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However, these reports preceded the definition of NK cells and our understanding of the molecular basis of PNH, prompting reassessment. Mosaicism in PNH[1] allows side-by-side functional comparisons of GPI^{+} and GPI^{neg} NK cells within individual patients, enabling the assessment of NK cell activity on a per cell basis (Figure 1; supplemental Figure 1, available on the *Blood* Web site). Despite reports of impaired activity,[3,4] the GPI-deficient NK cells were proficient at target cell-induced granule exocytosis (Figure 1A-B; supplemental Figure 1). Thus, early findings associating reduced NK cell activity with reduced LGL numbers rather than intrinsic cellular activity are correct.[4] The absolute number of NK cells (and more variably, other lymphocytes) is indeed reduced in PNH[7]; in our cohort of 39 patients, two thirds had NK cell counts below the reference range (Figure 1C), and NK cell numbers were not significantly correlated with neutrophil, monocyte, or platelet counts (supplemental Figure 2). The basis for reduced NK cell numbers in PNH is unclear, although this might be related to impaired chemotactic or homeostatic mechanisms, as we recently reported.[8] Although the activity of GPI-deficient NK cells is unimpaired, a reduction in absolute numbers of NK cells will reduce NK cell activity in the blood as a whole.

Clearly, PNH should not be classified as a functional NK cell deficiency (NKD). Classical NKD is characterized by ~1/10 the normal number of NK cells, and counts in most of our PNH patients exceeded this (Figure 1C). Furthermore, the term NKD is reserved for where "the impact upon NK cells need represent the major immunological abnormality in the patient."[6] In PNH, all hematopoietic lineages are affected because of the presence of *PIGA* mutations in hematopoietic stem cells.[1] More compelling is the clinical phenotype; the defining feature of NKD is the heightened susceptibility to viruses,[5,6] which has not been observed in PNH.[7,9,10] Instead, infection in PNH is bacterial in origin[10] and likely to be associated with neutropenia secondary to underlying bone marrow failure or associated with use of eculizumab, which increases the risk of infection with encapsulated bacteria normally eliminated by terminal complement components.[1] In summary, the low numbers of NK cells in PNH affect overall cytotoxicity, but this defect is not severe enough to manifest as heightened susceptibility to viral infection as seen in NKD.

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Contribution: R.J.K., A.H., and P.H. manage the PNH clinic and recruited the patient cohort; Y.M.E.-S. performed the experimental work; G.P.C. and Y.M.E.-S. designed the study; Y.M.E.-S., G.M.D., R.J.K., and G.P.C. analyzed the data; and G.P.C. wrote the article with input from all authors.

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To the editor:

Comparison of transplantation with reduced and myeloablative conditioning for children with acute lymphoblastic leukemia

Allogeneic stem cell transplantation (SCT) for patients with acute lymphoblastic leukemia (ALL) is mostly undergone with myeloablative conditioning (MAC) and it could be the major cause of short- or long-term complications such as endocrinologic disorders including hypogonadism or growth hormone-deficient short stature.[1,2] In recent years, SCT with reduced-intensity conditioning (RIC)

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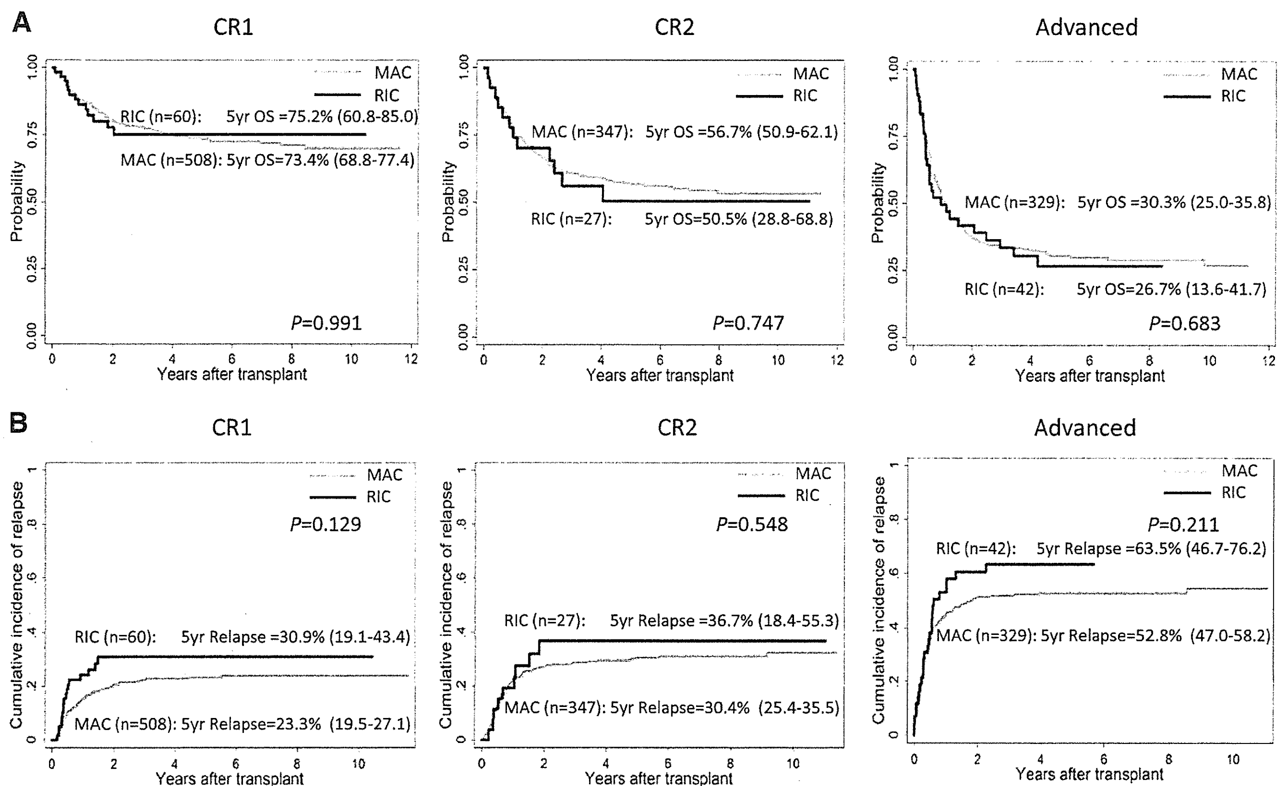

Figure 1. Probability of OS and cumulative incidence of relapse of children who underwent transplantation at CR1, CR2, and advanced stages with RIC and MAC regimen. (A) Probability of OS. (B) Cumulative incidence of relapse.

regimens was introduced for children who have pretransplant morbidity or are unable to tolerate a MAC regimen.[3-5] Although it has the possibility of reducing posttransplant late toxicities,[6] the exact clinical implications of a RIC regimen are still unclear. Therefore, in this study, we retrospectively compared the transplant outcomes with RIC and MAC regimens for children with ALL to determine the feasibility of SCT with a RIC regimen using the Transplant Registry Unified Management Program (TRUMP), a nationwide database established by the Japan Society for Hematopoietic Cell Transplantation (JSHCT).

