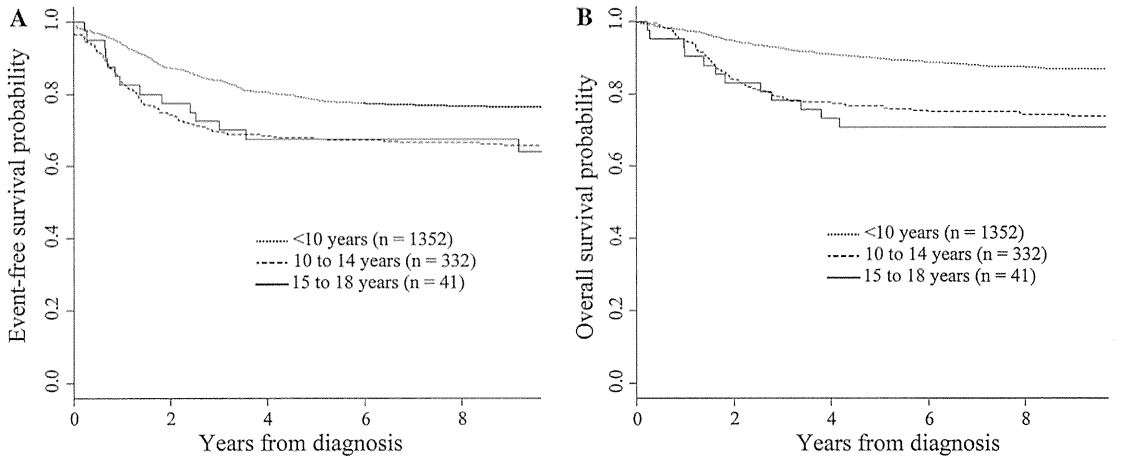

Fig. 1 Analysis of survival probability of patients aged 10 years or older. Estimated probability curves of **a** event-free survival and **b** overall survival are shown according to age groups

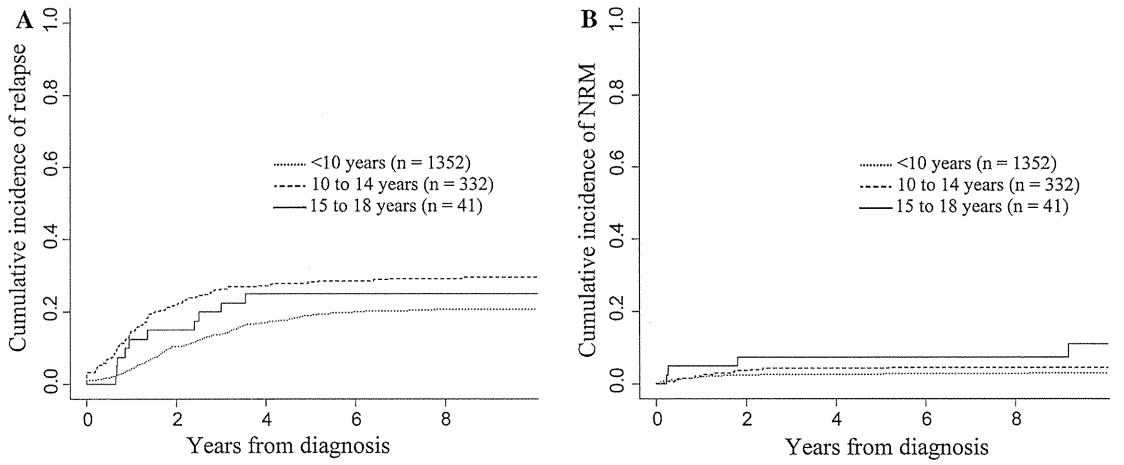

Fig. 2 Relapse and non-relapse mortality incidences of patients aged 10 years or older. Cumulative incidence curves of **a** relapse and **b** non-relapse mortality (NRM) are shown according to age groups

statistically associated with these two age groups, although CNS invasion at diagnosis was more frequently observed in the older age group. There was no isolated extramedullary relapse in older-adolescents, and only one patients with CNS-1 at initial evaluation suffered combined bone marrow and CNS relapse. Thus, the cumulative incidence of CNS-related relapse of older-adolescents was as low as 2.5 ± 2.5 % at 8 years after diagnosis. In the younger-adolescents, 7 (7.5 %) of 93 relapses were CNS-related, which resulted in the cumulative incidence of 2.1 ± 0.8 % at 8 years.

