal effect on decreasing the risk for systemic relapse (HR, 0.5; $P = .057$) in ALL, it tended to increase the risk for systemic relapse in AML (HR, 3.2; $P = .065$) (Supplementary Tables S1 and S2). In regard to iEMR, CR2+/active disease was significantly associated with a high risk of relapse in both groups (HR, 3.0 [$P < .001$] for ALL and HR, 3.3 [$P = .016$] for AML). Prior EMD was an independent risk factor for iEMR in patients with ALL (HR, 3.9; $P = .030$). Although TBI was a marginally high risk factor in AML (HR, 3.3; $P = .053$), there was a slightly significant association with decreased risk for iEMR in ALL (HR, 0.6; $P = .074$) (Supplementary Tables S3 and S4).

Treatment and Outcomes of First Systemic Relapse and iEMR after the First HSCT

Most relapsed patients received systemic treatment with curative intent (89.4% for systemic relapse and 93.3% for iEMR) combined with local radiation/surgery (14.8% for systemic relapse and 77.3% for iEMR) and DLI (6.1% and 8.0%, respectively) when feasible. One hundred twenty-six patients underwent second allogeneic HSCT, only 13 of whom had iEMR. Four patients with isolated BM relapse after the first transplantation received blinatumomab. Two of them achieved CR and then underwent a second alloHSCT, and they were alive without leukemia at their last follow-up. Of the 432 patients who initially relapsed, 185 (43.0%) achieved second CR (144 of 357 [40.3%] in systemic relapse versus 41 of 75 [54.7%] in iEMR; $P = .023$), 95 died of treatment-related causes (79 of 357 [22.1%] in systemic relapse versus 15 of 75 [20.0%] in iEMR; $P = .655$), and 256 died from progressive disease (213 of 357 [59.7%] versus 43 of 75 [57.3%]; $P = .729$). With a median follow-up of 18.3 months (range, 4.5 to 146.5 months), 82 of the 432 relapsed patients were alive, 75 of whom were leukemia-free. OS after first relapse was 26.0% (95% CI, 21.8% to 30.3%) at 1 year and 15.2% (95% CI, 11.4% to 19.1%) at 3 years. As shown in Figure 1B, the outcome was marginally better in patients with iEMR compared with those with systemic relapse (3-year OS, 16.5% [95% CI, 7.0 to 26.1] versus 15.3% [95% CI, 11.1 to 19.4]; $P = .089$). The univariate analysis found that relapse time after transplantation (23.1% for relapse at >6 months versus 8.2% for relapse at ≤6 months; $P < .001$) was associated with the 3-year survival rate after first transplantation. The multivariate analysis identified relapse at >6 months post-transplantation (HR, 0.5; $P < .001$) as