We analyzed 1334 children with ALL who underwent allogeneic SCT as the first transplant from January 2000 to December 2010 in Japan; they consisted of 1201 patients with a MAC regimen and 133 patients with a RIC regimen according to the intensity of the conditioning regimen. The definition of RIC or MAC was based on the internationally recognized criteria, in which MAC is defined as fractionated total body irradiation (TBI) of ≥8 Gy, a single TBI of ≥5 Gy, or busulfan of ≥8 mg/kg or ≥280 mg/m^2, and other regimens are categorized as RIC.[7] Patients were transplanted at first complete remission (CR1, n = 568), second complete remission (CR2, n = 374), or advanced stages (third or further remission and relapse, n = 371), and there was no significant difference between RIC and MAC in this regard (P = .125). The type of SCT according to the stem cell source was related bone marrow transplantation (n = 413), related peripheral blood stem cell transplantation (n = 89), unrelated bone marrow transplantation (n = 446), or unrelated cord blood transplantation (n = 386); the serological HLA disparity between donor and patient was none (n = 816) or mismatched (n = 486) for the graft-versus-host direction, and the number of mismatched transplants was significantly higher in RIC patients (P = .007).

At a median follow-up of 765 days, 5-year overall survival (OS) rates of patients with RIC and MAC were 52.4% and 56.1% (P = .525) and they were 75.2% and 73.4% at CR1 (P = .991), 50.5% and 56.7% at CR2 (P = .747), and 26.7% and 30.3% at more advanced stages (P = .683), respectively (Figure 1A). Five-year relapse-free survival rates were 43.0% and 52.4% in RIC and MAC (P = .070) and were 62.3% and 68.2% at CR1 (P = .249), 46.6% and 54.0% at CR2 (P = .520), and 14.9% and 27.0% at more advanced stages (P = .295), respectively. Relapse was observed in 434 patients (54 with RIC and 380 with MAC), which included 125 in CR1, 110 in CR2, and 194 in advanced stages at SCT. The cumulative incidence of relapse rates at 5 years were 43.1% in RIC and 33.6% in MAC (P = .020). The 5-year relapse rates of RIC and MAC at each disease status of SCT were 30.9% and 23.3% at CR1 (P = .129), 36.7% and 30.4% at CR2 (P = .548), and 63.5% and 52.8% at more advanced stages (P = .211), respectively (Figure 1B). Treatment-related mortality (TRM) among all patients was observed in 196 patients (18 with RIC and 178 with MAC) and the cumulative incidences of TRM at 5 years were 15.7% and 15.3% with RIC and MAC, respectively (P = .953). Neutrophil engraftment with absolute neutrophil count of ≥500/mm^3 was obtained in 944 patients (95 with RIC and 849 with MAC) and the cumulative incidence rates of neutrophil engraftment at day 100 were 91.7% with RIC and 96.2% with MAC (P = .498). After multivariate analysis adjusted by age at diagnosis, gender of patient, disease status at SCT, stem cell source, RIC/MAC, HLA compatibility, TBI, and cytogenetics, transplant outcomes with RIC and MAC regimens were not significantly different in OS (hazard ratio [HR] = 1.10, 95% confidence interval [CI] = 0.84-1.46, P = .488), relapse-free survival (HR = 1.25, 95% CI = 0.96-1.61, P = .093), relapse rates (HR = 1.11, 95% CI = 0.80-1.54, P = .530),

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TRM (HR = 0.89, 95% CI = 0.55-1.44, P = .621), and neutrophil engraftment (HR = 0.99, 95% CI = 0.79-1.26, P = .983).

In conclusion, the transplant outcomes of children with ALL who were given an RIC regimen in allogeneic SCT were not significantly different from those with an MAC regimen. Because this is a registry-based retrospective study and the number of patients with an RIC regimen is small, the results should be interpreted with caution. We need to proceed to a prospective study to prove the feasibility of SCT with an RIC regimen in children with ALL in order to reduce the transplant-related toxicities, especially in terms of the late effects after SCT.

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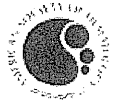

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Comparison of transplantation with reduced and myeloablative conditioning for children with acute lymphoblastic leukemia

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ORIGINAL ARTICLE

Acute megakaryoblastic leukemia with acquired trisomy 21 and *GATA1* mutations in phenotypically normal children

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Abstract *GATA1* mutations are found almost exclusively in children with myeloid proliferations related to Down syndrome (DS). Here, we report two phenotypically and cytogenetically normal children with acute megakaryoblastic leukemia (AMKL) whose blasts had both acquired trisomy 21 and *GATA1* mutation. Patient 1 was diagnosed with transient abnormal myelopoiesis in the neonatal period. Following spontaneous improvement of the disease, leukemic blasts increased 7 months later. He received less intensive chemotherapy, and he is now 6 years old in complete remission. Patient 2 was diagnosed with AMKL at the age of 18 months. Although he received intensive chemotherapy and a cord blood transplantation, he died without gaining remission. In both cases, trisomy 21 and *GATA1* mutation were detected only in leukemic blasts, but not in germline samples. Based on a literature review, we identified reports describing 14 non-DS AMKL with *GATA1* mutation and acquired trisomy 21. Of those, 12 cases were diagnosed during the neonatal period, whereas the remaining 2 cases were diagnosed at the age of 22 and 31 months, respectively.

Conclusion: These cases suggest that *GATA1* mutation may cooperate with the additional chromosome 21 in developing myeloid proliferations even in non-DS patients.

Keywords GATA1 · Acute megakaryoblastic leukemia · Trisomy 21 · Transient abnormal myelopoiesis

Abbreviations

AMKL Acute megakaryoblastic leukemia
BMA Bone marrow aspiration
CBC Complete blood count

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CBT	Cord blood transplantation
DS	Down syndrome
FCM	Flow cytometry
FISH	Fluorescent in situ hybridization
ML-DS	Myeloid leukemia associated with DS
PB	Peripheral blood
PHA	Phytohemagglutinin
TAM	Transient abnormal myelopoiesis
UPN	Unique patient number
WHO	World Health Organization

Introduction

Children with Down syndrome (DS) have a 10- to 20-fold increased risk of leukemia compared with non-DS children. In particular, the risk of developing acute megakaryoblastic leukemia (AMKL) is estimated to be 500 times higher in children with DS than in those without DS. Most patients with myeloid leukemia in DS (ML-DS) have a history of transient abnormal myelopoiesis (TAM) during their neonatal period. TAM is a unique clonal disease that occurs in approximately 10 % of DS neonates. It is characterized by proliferation of megakaryoblasts in the liver, spleen, and peripheral blood (PB) with a high incidence of spontaneous remission in which approximately 20 % of patients develop ML-DS within 4 years from initial diagnosis [12, 27]. Because of their unique clinical and biological characteristics, these two entities were categorized as "myeloid proliferations related to DS" in the World Health Organization (WHO) 2008 classification [22].

