(MLL-r) is responsible for approximately 80 % of ALL in infants, and is recognized as the most significant poor prognostic factor. Indeed, infants with MLL-r ALL bear unique characteristics, such as leukemia cells with CD10-negative immature B cell phenotype, high tumor burden at diagnosis, and dismal outcome with published event-free survival (EFS) rate of no more than 40 % even when treated with intensive chemotherapy with or without hematopoietic stem cell transplantation (HSCT) [2–4]. In contrast, MLL-r in children 1 year or older is rare, and their outcome and optimal treatment options remain controversial [5, 6]. Here, we report the clinical characteristics and outcome of children (≥1 year old at presentation) with MLL-r ALL who were enrolled in the studies conducted by the Tokyo Children's Cancer Study Group (TCCSG) from 1995 to 2009.

Patients and methods

Patients

Between November 1995 and May 2009, 1827 consecutive children with ALL who were 1 year or older at presentation were registered and treated in either of the five studies designated as TCCSG L95–14 ($n = 597$) [7, 8], L99–15 ($n = 754$) [9–11], L99–1502 ($n = 184$), L04–16 ($n = 135$), and L07–1602 ($n = 157$). The diagnosis of ALL was based on bone marrow morphology and cytochemical staining results. Each patient was evaluated with respect to the characteristics of the leukemic cells, including immunophenotype and cytogenetics. Rearrangement of *MLL* gene was detected mainly by karyotypic analysis with a G-banding technique. Split-signal fluorescence in situ hybridization (FISH), Southern blot analysis, real time PCR of several known MLL fusion transcripts were additionally tested in selected cases according to the physician's choice. Written informed consent, provided according to the Declaration of Helsinki, was obtained from the guardians of the patients, with institutional review board approval.

Treatment

Patients received different induction therapies according to their leukocyte (WBC) count and age at diagnosis; patients aged 1–6 years with WBC below 20,000/μL received four-drug induction regimen consisting of corticosteroid, vincristine, L-asparaginase, and anthracyclines, while patients with older age or higher WBC received five-drug induction with the inclusion of cyclophosphamide. Pre-phase of 7-day prednisolone monotherapy was introduced in the L99–15 study [9]. Following the initial induction therapy, patients with MLL-r ALL were stratified to the intensive high-risk post-remission therapy; regimen consisted of multiple high-dose cytarabine courses in L95–14, L99–15, and L99–1502 studies as illustrated in the supplemental table, and BFM95 high-risk block therapy in L04–16 and L07–1602 studies previously reported elsewhere [12]. All the MLL-r patients were allocated to undergo allogeneic HSCT at first remission. Allogeneic HSCT was performed at several stages according to the study: after the reinduction phase in the L95–14 study, after the second delayed intensification course in L99–15/L99–1502, and after the reinduction course (BFM protocol II) in the L04–16/L07–1602 studies. An add-on study evaluating minimal residual disease (MRD) targeting patient-specific immunoglobulin and T cell receptor gene rearrangements [13] were carried out along with the L99–15 study; however, the study failed because of low number of collected specimens.

Statistical considerations

The baseline characteristics and the clinical course of patients were analyzed using the χ^2 test or Fisher's exact test for categorical variables, and the Wilcoxon rank-sum test for continuous variables. EFS was defined as the time from the diagnosis of ALL to the last follow-up or the first event (failure to achieve remission, relapse, secondary malignancy, or any-cause death). Overall survival (OS) was defined as the time from the diagnosis of ALL to any-cause death. The probabilities of EFS (pEFS) and OS (pOS) were estimated using the Kaplan–Meier method. Standard errors (SE) were calculated using the Greenwood formula and curves were compared using the log-rank test. Cox proportional hazards regression model was used to identify the risk factors associated with the EFS rate. All analyses were performed using STATA® statistical software (version 11.0; StataCorp LP, College Station, TX). Follow-up data were actualized as of May 1, 2012.