The difference in the cumulative incidence of NRM at 8 years was also not statistically significant (4.6 ± 1.2 % for the younger-adolescents and 7.5 ± 4.2 % for the older-adolescents, $p = 0.15$). Although 11 patients who were aged <10 years died during induction therapy, death during induction therapy was observed in only one of younger-adolescents, and no older-adolescent died during induction therapy. Severe toxicity which resulted in discontinuation of protocol therapy occurred in 4 (1.2 %) of younger-adolescents (including 2 pancreatitis) and 1 (2.4 %) of older-adolescent (due to pancreatitis), whereas 13 (1.2 %) of patients who were aged <10 years could not continue because of toxicity (1 allergy and no pancreatitis).

In the younger-adolescents, 48 patients received allogeneic HSCT during the first CR, and 14 relapses and 5 NRM were observed, which resulted in EFS of 62.4 ± 7.0 %, whereas 3 relapses and no NRM after HSCT were observed in 11 older-adolescents who undertook allogeneic HSCT during the first CR, and EFS at 8 years of these patients was 70.0 ± 14.5 %.

Secondary neoplasms occurred in 4 patients (2 acute myeloblastic leukemia, 1 myelodysplastic syndrome, and

1 tongue carcinoma) among younger-adolescents and 1 patient (breast cancer) among older-adolescents.

Multivariate analysis of EFS did not reveal a statistically significant difference between the younger-adolescents and the older-adolescents (Table 3). Early response to treatment was a significant prognostic factor (hazard ratio for event was 1.81, $p = 0.02$). There was a high probability (86.7 ± 8.8 %) at 8 years of EFS for patients with VGPR in patients aged 15–18 years ($n = 16$).

Table 3 Multivariate analysis of the risk factors for event-free survival in patients aged 10 years or older

Characteristics	Hazard ratio (95 % CI)	*p* value
Patient age		
10–14 years	1	
15–18 years	0.92 (0.53–1.61)	0.77
Treatment		
L95-14	1	
L99-15	0.95 (0.63–1.43)	0.80
L99-1502/L04-16	1.30 (0.79–2.14)	0.30
WBC at diagnosis		
<100000 cells/μl	1	
≥100000 cells/μl	1.25 (0.76–2.04)	0.38
Immunophenotype		
Non-T	1	
T	1.04 (0.96–1.65)	0.85
PB blast on day 8		
<1000 cells/μl	1	
≥1000 cells/μl	1.81 (1.12–2.93)	0.015

WBC white blood cell, *PB* peripheral blood, *CI* cumulative incidence

Discussion

Several studies showed that pediatric-type intensive chemotherapy improves the prognosis of adolescents with ALL, but the outcome of adolescent aged 15 years or older is still not satisfactory [8]. However, there is no consensus on an optimum treatment strategy for these patients because of insufficient data. Therefore, accumulating clinical features is important to resolve this issue. The retrospective analysis of three consecutive TCCSG trials presented in this study demonstrates that long-term outcomes for children aged 15–18 years were comparable with those aged 10–14 years.

Older children with ALL generally exhibit high-risk factors such as high leukocyte count and T-cell phenotype. In this study, we show that patients aged 10 years or older had a higher frequency of the *TCF3-PBX1* fusion in our cohort and that the frequency was not significantly different between younger-adolescents and older-adolescents.