Acquired somatic mutations in the *GATA1* gene, which is an essential hematopoietic transcription factor, are detected in almost all TAM and subsequent ML-DS [23, 24]. This suggests that *GATA1* mutations may cooperate with the additional chromosome 21 in developing myeloid proliferations related to DS. So far, *GATA1* mutations have been reported almost exclusively in children with DS. The WHO 2008 classification defined TAM as "a unique disorder of DS newborns" [22]. However, several anecdotal reports have described that non-DS children also developed TAM and AMKL that harbored somatic mutations in *GATA1* accompanied by acquired trisomy 21 in their leukemic blasts [1–3, 7, 11, 14, 15, 17, 18, 20, 25].

Here, we report two cases of AMKL with *GATA1* mutation and acquired trisomy 21 that developed in children without phenotypic features of DS.

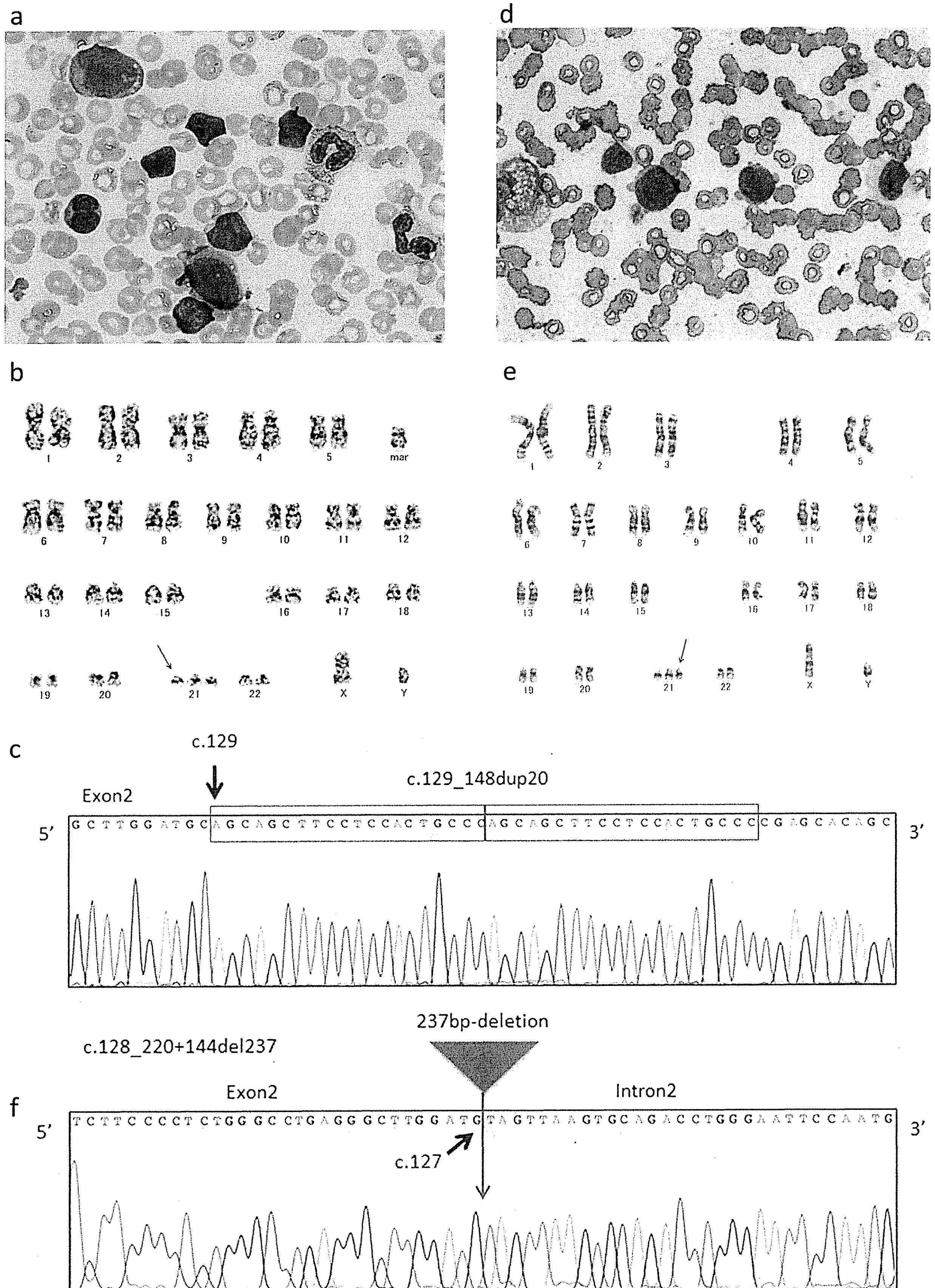
Case report

Patient 1 was a full-term male newborn with normal prenatal history who presented with purpura and petechiae throughout his body on the first day after birth. Physical examination revealed no evidence of hepatosplenomegaly, lymphadenopathy, testicular swelling, or phenotypic features of DS. Complete blood count (CBC) showed thrombocytopenia (17×10^9/L) and leukocytosis (24×10^9/L) with 57 % blasts and a bone marrow aspiration (BMA) that showed normocellularity, normal count of megakaryocytes (94/μL), and 12 % blasts that were medium-sized with basophilic cytoplasm and occasionally demonstrating blebs (Fig. 1a). Cytochemical staining of blasts were negative for myeloperoxidase. Flow cytometric (FCM) analysis showed that the blasts expressed CD7, CD13, CD33, CD34, CD41, and HLA-DR, but not glycophorin A. Trisomy 21 was detected by cytogenetic analysis without phytohemagglutinin (PHA) stimulation (Fig. 1b), whereas the karyotype was normal with PHA stimulation (Table 1). Fluorescent in situ hybridization (FISH) analysis for chromosome 21 showed a normal two-signal pattern in buccal epithelial cells.

Because of neonatal onset and presence of trisomy 21 in leukemic blasts, we examined *GATA1* gene mutations according to the procedure described previously [24]. Sequence analysis revealed a 20-bp duplication (c.129_148dup20; Fig. 1c). This mutation caused a frame shift that introduced a stop codon at codon 143 and produced a truncated GATA1 protein (GATA1s).