Results

Patient characteristics

The characteristics of the patients at diagnosis are reported in Table 1. We identified 25 MLL-r patients (1.3 %) among 1827 children with ALL; 5/597 (0.8 %) in L95–14 study, 17/754 (2.2 %) in L99–15 study, 1/184 (0.5 %) in L99–1502 study, 1/135 (0.7 %) in L04–16 study, and 1/157 (0.6 %) in L07–1602 study. The MLL-r ALL patients were young with a median age at diagnosis of 2 years (range 1–15 years) and presented with high WBC [median 27,690/μL (range 1800–1,113,000/μL)]. There was one patient with T cell ALL who was a 15-year-old female with *t*(6;11)(q27;q23) blasts. The other 24 patients had B cell precursor (BCP) phenotype, and CD10 were negative in 15/24 cases.

Table 1 Characteristics of children ≥1 year old with *MLL* gene rearranged ALL

	Overall, *n* (%)	*MLL-AF4*	Other MLL-r	*P* value*
Total no. of patients	25	12	13	
Sex				
Male	15 (60.0)	5 (41.7)	10 (76.9)	0.164
Female	10 (40.0)	7 (58.3)	3 (23.1)	
Age, years				
1–2	18 (72.0)	7 (58.3)	11 (84.6)	0.307
3–9	2 (8.0)	1 (8.3)	1 (7.7)	
10–15	5 (20.0)	4 (33.3)	1 (7.7)	
WBC count, $\times 10^9$/L				
<50	15 (60.0)	5 (41.7)	10 (76.9)	0.164
50–100	0 (0.0)	0 (0.0)	0 (0.0)	
≥100	10 (40.0)	7 (58.3)	3 (23.1)	
CNS disease				
CNS1	21 (84.0)	10 (83.3)	11 (84.6)	0.580
CNS2	1 (4.0)	0 (0.0)	1 (7.7)	
CNS3	1 (4.0)	1 (8.3)	0 (0.0)	
TLP+	2 (8.0)	1 (8.3)	1 (7.7)	
CD10				
Negative	15 (60.0)	10 (83.3)	5 (38.5)	0.001
Positive	8 (32.0)	0 (0.0)	8 (61.5)	
NA	2 (8.0)	2 (16.6)	0 (0.0)	
Immunophenotype				
BCP-ALL	24 (96.0)	12 (100)	12 (92.3)	0.967
T-ALL	1 (4.0)	0 (0.0)	1 (7.7)	
MLL subtype				
t(4;11)(q21;q23)/*MLL-AF4*	12 (48.0)	12 (100)		
t(9;11)(p22;q23)/*MLL-AF9*	4 (16.0)		4 (30.8)	
t(11;19)(q23;p13.3)/*MLL-ENL*	1 (4.0)		1 (7.7)	
t(1;11)(q32;q23)/*MLL-AF1*	1 (4.0)		1 (7.7)	
t(6;11)(q27;q23)/*MLL-AF6*	1 (4.0)		1 (7.7)	
Normal karyotype[a]	5 (20.0)		5 (38.5)	
Others[b]	1 (4.0)		1 (7.7)	

ALL acute lymphoblastic leukemia, *WBC* white blood cell, *CNS* central nervous system, *TLP+* traumatic lumbar puncture, *NA* not available, *BCP-ALL* B-cell precursor ALL, *T-ALL* T-cell ALL

* Comparison between *MLL-AF4* and other MLL-r

[a] MLL-r is confirmed by split signal FISH and/or southern blot

[b] 47,XY,+X,*t*(2;11),del(q22) was detected. MLL-r is confirmed by Southern blot

Karyotypic analysis showed 11 patients with *t*(4;11)(q21;q23), three with *t*(9;11)(p22;q23), one with *t*(11;19)(q23;p13.3), one with *t*(1;11)(p32;q23), and one T-ALL case with *t*(6;11)(q27;q23). 11q23 abnormalities could not be determined in eight cases [six with normal karyotype, one with abnormalities including *t*(2;11), and one with karyotypic failure]; however, MLL-r of these patients was confirmed by Southern blot and/or FISH. *MLL-AF4* and *MLL-AF9* were detected in each case by real-time PCR. Thus, among the 24 BCP-ALL cases, half of them ($n = 12$) had *t*(4;11)/*MLL-AF4*-positive ALL. There was no significant difference in background characteristics between

patients with *MLL-AF4*-positive ALL and those with other MLL-r ALL, except higher positive rate of CD10 expression on other MLL-r leukemic cells (Table 1).