Consistent with the outcomes of adolescent patients with ALL treated according to Dana-Farber Cancer Institute (DFCI) ALL Consortium Protocols [10], the EFS probability curves in TCCSG trials were superimposable for patients aged 10–14 and 15–18 years in our cohort and were reproducible for each treatment protocol. One of the distinctive features of DFCI treatment regimens is the use of intensive asparaginase (total doses of 525000–750000 U/m^2) and anthracycline (60–360 mg/m^2). Although asparaginase is one of the most important agents for the treatment of ALL, adolescent patients have increased rates of asparaginase-related toxicity such as pancreatitis and thrombosis [18, 19, 27]. In contrast, a significant feature of our treatment regimen was intensified

Table 4 Cumulative doses of chemotherapy during induction and consolidation therapy

	CALGB 8811	LALA 94	CCG 1882	FRALLE 93	DFCI 9101/0501	TCCSG L95-14/L99-15/1502
PSL (mg/m^2)	1260	840	1680	2540	1240–7240	2100–3480
DEX (mg/m^2)	140	320	210	140	0–900	84–740
VCR (mg/m^2)	22 (mg)	11.2 (mg)	19.5–37.5	10.5[b]	26	10.5–22.5
L-ASP (U/m^2)	84000	90000	90000–348000	132000	525000–750000	84000–244000
Anthracyclines[a] (mg/m^2)	202	396	86–158	249	60–360	155–243
CPA (mg/m^2)	4200	12500	3000–4000	0	0	2400–4600
Age	16–20 years ($n = 124$)	15–20 years ($n = 100$)	16–20 years ($n = 197$)	15–20 years ($n = 77$)	15–18 years ($n = 51$)	15–18 years ($n = 41$)
EFS	34 % at 7 years	41 % at 5 years	63 % at 7 years	67 % at 5 years	78 % at 5 years	67.5 % at 8 years
OS	46 % at 7 years	45 % at 5 years	68 % at 7 years	78 % at 5 years	81 % at 5 years	70.7 % at 8 years

Chemotherapy during maintenance is not included

[a] Anthracycline was calculated as adriamycin equivalent

[b] Eighteen and 12 mg/m^2 of vindesine (VDS) were scheduled in the FRALLE93 and the TCCSGL04-16, respectively

induction, including cyclophosphamide and block consolidation, with a relatively decreased total dose of asparaginase (84000–244000 U/m^2) and anthracycline (155–243 mg/m^2 of adriamycin equivalent) compared with the DFCI treatment regimen. Summary of total dose of chemotherapeutic agent and outcome of adolescent ALL is listed in Table 4 [10, 15, 16]. Our results demonstrate that TCCSG treatment regimens, which contained moderate doses of asparaginase and anthracycline, achieved comparable outcome for adolescents aged 15–18 years.

Of note, approximately 30 % of our older patients received HSCT during their first remission. Although a previous report suggests an advantage of allogeneic HSCT during the first remission in young adults [28, 29], pediatric-type chemotherapy can minimize the indication for allogeneic HSCT in adolescents [19, 30] to avoid acute and late complications. Our analysis showed that early response to treatment was a strong prognostic predictor in adolescents and the outcomes were excellent for patients with very good responses. Therefore, determining minimal residual disease kinetics [31] may be useful for developing a more refined stratification strategy, including limited indication for allogeneic HSCT.

According to the TCCSG registration data, only 41 (2.4 %) of 1,725 patients were aged 15–18 years. A population-based analysis from the Austrian study group included 6 % of 15 years or older ALL patients among 18 years or younger [11], and children's Oncology Group showed that their clinical trials included 7 % of 15 years or older among younger than 22-year-old ALL patients [8]. In most countries, the percentage of adolescents who enter clinical studies is lower compared with that of younger children [32–35]. Therefore, to clarify comprehensive clinical characteristics of adolescents with ALL, enrollment in clinical studies is essential. Collaboration between pediatric and adult study groups is required to obtain a comprehensive understanding of the characteristics of adolescents and young adults with ALL. More attention should be paid to young adults that are older than 18 years. A prospective collaborative trial is in progress in Japan [36].