The patient was diagnosed with TAM, and we decided to observe him carefully without chemotherapy because his general condition was well. PB blasts decreased, and he became transfusion independent 1 month after presentation, but the BM blast count remained around 10 %. At 7 months of age, PB blasts increased and infiltrated the skull which prompted us to initiate intensive chemotherapy [21]. Additional chromosomal abnormalities were not detected. Although circulating blasts and bone involvement disappeared immediately after administrating induction chemotherapy, profound myelosuppression with high fever occurred. Since severe hemophagocytosis following candidemia and sepsis caused by *Stenotrophomonas maltophilia* was observed both in the PB and BM, we diagnosed him with secondary hemophagocytic syndrome and administered chemoimmunotherapy consisting of dexamethasone, etoposide, and cyclosporine [19]. Finally, a BMA at 8 weeks after the initiation of induction therapy revealed normal hematopoiesis without evidence of blast or

Fig. 1 Characteristics of leukemic blasts. **a–c** Leukemic blasts obtained from Patient 1. **a** The blasts were medium-sized with basophilic cytoplasm and occasionally demonstrating blebs. **b** Cytogenetic analysis without PHA stimulation. *Arrow* shows trisomy 21. **c** GATA1 exon 2 mutation analysis revealed a 20-bp duplication (c.129_148dup20). **d–f** Leukemic blasts obtained from Patient 2. **d** The blasts showed high nucleo-cytoplasmic ratio and occasionally had blebs. **e** Cytogenetic analysis without PHA stimulation. *Arrow* shows trisomy 21. **f** GATA1 exon 2 mutation analysis revealed a 237-bp deletion (c.128_220+144del237)


a
d
b
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c.129
c.129_148dup20
Exon2
5'
GCTTGGATGC AGCAGCTTCCTCCACTGCCC AGCAGCTTCCTCCACTGCCC CGAGCACAGC
3'
237bp-deletion
c.128_220+144del237
f
Exon2
Intron2
5'
TCTTCCCCTCTGGGCCTGAGGGCTTGGATG TAGTTAAGTGCAGACCTGGGAATTCCAATG
3'
c.127

hemophagocytosis, and he was considered to be in hematological remission. *GATA1* mutation was no longer detected and cytogenetic analysis without PHA stimulation showed a normal karyotype. Because of severe myelosupression associated with hemophagocytosis after induction therapy, we decided to administer three courses of less intensive consolidation therapy utilizing the ML-DS protocol [9]. This patient remains in complete remission for 6 years after initial presentation, with normal growth and development. Neither trisomy 21 nor *GATA1* mutation is observed.

Patient 2 had no remarkable medical history until the age of 18 months when he developed hepatosplenomegaly and thrombocytopenia with circulating blasts. Physical examination revealed hepatosplenomegaly (liver 3 cm and spleen 8 cm below costal margin) without lymphadenopathy, testicular swelling, or phenotypic features of DS. The CBC showed leukocytosis (45×10^9/L) with 64 % blasts, anemia (Hb 8.0 g/dL) and thrombocytopenia (42×10^9/L), and BMA showed 43 % blasts. The blasts occasionally had blebs and were negative for myeloperoxidase (Fig. 1d). FCM analysis showed expression of CD7, CD13, CD33, CD34, CD42b, and HLA-DR, but was negative for glycophorin A. Cytogenetic analysis without PHA stimulation revealed trisomy 21 (Fig. 1e) and a partial deletion of the long arm at 11q23, whereas the karyotype with PHA stimulation was normal (Table 1). FISH analysis for chromosome 21 showed a normal two-signal pattern in buccal epithelial cells. Reverse transcription-polymerase chain reaction showed no evidence of *MLL* gene rearrangement. Because of the presence of acquired trisomy 21, we tested for *GATA1* gene mutations. Sequence analysis revealed a 237-bp deletion (c.128_220+144del237), which caused splicing error and produced GATA1s protein (Fig. 1f).

He was diagnosed with AMKL, but we suspended immediate initiation of intensive remission induction chemotherapy because of the presence of *GATA1* mutation. However, since he developed respiratory distress caused by prominent hepatosplenomegaly, we administered 24-h continuous infusion of low-dose cytarabine (100 mg/m_2). Six days after initiation of continuous cytarabine, PB blasts decreased to 1.5 %. Considering the presence of *GATA1* mutation and good response to cytarabine, we decided to treat him with less intensive chemotherapy for ML-DS [9]. After the second course of chemotherapy, PB blasts increased and a complex karyotype was observed (Table 1). He did not achieve remission even after additional four courses of more intensive chemotherapy and unrelated cord blood transplantation (CBT) with preconditioning regimen consisting of busulfan, fludarabine, and melphalan. The patient died of disease at 10 months after diagnosis.

Discussion

GATA1 is an essential hematopoietic transcription factor, which is required for the normal growth and maturation of both erythroid and megakaryocyte hematopoiesis. Germline mutations in the *GATA1* gene cause X-linked dyserythropoietic anemia with thrombocytopenia [13] or X-linked dyserythropoietic anemia with neutropenia [6], whereas acquired somatic mutations have been found in both TAM and ML-DS [23, 24].

Table 1 Cytogenetic analyses in cases 1 and 2

Source	Age at examination	PHA-stimulation	Karyotype
Case 1			
PB	0 years 0 months (day 12)	–	48,XY,+21,+mar[20]
PB	0 years 1 month	+	46,XY[20]
BM	0 years 0 months (day 21)	–	48,XY,+21,+mar[19]
BM	0 years 9 months (in remission)	–	46,XY[20]
Case 2			
PB	1 year 6 months	–	47,XY,+21[14]/46,XY[6]
PB	1 year 6 months	+	46,XY[20]
BM	1 year 6 months	–	47,XY,?del(11)(q23q23),+21[2]/47,idem,i(7)(q10)[2]/46,XY[16]
BM	1 year 7 months	–	46,XY[20]
BM	1 year 8 months	–	42,X,-Y,-2,-5,-8,del(11)(q23q23),-12,der(17)t(11;17)(q13:p13),+21[1]/46,XY[18]
BM	1 year 9 months	–	46,XY[20]
BM	1 year 9 months	–	47,XY,add(17)(p11.2),+21[5]/46,XY[15]
BM	1 year 10 months	–	47,XY,add(17)(p11.2),+21[18]/47,idem,t(1;20)(p32;q13.1)[1]/ 47,idem,t(11;15)(p15;q24)[1]

Table 2 Reported cases of *GATA1*-related TAM and AMKL in non-Down syndrome patients