Treatment outcome

Remission induction results

Initial prednisolone response, measuring peripheral blood leukemia blasts after 7-day prednisolone monotherapy, could be evaluated in 20 patients, and 18 were good responders (<1000 blasts/μL). Additionally, day 15 bone marrow response could be evaluated in 19 patients, and 14 were M1 marrow (<5 % bone marrow blasts), five were M2 (5–25 % blasts), and none was M3 (>25 % blasts). The overall remission induction rate was 96.0 % (24/25). One patient who failed to achieve remission, a 6-year-old boy with *MLL-AF4* positive ALL who presented with WBC count of 364,400/μL achieved his first remission after additional chemotherapy and received unrelated bone marrow transplantation (UBMT) thereafter. However, this patient developed renal cell carcinoma and died of ALL relapse 16 months after the initial diagnosis.

Analysis of overall outcome

Of the 24 patients in remission, 19 received allogeneic HSCT in 1CR; five from sibling bone marrow, one from sibling cord blood, ten from unrelated bone marrow, and three from unrelated cord blood. Twelve cases remained in continuous complete remission (CCR) after receiving allogeneic HSCT, three relapsed, and four, all of whom transplanted from unrelated donor, died in remission [two because of thrombotic microangiopathy (TMA) and two of interstitial pneumonia]. Two patients relapsed during therapy; one died and one in CCR after HSCT in second remission. Three patients did not undergo allogeneic HSCT, two with chemotherapy only and one with autologous peripheral blood stem cell transplantation (PBSCT), and all are in CCR. The 5-year pOS and pEFS for all 25 patients were 64.0 % (SE 9.6 %) and 60.0 % (SE 9.7 %), respectively, after a median follow-up of 7.5 years (range 0.5–11.0 years) (Fig. 1). Information regarding late complications was not available except secondary malignant neoplasms. One patient developed renal cell carcinoma as described previously, but other late effects in 16 live patients could not be evaluated in this study.

The prognostic impact of several potential risk factors (Table 2) was determined. In the univariate analysis, 5-year pEFS of patients with CD10 positive ALL was significantly worse compared to that of CD10 negative cases. However, further analysis with a Cox regression model indicated that none of these factors exerted independent predictive strength (data not shown).

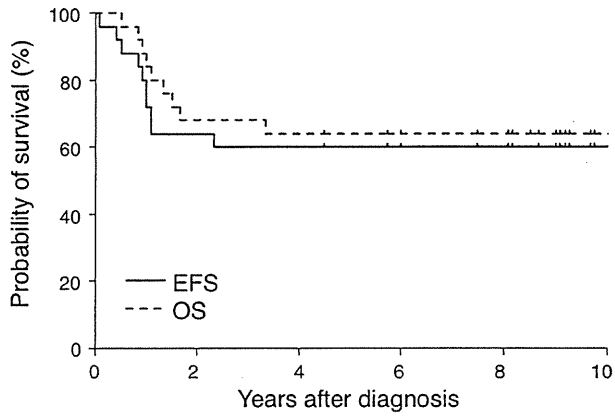

Fig. 1 Event-free survival (EFS) and overall survival (OS) rates for children (≥1 year old) with *MLL*-rearranged ALL treated with Tokyo Children's Cancer Study Group ALL protocols

Table 2 Five-year EFS by selected prognostic features for children ≥1 year old with *MLL* gene rearranged ALL