Adverse events are generally more problematic in older patients, such as osteonecrosis, thrombotic events, and infection [10, 19]. It is very important to assess how these adverse events affect the quality of life by conducting prospective studies to avoid poor medical compliance of older children.

In conclusion, we suggest that the clinical characteristics and treatment outcomes of adolescents aged 15–18 years are similar to those of children aged 10–14 years. Therefore, our treatment backbone, intensive induction, and block-type consolidation can be adopted for adolescents, although further prospective studies and biological investigations are required for treatment optimization, including a minimized indication for administering allogeneic HSCT.

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

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An Overall Characterization of Pediatric Acute Lymphoblastic Leukemia with *CRLF2* Overexpression

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For an overall characterization of pediatric B-cell precursor acute lymphoblastic leukemia (BCPALL) with *CRLF2* overexpression (OE), we conducted genetic analysis of *CRLF2* in 167 pediatric BCPALL patients. *CRLF2* OE was detected in 30 (18%) of 167 patients, the *P2RY8-CRLF2* fusion was identified in only 3 (1.8%) of 167 patients, all of which demonstrated *CRLF2* OE. Moreover, *CRLF2* gain was identified in 18 (11%) of 167 patients. Messenger RNA sequencing revealed a novel fusion transcript, *CSF2RA-CRLF2*, in a case with *CRLF2* OE, suggesting that this fusion is associated with *CRLF2* OE. In survival analysis, no significant differences in 5-year event-free survival (EFS) and overall survival were observed between patients with and without *CRLF2* OE (70.7 vs. 75.4%, log rank $P = 0.68$ and 96.4 vs. 82.1%, log rank $P = 0.11$, respectively). However, a significant difference in 5-year EFS between *CRLF2* OE patients with and without *IKZF1* deletion was observed (44.4 vs. 83.1%, log rank $P = 0.02$). In multivariate analysis, only *IKZF1* deletion was a significant predictor of inferior OS (hazard ratio: 2.427, $P = 0.04$).These findings suggest that *CRLF2* OE is not an independent prognostic factor in pediatric BCPALL.

INTRODUCTION

Approximately 20% of pediatric acute lymphoblastic leukemia (ALL) patients still have a poor outcome, despite progress in intensified therapies and the development of detailed risk classifications (Pui et al., 2011). To improve the prognosis of high risk pediatric ALL, the identification of novel prognostic factors and therapeutic targets are required. As patients who lack recurrent cytogenetic abnormalities are known to be at higher risk of poor outcome, *IKZF1* deletion (Mullighan et al., 2009b; Kuiper et al., 2010; Waanders et al., 2011), *CRLF2* overexpression (OE), and *BCR-ABL1*-like gene expression profile (GEP; Den Boer et al., 2009; Harvey et al., 2010b; Roberts et al., 2012) have recently been intensively studied as potential prognostic factors.

Previous studies have described the prognostic significance of *CRLF2* OE and *CRLF2* rearrangements, including *P2RY8-CRLF2* and *IgH-CRLF2*

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(Mullighan et al., 2009a; Cario et al., 2010; Harvey et al., 2010a; Ensor et al., 2011; Chen et al., 2012; Palmi et al., 2012). However, it is controversial whether alterations of *CRLF2* can be considered independent indicators of poor prognosis. To address this issue, multivariate analysis of these adverse genetic alterations, including *CRLF2* OE, *IKZF1* deletion, and *BCR-ABL1*-like GEP, in uniformly treated patients will be required to evaluate their independent prognostic value (Harvey et al., 2010a; Ensor et al., 2011; Schwab et al., 2013; van der Veer et al., 2013; Yamashita et al., 2013). Here, to assess the independent prognostic value of *CRLF2* OE and genetic alterations involving *CRLF2*, we conducted genetic analysis of *CRLF2* in 167 pediatric BCPALL patients treated according to the Japan Association of Childhood Leukemia Study (JACLS) ALL02 protocol.