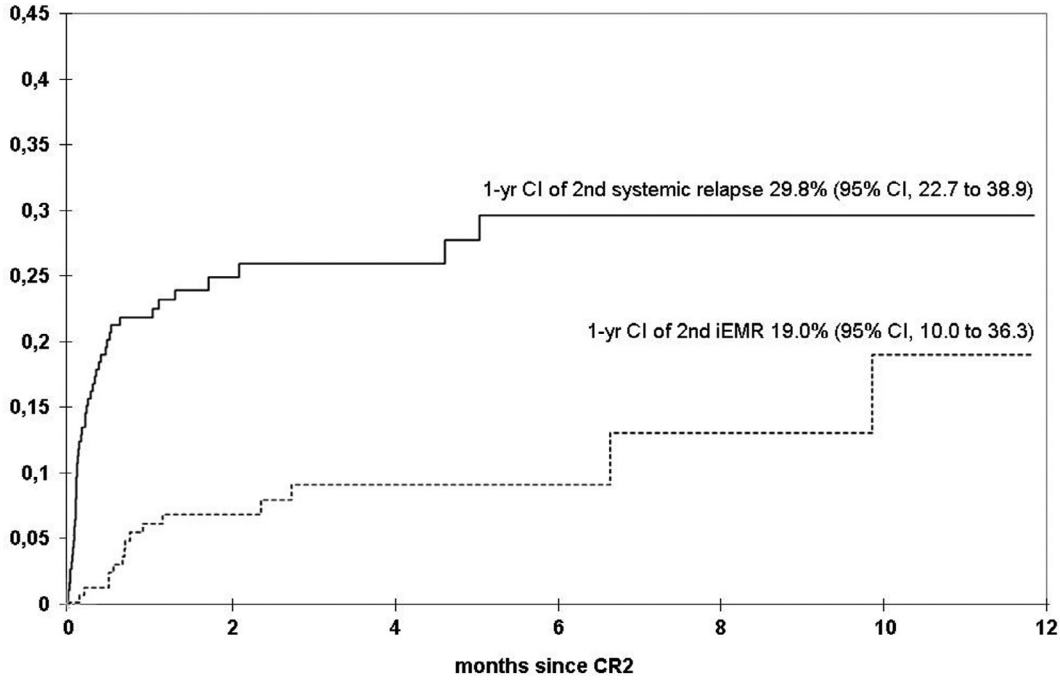

Figure 2. Cumulative incidence of second relapse by relapse type.

an independent factor only for predicting better 3-year OS after first relapse (Table 5).

Outcomes of Patients Experiencing Additional Relapse Events

Among the 185 patients who achieved CR2, 63 had a second relapse as either a systemic relapse (n = 46) or an iEMR (n = 17). The cumulative incidence of second relapse at any site after transplantation at 1 year was 48.8% (95% CI, 37.0% to 64.2%). The cumulative incidences of systemic and iEMR sites at 1 year were 29.8% (95% CI, 22.7% to 38.9%) and 19.0% (95% CI, 10.0% to 36.3%), respectively (Figure 2).

Of the 357 patients who had an initial systemic relapse, 46 relapsed again in BM only (n = 31) or combined with EMR (n = 9) as second systemic relapse (40 of 46; 86.9%) or isolated EM sites (n = 6) as second iEMR (6 of 46; 13.8%). Several patients who experienced a second systemic relapse achieved remission again (4 of 40 for systemic relapse, 2 of whom underwent a third HSCT), and patients with an initial systemic relapse who had a second iEMR had a better chance of achieving a third remission (4 of 6) (Figure 3). Twelve of 357 patients who experienced an initial systemic relapse and could not achieve CR2 by salvage chemotherapy underwent a second allo-HSCT with active disease status. One of them was alive and had no evidence of disease (NED) at 10 months post-transplantation, and the others died due to progressive disease (n = 7) or transplantation-related mortality (n = 4). In addition, 1 patient with t(9;22) who did not achieve CR2 was alive with disease and being treated with tyrosine kinase inhibitors at the time of this report.

Among the 75 patients who had an initial iEMR, 17 relapsed again (systemic relapse, n = 7; BMR, n = 6; combined relapse, n = 1) as second systemic relapse (7 of 17; 41.2%), with 10 experiencing second iEMR (10 of 17; 58.8%). Only 1 patient in this group achieved CR3 (0 of 7 in systemic relapse versus 1 of 10 in iEMR) (Figure 4). A second iEMR event was more common after a first iEMR event than after a first systemic relapse event (10 of 17 [58.8%] versus 6 of 46 [13.0%]; P = .001).

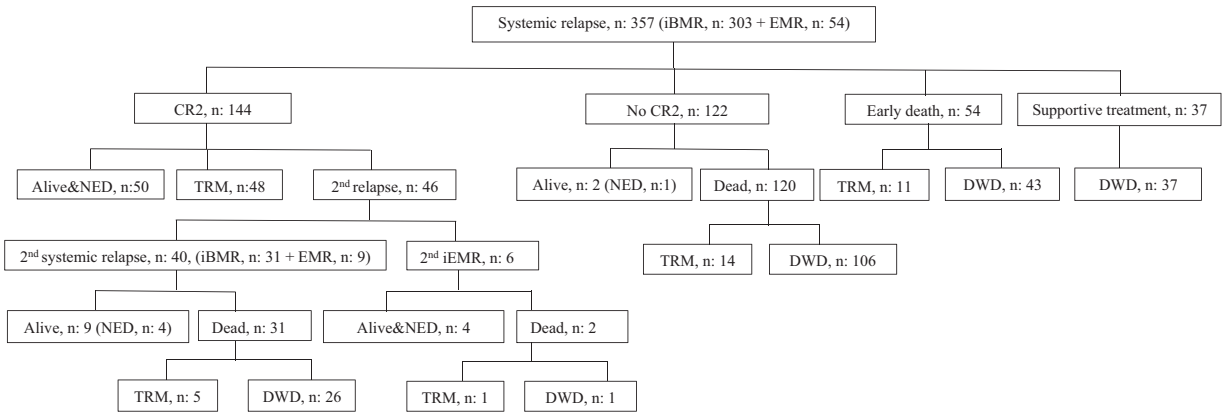

Figure 3. Outcomes after initial systemic relapse.