	No. of patients	5-year EFS, % (SE)	*P* value
Age, years			
1–2	18	66.6 (11.1)	0.188
3–15	7	42.8 (18.7)	
WBC count, × 10^9/L			
<100	15	60.0 (12.6)	0.905
≥100	10	60.0 (15.4)	
CNS disease			
CNS2, CNS3, or TLP+	4	75.0 (21.6)	0.581
CNS1	21	57.1 (10.7)	
CD10			
Negative	15	80.0 (10.3)	0.009
Positive	8	25.0 (15.3)	
MLL subtype			
t(4;11)(q21;q23)/*MLL-AF4*	12	75.0 (12.5)	0.237
Others	13	46.1 (13.8)	
Prednisolone response			
PGR	18	55.5 (11.7)	0.803
PPR	2	50.0 (35.3)	

CNS central nervous system, *EFS* event-free survival, *PGR* prednisolone good responder, *PPR* prednisolone poor responder, *SE* standard error, *TLP+* traumatic lumbar puncture

Outcome according to the MLL-r subtypes

Clinical characteristics and outcome of children with *MLL-AF4*-positive ALL are shown in Table 3. Notably, 9

Table 3 Clinical characteristics and outcome of children with t(4;11)/*MLL-AF4* positive ALL

	Sex	Age (year)	WBC ($\times 10^9$/L)	CNS disease	CD10	Karyotype	Protocol	PSL response	Day15 BM response	CR	HSCT in 1CR	Outcome
1	F	1	1.8	CNS1	neg	46XX, *t*(4;11)(q21;q23)	L95–14	ND	ND	Yes	sibBMT	CCR
2	F	14	18.3	CNS1	neg	46XX, *t*(4;11)(q21;q23)	L95–14	ND	ND	Yes	No	CCR
3	F	13	388.5	CNS1	neg	46XX, *t*(4;11)(q21;q23)	L95–14	ND	ND	Yes	APBSCT	CCR
4	M	12	414.6	CNS1	neg	47XY, +X, *t*(4;11)(q21;q23)	L95–14	ND	ND	Yes	sibBMT	CCR
5	F	1	25.1	TLP+	neg	48XX, +X, *t*(4;11)(q21;q23), +21	L99–15	PGR	M1	Yes	UCBT	CCR
6	M	6	364.4	CNS1	neg	47XY, +X, *t*(4;11)(q21;q23)	L99–15	PGR	M1	No	–	RCC, died of ALL
7	M	1	174	CNS1	neg	46XY, *t*(4;11)(q21;q23)	L99–15	PGR	M1	Yes	UBMT	CCR
8	F	1	1113	CNS3	neg	46XX, *t*(4;11)(q21;q23)	L99–15	PGR	M1	Yes	UCBT	CCR
9	M	2	15.8	CNS1	neg	47XY, +X, *t*(4;11)(q21;q23)	L99–15	PGR	M1	Yes	UBMT	Died of TMA
10	F	2	146.3	CNS1	neg	ND	L99–1502	PPR	M1	Yes	UBMT	CCR
11	M	11	320	CNS1	ND	46XY, *t*(4;11)(q21;q23)	L04–16	PGR	M1	Yes	–	Relapse, died of ALL
12	F	2	24.2	CNS1	ND	46XX, del(7)(p15), *t*(4;11)(q21;q23)	L07–1602	PGR	M1	Yes	sibBMT	CCR

ALL acute lymphoblastic leukemia, *APBSCT* autologous peripheral blood stem cell transplantation, *BM* bone marrow, *CCR* continuous complete remission, *CNS* central nervous system, *CR* complete remission, *F* female, *HSCT* hematopoietic stem cell transplantation, *M* male, *ND* no data, *neg* negative, *PGR* prednisolone good responder, *PPR* prednisolone poor responder, *PSL* prednisolone, *RCC* renal cell carcinoma, *sibBMT* sibling donor bone marrow transplantation, *TLP+* traumatic lumbar puncture, *TMA* thrombotic microangiopathy, *UBMT* unrelated bone marrow transplantation, *UCBT* unrelated cord blood transplantation, *WBC* white blood cell