MATERIALS AND METHODS

Patient Cohort and Samples

BCPALL patients, from whom DNA and RNA extracted from diagnostic bone marrow or peripheral blood samples was available, were included in this study ($n = 167$). All patients were treated according to the JACLS ALL02 study protocol (Suzuki et al., 2010; Hasegawa et al., 2011). Diagnosis of BCPALL was based on morphological findings in bone marrow aspirates and immunophenotype analyses of leukemic cells by flow cytometry. Conventional cytogenetic analyses using G-banding and molecular studies were part of the routine work-up (Suzuki et al., 2010; Hasegawa et al., 2011). Bone marrows smears were examined by microscopy on days 15 and 33 (end of the induction phase) to evaluate treatment response. M1, M2, and M3 marrow were defined as fewer than 5, 5–25, and more than 25% blast cells in the bone marrow aspirate, respectively. Cases of *BCR-ABL1*-positive, infant, and Down syndrome-associated ALL were excluded from this study. Informed consent was obtained from the guardians of patients, according to the Declaration of Helsinki and treatment and genetic study protocols were approved by the institutional review boards of the participating institutes.

Detection of *CRLF2* OE, *P2RY8-CRLF2* Fusion, and F232C Mutation in *CRLF2*

Real time quantitative polymerase chain reaction (RQ-PCR) of *CRLF2* was conducted as previously described (Asai et al., 2013). *CRLF2*-OE was defined as an expression level 10-fold or greater than the median expression value in all patients (Harvey et al., 2010a; Yamashita et al., 2013). The *P2RY8-CRLF2* fusion was detected by reverse transcriptase (RT)-PCR or the SALSA multiplex ligation-dependent probe amplification (MLPA) kit P335-A4 (MRC Holland, Amsterdam, Netherlands), as previously described (Asai et al., 2013). The presence of the *CRLF2* F232C point mutation was detected by direct sequencing in cases with *CRLF2* OE, as previously described (Asai et al., 2013).

Determination of *IKZF1* Deletion, *CRLF2* Gain, and *JAK2* Mutation

IKZF1 deletions and copy numbers of *CRLF2* were determined by MLPA analysis, as previously described (Schwab et al., 2010; Asai et al., 2013). Screening of *JAK2* exons 16, 20, and 21 (gene accession number NM 004972) mutations was performed in patients harboring *IKZF1* deletions, as described previously (Asai et al., 2013).

Messenger RNA Sequencing (mRNA-seq)

For the determination of unrevealed genetic alterations causing *CRLF2* OE, mRNA-seq was performed in seven *CRLF2* OE patients harboring *IKZF1* deletions, where sufficient RNA samples were available, according to previously described methods (Masuzawa et al., 2014).

5′-Rapid Amplification of cDNA End (5′-RACE)

For determination of unknown 5′ partners of *CRLF2*, 5′-RACE was performed in nine of 10 *CRLF2* OE patients without *P2RY8-CRLF2* fusion and *CRLF2* gain, where mRNA-seq was not performed, according to the manufactured protocol (5′-Full Race Core Set, Takara Bio, Tokyo, Japan). The primer pairs used in this analysis are listed in Supporting Information Table 1.