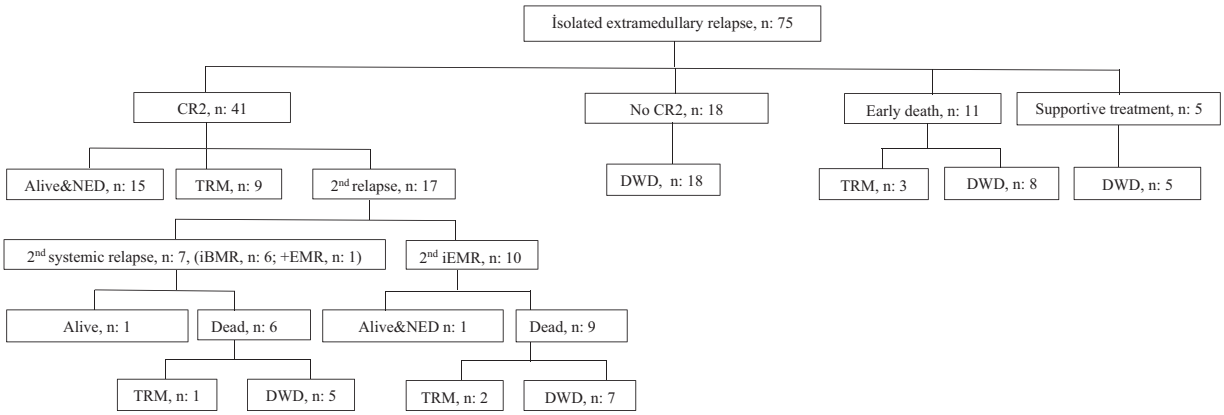

Figure 4. Outcomes after initial iEMR.

At the time of this report, 15 of the 63 patients who experienced a second relapse were alive and 9 were leukemia-free, with a median follow-up of 6.1 months (range, 0.2 to 118.8 months) for the patients who were alive. Two of the 15 surviving patients after a second relapse underwent a third transplantation and were leukemia-free at 20 months and 25 months post-transplantation. Patients who had a second iEMR event survived significantly longer than those with a second systemic relapse (median survival, 11.17 months [range, 6.57 to 15.77 months] versus 2.00 months [range, 0.67 to 3.34 months]; P = .004) (Supplementary Table S5).

DISCUSSION

In our large pediatric population with long-term follow-up, we compared incidence, risk factors, and outcomes of iEMR and systemic relapse. Our data show an overall 5-year cumulative incidence of relapse of 30.3%, a systemic relapse rate of 24.8%, and an iEMR rate was 5.5%, accounting for 17.3% of all first relapse events. The CNS seemed to be the most common EM, followed by the testicles. iEMR developed significantly later than systemic relapse. CR2+/active disease at allo-HSCT and any prior EMD in the course of therapy before transplantation were independent risk factors associated with the increased cumulative incidence of iEMR, whereas systemic relapse was more frequent in patients with CR2+/active disease at allo-HSCT and those undergoing transplantation with MSD. cGVHD was an independent factor for a lower risk of systemic relapse. Patients with first iEMR survived marginally longer than those with first systemic relapse.