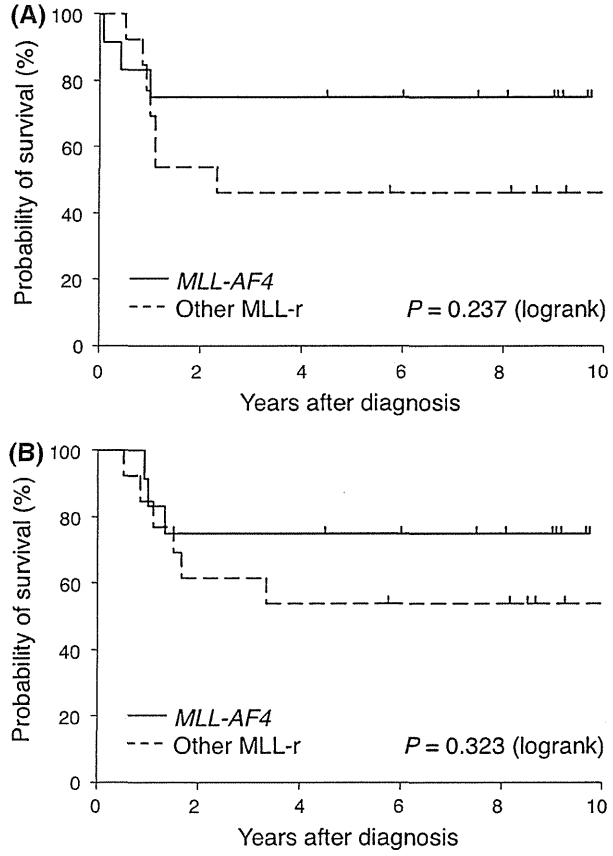

Fig. 2 **a** Event-free survival (EFS) and **b** Overall survival (OS) rates for children (≥1 year old) with *MLL-AF4* positive ALL and other MLL-r ALL treated with Tokyo Children's Cancer Study Group ALL protocols

out of 12 children are alive without any events and their 5-year pEFS and pOS was 75.0 % (SE 12.5 %). In contrast, 5-year pEFS and pOS of the 13 children with other MLL-r ALL were 46.1 % (SE 13.8 %) and 53.8 % (SE 13.8 %), respectively (Fig. 2). The number of each MLL-r subtype other than *MLL-AF4* is too small to analyze separately; however, there were four children with *MLL-AF9*-positive ALL and all of them were enrolled in the L99–15 study. Of the four patients, three underwent allogeneic HSCT in 1CR, two are alive (one with chemotherapy only and one with UBMT) and two died (one of CNS leukemia recurrence and one of HSCT-related interstitial pneumonia). Table 4 summarizes the information on the treatment for *MLL-AF4* and other MLL-r ALL patients. There was a trend of longer median days from diagnosis to HSCT in the patients with other MLL-r ALL, which could be attributed to the higher rate of HSCT from unrelated bone marrow donor compared with patients with *MLL-AF4*-positive ALL, although the difference was not statistically significant. Leukemia-related death was observed in two patients with *MLL-AF4* and three with other MLL-r. However, while there was only one HSCT-related death (TMA) in *MLL-AF4* patients, four HSCT-related deaths (TMA in one, interstitial pneumonia in two, and pneumocystis pneumonia in one) occurred in other MLL-r patients.

Discussion

In our cohort of 1827 children with ALL whose age was 1 year or older at their presentation, only 1.3 % was found to have *MLL* gene rearrangement (MLL-r), which is far less frequent compared to that in infants with ALL. However, it should be noted that there are potential risks of underestimation for detecting MLL-r, because up to 16 % of "true" MLL-r patients could be missed with only conventional cytogenetic tests unless molecular methods such as split signal FISH or Southern blotting are applied [14].