UPN	Sex	Diagnosis of MPD[a]	Cytogenetics of leukemic blasts	Cytogenetics or FISH analysis on germline sample[b]	*GATA1* exon2 mutation(RNA)	Antileukemic therapy	Clinical status	Reference
1	Male	6 days	47,XY,dup(6)(q25.3q26),+21	46,XY,dup(6)(q25.3q26) (PHA)	c.137ins57	None	CCR	[2]
2	Female	1 day	48,XX,+21,+21	46,XX (skin)	c.90_91delAG	None	CCR	[17]
3	Male	1 day	46,XY[10]/47,XY,+21[10]	46,XX (skin)	c.85G>T	None	CCR	[3]
4	Female	4 days	47,XX,+21	46,XX (BM in CR)	c.119G>T	None	CCR	[11]
5	Male	1 day	47,XY,+21	46,XY (PHA) No trisomy21 by FISH (buccal)	c.187_188ins8	None	CCR	[15]
6	Male	1 day	47,XY,+21	46,XY (BM in CR)	c.195_196ins7	None	CCR	[20]
7[c]	Female	1 month 7 months	47,XX,+21[16]/46,XX[4] (at 1 month) 47,XX,+21[14]/47,idem,del(5)(p13)[3]/46,XX[8] (at 7 months)	No trisomy21 by FISH (buccal)	c.150delG	Intensive Cx	CCR	[7]
8[c]	Female	7 days 24 months	47,XX,+21(at 7 days) 47,XX,der(10)t(1;10)(q32.3;q26)ins(10;?)(q26;?)x2,+21(at 24 months)	46,XX (skin)	c.97del35	Intensive Cx	CCR	[2]
9[c]	Female	1 day 17 months	47,XX,+21(in TMD) (at 1 day) 47,XX,der(9)t(1;9)(q25;q34),+21[14]/46,XX[11] (at 17 months)	46,XX (BM in CR)	c.49C>T	Intensive Cx	CCR	[20]
10	Male	1 month	47,XY,+21	46,XY (BM in CR)	c.1A>G	Less intensive Cx	CCR	[1]
11	Male	1 day	47,XX,+21	46,XX (skin) No trisomy21 by FISH (buccal)	c221G>C	Less intensive Cx	CCR	[25]
12	Male	1 day 7 months	See Table 1	No trisomy21 by FISH (buccal)	c.129_148dup20	Both Intensive and less intensive Cx	CCR	Case1
13	Male	1 day	47,XY,+21	46,XY (skin)	c.142insGTGTG	NA	Dead[e]	[1]
14[d]	Male	22 months	47,XY,+21,del(20)(q?)	46,XY (skin)	c.217-218delCC	Intensive Cx+BMT	CCR	[18]
15[d]	Male	31 months	48,XY,+8, +21	NA (Identical twin of UPN 14)	c.217-218delCC	Intensive Cx+BMT	CCR	[18]
16	Male	18 months	See Table 1	No trisomy21 by FISH (buccal)	c.128_220+144del237	Intensive Cx+CBT	DOD	Case2

NA not available, *BM* bone marrow, *PB* peripheral blood, *CCR* continuous complete remission, *DOD* dead of disease, *Cx* chemotherapy, *BMT* BM transplantation, *CBT* cord blood transplantation, *UPN* unique patient number, *MPD* myeloproliferative disease

[a] MPD, including TAM and AMKL

[b] Skin, sample obtained from skin fibroblast; buccal, sample obtained from buccal epithelium; PHA, cytogenetics with PHA stimulation; BM in CR, bone marrow sample in clinical remission

[c] Cases 7–9 were diagnosed AMKL in neonatal period. They achieved remission spontaneously, but relapsed thereafter.

[d] Patients 14 and 15 are identical twins

[e] Death due to complication (details were not given)

Including the 2 patients described, 16 cases of *GATA1* mutation-related TAM and AMKL in phenotypically and cytogenotypically normal children have been reported thus far (Table 2). Of these 16 cases, 13 were diagnosed during the first few months of life similar to what is observed for TAM in DS. In 6 cases (unique patient number, UPN 1-6), the blasts disappeared spontaneously without chemotherapy, and the patients did not develop subsequent AMKL [2, 3, 11, 15, 17, 20]. Another 3 cases (UPN 7-9) attained spontaneous remission, but blasts increased again at 6 to 24 months after diagnosis [2, 7, 20] similar to the pattern seen in TAM progressing to ML-DS [22]. These 3 cases received AML-type chemotherapy and remained in complete remission. Other 2 cases (UPN 10 and 11) received less intensive chemotherapy for TAM [1, 25]. Patient 1 reported here (UPN 12) followed a unique course as described above. One case (UPN 13) was diagnosed with AMKL at premature birth and died from non-leukemia related complications (details were not described) [1]. We speculate that this case may have been a severe form of TAM that often experience fatal complications [12]. The remaining 3 cases were diagnosed at an age between 1 and 3 years (UPN 14-16). Stark et al. reported a case of identical twins developing AMKL without history of TAM, and both received allogeneic BM transplant from their complete-HLA-matched father after chemotherapy resulting in complete remission (UPN 14, 15) [14, 18]. In contrast, patient 2 presented in this current report showed resistance to intensive chemotherapy and CBT and succumbed to disease.

Approximately 20 % of patients with TAM develop ML-DS and 10 % die mostly due to liver failure or coagulopathy [4]. Klusmann et al. reported that 19 % of patients with TAM required treatment with low-dose cytarabine because of severe symptoms [8]. Of 13 non-DS cases who were diagnosed with TAM in our literature review, 4 patients developed subsequent myeloid leukemia; one patient died; and 2 patients received less intensive chemotherapy. Although the number of cases was limited, clinical course of non-DS TAM appeared similar to that of TAM in DS.

Children with ML-DS harboring *GATA1* mutations show better response to chemotherapy and more favorable prognosis compared to non-DS children with AML [9, 22]. Based on our literature review, 7 non-DS cases were diagnosed with AMKL harboring *GATA1* mutation after the first 6 months of life. Of those, 6 cases remained in remission after receiving varying treatments such as intensive chemotherapy and BM transplantation. Only patient 2 presented here had resistant disease and died without attaining remission even after CBT. It remains to be elucidated whether myeloid leukemia with *GATA1* mutation in non-DS children also shows good response to less intensive chemotherapy similar to ML-DS.

TAM and ML-DS uncommonly occurs in phenotypically normal children with trisomy 21 mosaicism [22]. AMKL in children with mosaic DS have also been reported to show favorable outcome. Kudo et al. reported 7 cases with AMKL and mosaic DS in which trisomy 21 was confirmed in skin fibroblasts or lymphocytes in remission [10]. About half of them had mental retardation or dysmorphic features. Cytogenetic analysis using various types of cells such as skin fibroblasts, buccal mucosa, or remission marrow is necessary in order to rule out trisomy 21 mosaicism; however, it is theoretically impossible to diagnose patients with mosaic DS with very low frequency of trisomy 21 cells using classical assays such as G-banding or FISH. Although SNP microarray analysis may be helpful in detecting low-level mosaicism, it is not utilized routinely. None of 16 cases with *GATA1* mutation-related TAM and AMKL in "apparently normal children" mentioned above showed phenotypic features of DS or trisomy 21 in their germline samples. From a clinical point of view, it would be acceptable to consider these patients as "non-DS patients".