Earlier studies, most of which included adult patients only or adult patients together with pediatric patients, reported that iEMR developed in 3.1% to 9.7% of patients with acute leukemia who underwent allo-HSCT, accounting for 9.6% to 25.5% of all first relapse events after transplantation [3-9,11]. We found iEMR rates of 5.5% and 17.3% for first relapses, which is in line with recent reports. However, in a retrospective European Society for Blood and Marrow Transplantation survey conducted more than 20 years ago, the incidence of EMR was only 0.65% in patients with AML after allo-HSCT [12]. The higher incidence in more recent reports could be due to several reasons. First, improvements in transplantation procedures, such as alternative donors and decreased transplantation-related mortality, and the resulting increase in post-transplantation survival might have enabled HSCT recipients to survive long enough to develop an EMR [3]. Second, the iEMR group had a longer relapse-free time compared with the systemic relapse group [5]. Although some studies did not confirm this finding [7,11], EMR has consistently been reported to occur later than systemic relapse [3,5,6,9], similar to our findings of 7.19 months versus 5.88 months post-transplantation, respectively. Harris et al. [14] suggested that this difference is because EM sites harbor the asymptomatic growth of leukemia for a prolonged period. Some recent studies have illustrated the challenges associated with relapsed or refractory acute leukemia complicated by EMR after exposure to cellular therapy such as DLI [15], immunotherapy such as blinatumomab [16], and second transplantation [17-19]. iEMR occurred more frequently at the second relapse than at the first relapse, confirming that the incidence of iEMR increases as patients live longer with current treatment modalities. In light of this information, unlike in past decades, EMR is one of the most important causes of transplantation failure in the modern era.

EMR occurs in diverse sites, including the CNS, testicles, bone, skin, and soft tissue, in which the graft-versus-leukemia (GVL) effect is less active than in BMR [19], suggesting that the GVL effect is one of the mechanisms responsible for the increased frequency and widespread distribution of EMR after allo-HSCT [2,20]. The CNS seems to be the most common EM involvement site in ALL patients [7,9]. We found that the CNS, either isolated or combined with other sites, was the most common EMR site for both ALL and AML.

Disease status at transplantation was an independent risk factor for both systemic relapse and iEMR, consistent with previous studies [3,5,7,12]. As expected, late-phase disease (CR2 +/active disease) was associated with a 3.4-fold greater risk for systemic relapse compared with transplantation at CR1 in pediatric patients, as reported previously [21,22]. We found that the risks of both systemic relapse and iEMR increased even in patients with CR2 or beyond. In recent years, quantification of MRD before transplantation has been used for post-transplantation relapse prediction and has shown great relevance [23]. Unfortunately, we could not analyze MRD as a risk factor, as there was no standardization for MRD measurement in our country until recently.

Prior EMD was one of the most predictive risk factors for iEMR after HSCT in many studies [5,7,9,13]. In our study, approximately 20% of patients with iEMR had EMR before HSCT, which was a significant factor for an increased cumulative incidence of iEMR after transplantation (HR, 2.3; $P = .007$). Shem-Tov et al. [5] reported that most of the patients with iEMR had EMD before transplantation at the same site, but this was much less frequent for CNS disease, possibly related to the therapeutic approach.

There are currently no data on the role of the conditioning regimen in determining EMR. Previous studies showed paradoxical results regarding the effect of conditioning regimen on EMR. A retrospective study found a higher incidence of iEMR in patients receiving a busulfan/cyclophosphamide (Bu/Cy) regimen compared with those receiving a TBI-containing regimen [24]; however, other studies did not show such a difference [25,26]. A Chinese study found a higher incidence of EMR in patients with AML who received TBI-based conditioning compared with those receiving a Bu/Cy regimen [7]. In the present study, we found a marginal protective effect of TBI on both systemic relapse (HR, 0.5; P = .057) and EMR (HR, 0.6; P = .074) in patients with ALL. It could be speculated that the conditioning regimen with chemotherapy only does not have as much of an advantage for sanctuary sites compared with TBI. The FORUM trial showed that the standard of care is remains TBI for patients with ALL [27]. The finding of TBI as an increased risk factor in both systemic relapse and iEMR in patients with AML, although not statistically significant, indicates that the biology of these acute leukemias differs.

The protective role of cGVHD in iEMR remains controversial in the literature. Some studies have reported a protective effect of cGVHD for EMR [7], whereas others have reported no effect [3,4,5,7,9,13]. We found that cGVHD had no protective role in iEMR but did protect against systemic relapse, suggesting that the pathogenesis of iEMR differs from that of systemic relapse and identifying EM sites as potential sanctuary sites for leukemic cells. This could be due to the greater homing of donor-derived cytotoxic T lymphocytes to the bone marrow than EM sites [28]. In addition, the intrinsic tendency of leukemic cells to invade EM sites may help leukemic cells escape immune surveillance by cytotoxic T cells [29].