Pui et al. published the largest report that focused on children with MLL-r ALL, which reviewed 497 children and young adults with ALL and 11q23 abnormalities from 11 study groups and single institutions from 1983 to 1995 [5, 6]. In this report, outcome of children (≥1 year old) was significantly better than infants (<1 year old), and children with *MLL-AF4*- and *MLL-AF9*-positive ALL fared significantly worse, but there was no difference among *MLL* subtypes in infants. In fact, the outcome of children with *MLL-AF4*-positive ALL in recently completed clinical trials continues to be dismal; pEFS is 35 ± 11 % in 6 years in the BFM90 study ($n = 22$) [15], 40 ± 9.8 % in 6 years in the BFM95 study ($n = 25$) [12], and 43.8 ± 12.4 % in 5 years in the AIEOP ALL2000 study ($n = 16$) [16]. In that sense, it is notable that 9 out of 12 children with *MLL-AF4* positive ALL in our cohort survived with no events. Several factors contributing to the favorable outcome of *MLL-AF4* positive ALL children in the present report could be speculated. One is the intensive use of high-dose cytarabine in the TCCSG studies. High sensitivity of MLL-r leukemia cells to cytarabine is well recognized from in vitro study of infant ALL cells [17], which could be attributed to the high expression of the human equilibrative nucleoside transporter 1 (hENT1) that transports cytarabine across the cell membrane [18]. Another is high success of allogeneic HSCT in our cohort. Although one patient died of TMA after UBMT, seven out of eight children who underwent allogeneic HSCT in first remission survived in CCR. However, it should be noted that allogeneic HSCT could cause severe late effects that is not evaluated in the present analysis. Moreover, two patients who did not receive allogeneic HSCT (one with chemotherapy only and the other with autologous PBSCT) are alive in CCR. In the current ongoing childhood BCP-ALL study in Japan (designated as ALL-B12) conducted by the Japanese Pediatric Leukemia/

Table 4 Treatment of children ≥1 year old with *MLL* gene rearranged ALL

	Overall, *n* (%)	*MLL-AF4*	Other MLL-r	*P* value*
Total no. of patients	25	12	13	
Treatment regimen				
L95–14	5 (20.0)	4 (33.3)	1 (7.7)	0.058
L99–15/L99–1502	18 (72.0)	6 (50.0)	12 (92.3)	
L04–16/L07–1602	2 (8.0)	2 (16.6)	0 (0.0)	
Conditioning for HSCT in 1CR				
TBI (12 Gy) + ETP + CY	14 (70.0)	6 (66.6)	8 (72.7)	0.844
Non-TBI	6 (30.0)	3 (33.3)	3 (27.2)	
Donor for HSCT in 1CR				
Sibling bone marrow	5 (25.0)	3 (33.3)	2 (18.1)	0.409
Sibling cord blood	1 (5.0)	0 (0.0)	1 (9.0)	
Unrelated bone marrow	10 (50.0)	3 (33.3)	7 (63.6)	
Unrelated cord blood	3 (15.0)	2 (16.6)	1 (9.0)	
Autologous	1 (5.0)	1 (8.3)	0 (0.0)	
Days from diagnosis to HSCT in 1CR				
Median	224	196	228	0.653
Range	94–425	94–336	96–425	

ALL acute lymphoblastic leukemia, *HSCT* hematopoietic stem cell transplantation, *1CR* first complete remission, *TBI* total body irradiation, *ETP* etoposide, *CY* cyclophosphamide

* Comparison between *MLL-AF4* and other MLL-r

Lymphoma Study Group, only *MLL-AF4*-positive ALL patients with poor prednisolone response or patients with high level of MRD after early intensification course are eligible for allogeneic HSCT.