Our results reconfirm that *GATA1* mutation cooperates with trisomy 21 in developing myeloid proliferations since this combinatorial aberration was also found in "apparently normal children". However, it is still unknown why children harboring trisomy 21 are likely to gain *GATA1* mutations or how these mutations and trisomy 21 work together in leukemogenesis. In children with DS, it has been elucidated that several genetic events, such as dysregulation of the cohesin complex, accumulate during progression from TAM to ML-DS [16, 26]. Mechanistic insights may be achieved by comparing the genetic landscapes of *GATA1* mutation-related myeloid proliferations between DS and non-DS. Based on the limited number of cases of non-DS AMKL examined thus far, the coincidence of trisomy 21 and *GATA1* mutation appears uncommon [1, 5, 26]. However, since *GATA1* mutation is not examined routinely in non-DS AMKL patients, the frequency of mutation among these types of patients is unknown; systematic examination in a large cohort seems warranted. If leukemic blasts of non-DS AMKL patient show trisomy 21, it seems reasonable based on current evidence that *GATA1* mutations should be examined.

Conflict of interest There are no conflicts of interest to report.

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ORIGINAL ARTICLE

Outcome of pediatric acute lymphoblastic leukemia with very late relapse: a retrospective analysis by the Tokyo Children's Cancer Study Group (TCCSG)

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Abstract Relapse period is strongly associated with second relapse risk in relapsed acute lymphoblastic leukemia (ALL) in children. In this context, the treatment outcome of very late relapse should be better; however, data regarding very late relapse is limited. We retrospectively analyzed the outcomes of two consecutive Tokyo Children's Cancer Study Group (TCCSG) ALL trials (1995–2004) with a focus on late relapse, which was divided into two categories: late relapse (6–24 months from the end of therapy, $n = 48$) and very late relapse (>24 months from the end of therapy, $n = 57$). Forty-three patients (29 late relapse and 14 very late relapse) received allogeneic hematopoietic stem cell transplantation (HSCT) at second remission. The event-free survival (EFS) probabilities of late relapse and very late relapse were 54.5 ± 7.3 and 64.8 ± 6.8 % at 7 years, respectively ($P = 0.36$), and were not significantly different. However, the second relapse incidence of late relapse (34.7 ± 7.1 %) was higher than that of very late relapse (15.5 ± 5.1 %, $P = 0.03$). The second relapse risk was low for very late relapse ALL, which suggests that these patients should be treated without allogeneic HSCT.

Keywords Acute lymphoblastic leukemia · Relapse · Very late · Children

Introduction

Current clinical trials have cured more than 80 % of children with acute lymphoblastic leukemia (ALL), and one of the main reasons for this outcome was the adaptation of a risk stratification strategy using biological features and

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early treatment responses [1–6]. However, relapse remains the most common treatment failure. Salvage therapy for relapsed pediatric ALL typically consists of intensive chemotherapy including allogeneic hematopoietic stem cell transplantation (HSCT) as a consolidation treatment for most relapsed patients, who had a survival probability of 40–60 % after the first relapse [7–9].

Relapse risk-oriented stratification has also contributed to improving the outcome of relapsed childhood ALL [9, 10]. Previous studies demonstrated that the risk of second relapse could be estimated by the relapse period, relapse site, immunophenotype [11–13], and early responses to re-remission induction therapy. Thus, most clinical trial groups for pediatric ALL have adopted a combination of these factors in order to treat relapsed ALL [12, 14, 15].

The relapse period is considered the most important of these risk factors, and the Berlin–Frankfurt–Munster (BFM) group divided the relapse period into three categories: very early (earlier than 18 months from the diagnosis), early (later than 18 months from the diagnosis and earlier than 6 months from the end of the treatment), and late (later than 6 months from the end of the treatment), and showed that late relapse was associated with a good prognosis. The Pediatric Oncology Group also stratified ALL that relapsed within 6 months after the cessation of treatments as being at a higher risk of second relapse [16]. In this context, the second relapse risk of "very late" relapsed ALL should be lower, as suggested by previous studies [17–19]. However, the number of patients analyzed has been limited due to the rarity of very late relapse, and data regarding long-term outcomes is currently insufficient to establish a standard treatment for patients with very late relapse. Thus, we retrospectively analyzed the long-term outcomes of late relapsed ALL patients who were enrolled in two consecutive Tokyo Children's Cancer Study Group (TCCSG) clinical trials, with a focus on children with very late relapse.

Patients and methods

Patients

In order to identify patients with very late relapse, a total of 1,605 newly diagnosed ALL patients (1–18 years) enrolled in the two consecutive TCCSG trials, L95-14 [20] (between 1995 and 1999, $n = 597$), L99-15/1502 [21–23] (between 1999 and 2004, $n = 754/254$) were reviewed as of June 2013. Their front-line treatment schedules and findings have already been reported [20–23], and the duration of chemotherapy was 24 months for all patients except standard risk patients enrolled in L99-15/1502 with 36 months of treatment. The treatment strategy after relapse was decided by each physician.

In the present study, we defined "late relapse" and "very late relapse" as relapse at 6–24 months and later than 24 months after the end of the treatment, respectively (Fig. 1a). Patients who received HSCT at their first remission were excluded.

Statistical analysis

Fisher's exact test was used to compare differences in the distribution of clinical features among each group. The duration of event-free survival (EFS) was defined as the time from relapse to either treatment failure (second relapse, death, or the diagnosis of secondary cancer) or to the final day of observations that confirmed the patient was failure free. Overall survival (OS) was defined as the time from relapse to death from any cause or the time of the last follow-up. The probabilities of EFS and OS were estimated using the Kaplan–Meier analysis, and the significance of differences was evaluated using the log-rank test.

The cumulative incidences of relapse were estimated taking into account the competing events of death without second relapse and development of a secondary malignancy. To determine the cumulative incidence of non-relapse mortality (NRM), relapse and the development of secondary malignancies were considered as competing risk factors. Gray's test was used to assess the significance of the relapse period on the cumulative incidences. All statistical analyses were performed using the R software 2.13.0 (The R Foundation for Statistical Computing, Vienna, Austria). A 2-sided p value less than 0.05 was considered significant.

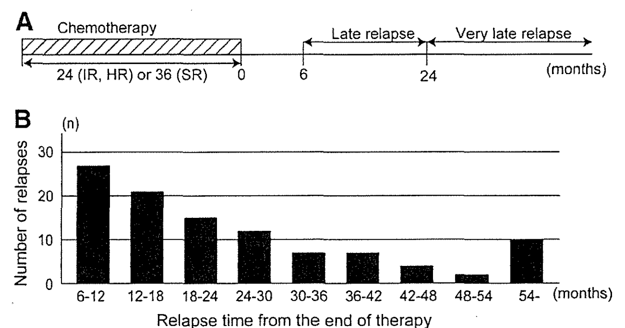

Fig. 1 Definition and distribution of late and very late relapse. **a** Definition of late and very late relapse. Late and very late relapse were defined as 6–24 months and later than 24 months after the end of chemotherapy, respectively. In TCCSG clinical trials, which were included in this analysis, the duration of the chemotherapy regimen was 24 (intermediate and high risk) or 36 (standard risk) months. **b** Distribution of the late and very late relapse periods

Results

Patients

Patient characteristics are summarized in Table 1. A total of 105 patients relapsed 6 months or later after the end of therapy, including 48 who were late relapse patients (6–24 months) and 57 who were very late relapse patients (later than 24 months). The distribution of the relapse period is shown in Fig. 1b. Excluding 2 patients (1 late relapse and 1 very late relapse) due to insufficient follow-up data, the median follow-up period of surviving patients after relapse for the 103 patients was 7.3 years. Although 3 patients (1 patient with late relapse, and 2 patients with very late relapse) died during remission induction chemotherapy, all other patients achieved second remission.