There is no established standard of care for EMR after allo-HSCT. Considering the generally overall poor response to local treatment only [30], systemic therapy combined with local therapy should be considered, particularly for patients with good performance status. In our daily practice, we used systemic chemotherapy combined with local therapy to reduce tumor burden followed by further immunotherapy, corresponding to a second transplantation or DLI in patients with a good performance status. With modern immune and cellular therapies such as a bispecific T-cell engager antibody (blinatumomab) and chimeric antigen receptor (CAR) T cell therapy, significant advances have been made in treating patients with post-transplantation relapse, as in our two patients with post-transplantation relapse who underwent a second allo-HSCT by bridging with blinatumomab. CAR T cell therapy not only can traffic to EM sites to eradicate leukemia cells, but also can persist in PB and BM treatment and prevent systemic relapse. CAR T cell therapy has promising safety and efficacy in treating EM leukemia [31]. Although the effect of blinatumomab has not been well elucidated in patients with EMR, the treatment outcome is considered ineffective compared with that in patients with hematologic relapse without EMR [17,32]. However, EMD is usually associated with BMR in these studies. In one study, the use of blinatumomab in 2 ALL patients with iEMR provided good remission and a safe bridge to allo-HSCT [33]. The authors suggested that isolated EMR might be a different disease entity than EMD with BM relapse, and that blinatumomab could be a good treatment option for ALL patients with iEMR [33]. Although the immune effect probably prevents systemic relapse rather than EMR, recent studies have shown that certain immune modulators, such as ipilimumab, an immune checkpoint blocking agent targeting cytotoxic T lymphocyte-associated protein 4, will be successful in the treatment of EMR [34].

The 3-year OS for patients with first relapse after allo-HSCT in this series was 15.2%. Relapse by 6 months post-transplantation was independently associated with better outcomes. Although some studies reported that iEMR had a better prognosis than systemic relapse [3,5,14,27], others found a similar OS in these groups [7,8]. In our series, the estimated 3-year OS was slightly better in patients with iEMR than in those with systemic relapse.

Shem-Tov et al. [5] argued that EMR almost always results in systemic relapse in patients who do not undergo transplantation [30], which is not necessarily the case for post-transplantation relapse. They showed that patients with a first EMR continued to have recurrent EMR events, but most did not develop BM involvement. In contrast to this Israeli study, which included only adult patients, we observed that patients with systemic relapse as their first relapse tended to have systemic relapse as their second relapse (86.9% for second systemic relapse versus 13.0% for second iEMR), but those with first iEMR developed further systemic or iEMR relapse events with approximately similar frequencies (58.8% for second iEMR versus 41.2% for second systemic relapse). This discrepancy could be related to the difference in the biology of the disease between these cohorts, including different age groups. Although our follow-up period was very short, we observed a better prognosis in patients with a second iEMR compared with those with systemic relapse.

The major limitations of this study are related to its retrospective nature. Nonetheless, several conclusions can be drawn. The number of subjects in this study was sufficiently large. Owing to the low incidence of EMR, correlation analysis performed on a relatively large group will yield reliable results and more accurate parametric analysis. Another limitation of this study is that we could not include the MRD status of our patients.

In conclusion, the results presented herein demonstrate that iEMR is a frequent and significant relapse event that is challenging for transplant physicians to treat owing to a lack of standardized regimens. Compared with systemic relapse, EMR manifests clinically later after HSCT and is associated with a slightly better prognosis. CNS relapse is the most common subtype of EMR. Disease status at transplantation and prior EMD are independent risk factors for iEMR after HSCT. In the era of immune and cellular therapies, clinicians must be aware of and ensure the early diagnosis of EMR to prolong patient survival. Additional similar analyses using a more contemporary population of patients with available MRD assessments and more novel immunotherapy options are needed to identify the best treatment approaches for patients with post-transplantation relapse.

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Authorship statement: V.H. designed the study, drafted the manuscript, and performed the statistical analyses; V.U. created the figures and was involved in study design and data collection; G.Ö., K.Y., S.A., A.K., İ.O.B., B.Ş.K., C.B., T.İ., D.A., Ü.K., G.T.K., A.Y., M.G., S.K., G.T.K., M.K., F.V.O., M.E., Z.K., O.G., Y.Y., N.Ö., B.A., Ö.T., C.A., B.A., G.S., D.A., M.S.E., E.Z., and E.P. were

involved in study design and data collection; and all authors were involved in manuscript preparation and approved the final version of the manuscript.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jtct.2021.06.023.

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