Patients with other subtypes of MLL-r are rare. Compared to the children with *MLL-AF4*-positive ALL, children with other MLL-r ALL had a trend of higher male predominance, lower age, and lower WBC count at diagnosis, although not statistically significant. Clearly, higher percentage of CD10 positive phenotype was observed in the other MLL-r ALL patients. Compared to *MLL-AF4*, it has been reported in an infant ALL study that the frequency of CD10 positivity is higher in *MLL-AF9,* and is associated with more mature immunoglobulin rearrangement pattern [19]. In fact, three out of four patients with *MLL-AF9*-positive ALL in our cohort had CD10-positive phenotype. While EFS and OS rate of children with *MLL-AF4*-positive ALL was favorable, that of children with other MLL-r ALL was poor with 46.1 % (SE 13.8 %) and 53.8 % (SE 13.8 %) in 5 years, respectively. It seems that the poor outcome of children with other MLL-r ALL has contributed to the poorer EFS rate of CD10-positive MLL-r ALL because all the evaluable cases with *MLL-AF4*-positive ALL had CD10-negative phenotype (Table 2). Because there was only one early relapse among the 13 patients with other MLL-r ALL, we speculate that the intensive use of high-dose cytarabine itself had favorable effect on other MLL-r cases as well as *MLL-AF4*-positive cases. Rather, poor final outcome in other MLL-r patients could be attributed to other factors such as higher incidence of HSCT-related deaths, inappropriate timing of HSCT for the relapsed cases, and so on. Causes of death in other MLL-r ALL were leukemia relapse in three cases and HSCT-related death in four cases (two because of interstitial pneumonia, one because of TMA, and one because of pneumocystis pneumonia). As there were two leukemia deaths and only one HSCT-related death in patients with *MLL-AF4*-positive ALL, it is likely that higher incidence of HSCT-related deaths adversely affected the poorer outcome in patients with other MLL-r ALL. Moreover, this might be attributed to the higher prevalence of young age children in other MLL-r ALL considering the fact that four out of five overall HSCT-related deaths occurred in patients 2 years old or younger. The timing of HSCT might have influenced on leukemia relapse among other MLL-r ALL patients: days from diagnosis to HSCT was 249, 257, and 425, respectively, in the three patients with other MLL-r ALL who had relapsed after HSCT, while no relapse occurred in patients who were transplanted within 240 days after diagnosis regardless of *MLL* subtypes. In addition, one of the three relapsed cases had T cell phenotype, which is generally considered to have poorer outcome compared to B cell precursor ALL. Undoubtedly the number of patients analyzed in this report is relatively small. Therefore, the potential bias could not be excluded. In any case, it is unacceptable that nearly half the treatment failure after allogeneic HSCT consisted of transplant-related complications.

As mentioned previously, late effects other than secondary malignant neoplasms could not be evaluated in this study because the data were not available in the present cohort. However, considering the fact that the majority of the non-infant patients with MLL-r ALL were young (18/25 cases were 1–2 years old at diagnosis) and many of these patients (8/13 live cases whose age were 1–2 years at diagnosis) have received HSCT with TBI-conditioning, the quality-of-life in these patients is an important issue. In the previous report on infants with MLL-r ALL, severe growth impairment was observed in nearly 60 %, especially in those who received TBI-based conditioning [14]. At present, it is not clear whether TBI adversely affects the growth and other late complications in children 1–2 years old as it does on infants. Nonetheless, considering the potential risk of developing severe late effects, the restriction of HSCT indication and development of non-TBI conditioning for those who need transplantation should be explored.

Recent research has revealed unique mechanism of MLL-r leukemogenesis, that the aberrant epigenetic regulation induced by MLL fusions via the histone H3 lysine 79 (H3K79) methyltransferase DOT1L brings about unique profile of gene expression, thus leading to leukemia [1, 20]. Clinical development of DNA methyltransferase inhibitors and/or histone deacetylase inhibitors is currently in progress [21]. Direct inhibition of DOT1L is also in clinical development [21–23]. These novel therapeutic approaches would be necessary to improve the outcome of infants and children with MLL-r ALL and might replace the position of allogeneic HSCT.

In conclusion, favorable outcome was observed in children 1 year or older with *MLL-AF4*-positive ALL treated with intensive chemotherapy followed by allogeneic HSCT. However, this strategy did not benefit children with other MLL-r subtypes and relatively high incidence of transplant-related death was an issue. Therefore, indication of HSCT in first remission for children with MLL-r ALL should be restricted to truly unfavorable risk cases identified by more accurate risk-stratifying tools such as MRD.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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