Outcomes after relapse

Second relapse occurred in 24 patients at a median of 556 (range 175–1,379) days after the first relapse, and 16 patients died before the second relapse, including 3 patients who died before they achieved second remission. OS and EFS at 7 years after the first relapse were 67.2 ± 4.9 and 60.2 ± 5.0 %, respectively, and the relapse and non-relapse mortality incidences were 24.4 ± 4.4 and 15.4 ± 3.7 %, respectively.

EFS of late relapse patients and very late relapse patients were 54.5 ± 7.3 and 64.8 ± 6.8 % at 7 years, respectively, and this difference was not significant ($p = 0.36$, Fig. 2a). However, the incidence of relapse was significantly higher in late relapse group (34.7 ± 7.1 %) than in very late relapse group (15.5 ± 5.1 %) ($p = 0.031$, Fig. 2b), while the non-relapse mortality rates of late relapse patients and very late relapse patients were 10.8 ± 4.6 and 19.8 ± 5.8 %, respectively ($p = 0.19$, Fig. 2c). Of 4 T-ALL with very late relapse, 2 were alive in CR, 1 died before relapse, and 1 patients suffered relapse.

As with salvage treatment for the first relapse, 29 (60.4 %) of 48 late relapse patients and 14 (24.6 %) of 57 very late relapse patients underwent allogeneic HSCT at the second complete remission (CR2). A median time from the first relapse to HSCT was 201 days for late relapse and 181 days for very late relapse. Second relapse occurred in 9 patients after the HSCT, whereas 8 patients died before second relapse. When the 43 patients who received HSCT in CR2 were censored at the transplantation time in order to focus on the outcomes of patients treated with chemotherapy, EFS of very late relapse patients (73.6 ± 7.0 %) was significantly higher than that late relapse patients (37.4 ± 11.8 %) ($p = 0.027$, Fig. 3a). The EFS superiority of very late group was reproduced when limited to the patients who were treated with chemotherapy only ($p = 0.009$, Fig. 3b).

Table 1 Characteristics of patients who relapsed at 6 months or later

Characteristics	Late relapse	Very late relapse	p
Total	48	57	
Gender (n)			0.42
Male	33	34	
Female	15	22	
Age at diagnosis (years)			0.38
Median	4	5	
Range	1–14	1–14	
WBC at diagnosis (/ul)			0.29
Median	9,300	7,800	
Range	300–318,000	1,000–282,000	
Lineage			0.37
Non-T	46	52	
T	1	4	
Cytogenetics (n)			0.73
Normal karyotype	25	29	
High hyperdiploid	10	9	
Others/not known	13	19	
Study (n)			0.68
L95-14	14	19	
L99-15/1502	34	38	
Relapse site			0.25
BM only	38	43	
EM only	4	6	
Combined BM and EM	6	4	
Data not available	0	4	

Late relapse and very late relapse were defined as relapse at 6–24 months from the end of therapy, and at 24 months or later from the end of therapy, respectively

WBC white blood cell count, *BM* bone marrow, *EM* extramedullary

Among the 57 very late relapse patients, 16 patients relapsed at later than 48 months after the end of the treatment, 14 had bone marrow-related relapse, and 2 had isolated extramedullary relapse. All of these extremely late relapse patients were alive without a second relapse at a median follow-up duration of 4.7 years after the first relapse, although only 1 patient received allogeneic HSCT during CR2.

Although most of the first relapse sites were related to the bone marrow, 10 relapses were isolated to extramedullary regions. All 10 isolated extramedullary relapse patients survived without events, although only 1 patient underwent allogeneic HSCT. The cumulative incidence of second relapse in bone marrow-related relapse was 28.5 ± 5.0 %. When limited to very late relapse patients, the cumulative incidence of relapse in bone marrow-related relapse patients ($n = 47$) was 19.0 ± 6.2 %. The second relapse site was the bone marrow in 20 patients, isolated

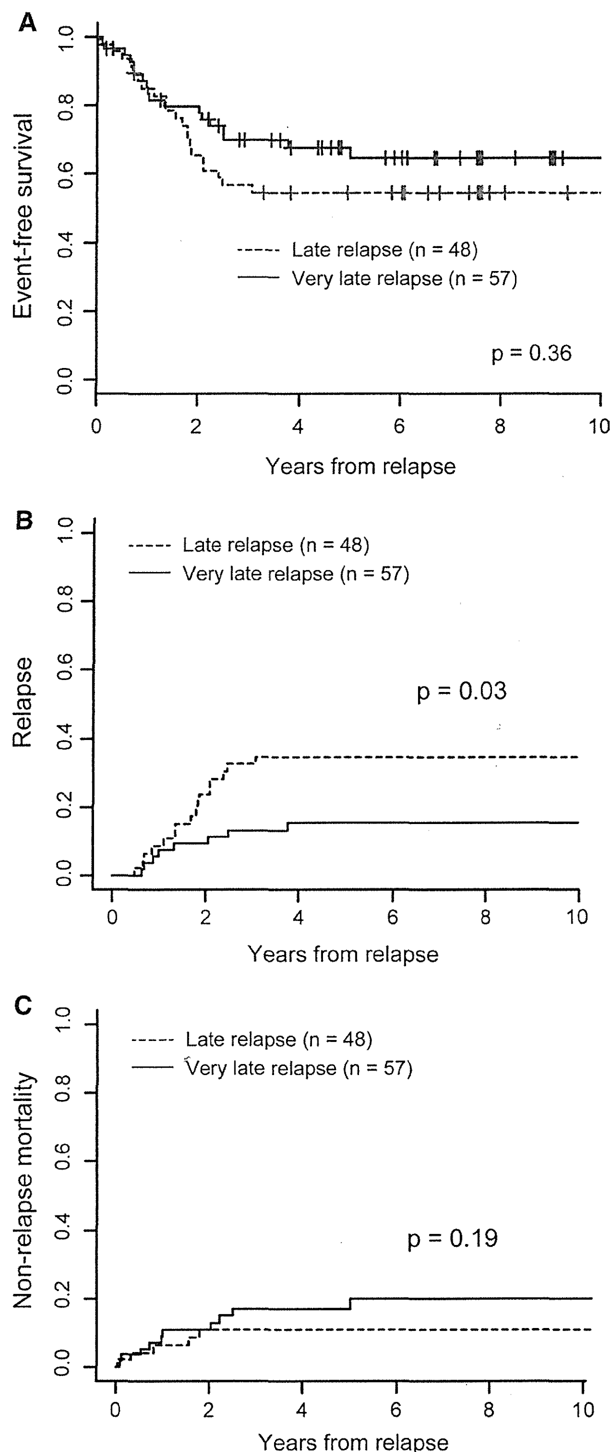

Fig. 2 Outcomes of late and very late relapsed ALL. **a** Event-free survival of relapsed patients. **b**, **c** Cumulative incidence of relapse and non-relapse mortalities

extramedullary region in 3 patients (2 bone and 1 mammary gland), and combined in 1 patient (bone marrow and the central nervous system).

Discussion

Advances in treatment strategies have reduced the relapse rate of pediatric ALL to approximately 10 %. The relapse period is a potent risk factor for second relapse, with previous studies reporting that the later relapse occurs, the better the outcome of salvage chemotherapy [11]. Accordingly, very late relapse is considered to be a very good risk factor for relapsed ALL; however, only limited evidence is available because of the small number of patients [17, 18]. In our study, we demonstrated that very late relapse was associated with a lower relapse incidence than that of late relapse.

The relapse site has also been linked to the outcomes of relapsed ALL, and isolated extramedullary relapse is considered to be a good prognostic factor. The BFM group classification for relapsed ALL categorized late and isolated extramedullary relapse as the lowest risk group, and our results confirmed that late and isolated extramedullary relapse could be salvaged without HSCT. Although relapse including bone marrow was considered as a higher risk of relapse, the incidence of bone marrow-related relapse in our cohort was not high when it occurred very late. Extremely late relapse (later than 48 months) in particular had excellent outcomes, and was assumed to be curable without allogeneic HSCT. However, of note, a study reported that relapse period usually depends on the prior treatment, and extremely late relapse might become much fewer by recent intensive chemotherapeutic strategy [24].

The effectiveness of risk-adapted strategies is well recognized not only in de novo ALL, but also relapsed ALL, and excess intensified treatments such as allogeneic HSCT should not be adapted to avoid unnecessary morbidity and non-relapse mortality. In our cohort, of 14 patients with very late relapse and receiving HSCT, 5 patients died before relapse, while only two patients relapsed, which suggested that allogeneic HSCT for very late relapsed ALL may lead to higher non-relapse mortality rates in spite of a lower relapse incidence. Recent studies showed that measuring minimal residual disease (MRD) was useful for stratifying relapsed ALL [9, 10]. Although data regarding MRD was unfortunately not available in our patients, MRD kinetics after salvage treatments may be used to predict a high-risk subgroup in very late relapsed ALL.

Interestingly, some studies suggested that a certain portion of late relapse were not reoccurrence of the first leukemic cells, but independent development from preleukemic clones which had existed at the first onset, especially ALL with *ETV6-RUNX1* [25–27], although data regarding this fusion was not available in our cohort. The findings are consistent with our results that very late relapse is associated with lower relapse risk as relapsed ALL, although we unfortunately could not compare molecular profile of leukemic cells at diagnosis and at relapse.

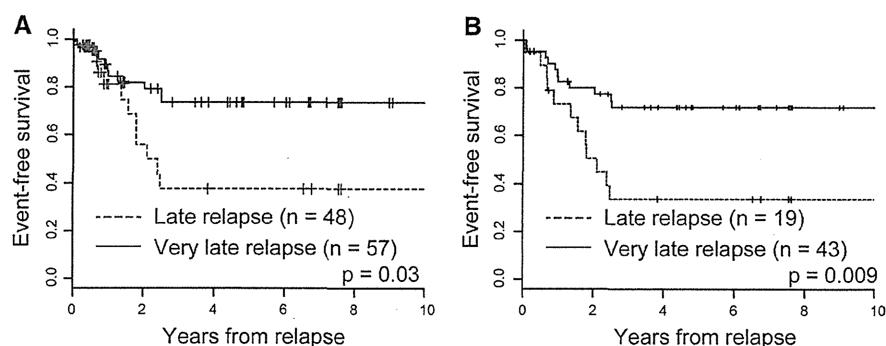

Fig. 3 Outcome of late and very late relapsed ALL with focus on chemotherapy. Event-free survival of late relapse and very late relapse patients: **a** with censoring of allogeneic HSCT during the second remission, and **b** when omitting patients who received allogeneic HSCT during the second remission

This study had some limitations which may have introduced bias, mainly due to the nature of a retrospective and uncontrolled study. For example, salvage strategies were not uniform, including the indication of allogeneic HSCT. Further prospective studies regarding very late relapse in a larger cohort are required even though it will require a long commitment.

In conclusion, our results demonstrated that the risk of second relapse in very late relapsed ALL was lower than that of late relapsed ALL, and suggested that these patients should be treated without allogeneic HSCT even when first relapse occurs in the bone marrow.

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Conflict of interest All authors had no conflict of interest to disclose.

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ORIGINAL ARTICLE

Favorable outcome in non-infant children with *MLL-AF4*-positive acute lymphoblastic leukemia: a report from the Tokyo Children's Cancer Study Group

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Abstract Unlike acute lymphoblastic leukemia (ALL) in infants, *MLL* gene rearrangement (MLL-r) is rare in ALL children (≥1 year old). The outcome and optimal treatment options for MLL-r ALL remain controversial. Among the 1827 children enrolled in the Tokyo Children's Cancer Study Group ALL studies L95–14, L99–15, L99–1502, L04–16, and L07–1602 (1995–2009), 25 MLL-r ALL patients (1.3 %) were identified. Their median age and leukocyte count at diagnosis was 2 years old (range 1–15 years) and 27,690/μL (range 1800–1,113,000/μL), respectively. All but one patient achieved complete remission (CR) after induction therapy, and 19 underwent allogeneic hematopoietic stem cell transplantation (HSCT) in first CR according to the protocol. The 5-year event-free survival (EFS) and overall survival (OS) rate were 60.0 % [standard error (SE), 9.7 %] and 64.0 % (SE 9.6 %), respectively. Notably, 9/12 cases with *MLL-AF4*-positive ALL are alive in continuous CR with a 75.0 % (SE 12.5 %) EFS rate. The causes of treatment failure were as follows: one induction failure, five relapses, and five transplant-related deaths. With intensive chemotherapy and allogeneic HSCT, favorable outcome of children (≥1 year old) with *MLL-AF4*-positive ALL was observed. However, considering the risk of acute and late toxicities associated with HSCT, its indication should be restricted.

Keywords Acute lymphoblastic leukemia · MLL · Children · Hematopoietic stem cell transplantation

Introduction

Translocations involving chromosome band 11q23 leading to rearrangement of the *MLL* gene (encoding mixed-lineage leukemia, also called *KMT2A*) is frequently found in acute lymphoblastic leukemia (ALL) in infants younger than 1 year old and therapy-related leukemia that develops in patients treated with topoisomerase II inhibitors for other malignancies [1]. In particular, *MLL* gene rearrangement

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