Statistical Analyses

Estimation of survival distributions was performed by the Kaplan–Meier method and differences compared using a log rank test. A P value <0.05 (two-sided) was considered significant. Event-free survival (EFS) was defined as the time from diagnosis to any event (death from any cause, relapse, secondary malignancy, or failure to respond to therapy). Overall survival (OS) was defined as time from diagnosis to death of any

TABLE 1. Association of Genetic Features with *CRLF2* Overexpression

	CRLF2 overexpression				*P* value
	Yes%		No%		
Total	30		137		
Karyotype					<0.01
No fusion genes	26	86.7	80	58.4	
(Normal karyotype)	8	26.7	31	22.6	
(Hyperdiploid/triple trisomy)[a]	8	26.7	7	5.1	
(Others)[b]	6	20.0	29	21.2	
(Undetermined)	4	13.3	13	9.5	
Fusion genes	4	13.3	57	41.6	
(*ETV6-RUNX1*)	0	0.0	25	18.2	
(*TCF3(E2A)-PBX1*)	0	0.0	24	17.5	
(11q23/*MLL* fusion)	2[c]	6.7	8	5.8	
(*P2RY8-CRLF2*)	3[c]	10.0	0	0.0	
CRLF2 gain	11	36.7	7	5.1	<0.01
IKZF1 deletion	9	30.0	16	11.7	0.02

[a]Triple trisomy indicates trisomy 4, 10, and 17.
[b]Karyotype other than normal karyotype, hyperdiploid, triple trisomy, and 11q23 abnormality, showing negative results after screening for the chimeric fusions described in the text.
[c]One patient had both *MLL-AFF1* and *P2RY8-CRLF2* fusions.

cause or last follow-up. Patients with no events of interest were censored at the date of last contact. Hazard ratios for probability of relapse between subgroups were calculated using a univariate Cox model. Multivariate analysis was performed using a Cox regression model, adjusted for other risk factors, including age at diagnosis, initial WBC count, NCI risk, M3 bone marrow status at day 15, and *IKZF1* deletion. Other comparisons were performed using the χ^2, Fisher exact, and Mann–Whitney *U* tests, as appropriate.

RESULTS

Genetic Features of Patients with *CRLF2* OE

The chromosomal and genetic features of the 167 patients included in this study are provided in Table 1. *CRLF2* OE was identified in 30 (18%) of patients. Recurrent fusion genes, including *ETV6-RUNX1* and *TCF3-PBX1*, were absent in patients with *CRLF2* OE (0 vs. 36%, $P < 0.01$), consistent with previous reports (Russell et al., 2009; Yoda et al., 2010; Ensor et al., 2011; Palmi et al., 2012; Schwab et al., 2013). In contrast, two of the 10 cases with chromosome 11q23 abnormalities showed *CRLF2* OE. A *P2RY8-CRLF2* fusion was identified in three patients, one of whom also harbored a *MLL-AFF1* fusion (Table 1). Thus, 26 of 30 patients with *CRLF2* OE did not carry recurrent fusion genes. Karyotype was determined by G-banding analysis in 22 of the 26 *CRLF2*-OE patients without recurrent chromosomal abnormalities (Table 1). Eight of these were identified as having normal karyotypes, a similar percentage to that of patients without *CRLF2* OE (36 vs. 46%, $P = 0.42$). Interestingly, a high hyperdiploid (HHD) karyotype was identified in eight of the remaining 22 patients, which was significantly more than in the non-*CRLF2* OE patients (36 vs. 10%, $P = 0.005$). In terms of genetic alterations related to high risk BCPALL, MLPA analysis revealed *IKZF1* deletions in 25 (15.0%) of 167 patients. *IKZF1* deletion was more common in *CRLF2* OE than in non-OE patients (30 vs. 12%, $P = 0.02$, Table 1, Fig. 1). *JAK2* activating mutations were not identified in any of the 25 patients with *IKZF1* deletions.

Genetic Alterations Related to *CRLF2* OE

CRLF2 gain was identified in 18 (11%) of 167 patients by MLPA analysis and was significantly more common among *CRLF2* OE patients than non-OE patients (11/30 [37%] vs. 7/137 [5%], $P < 0.01$; Table 1). In addition, none of the patients with *CRLF2* gain had a deletion of *IKZF1*. Interestingly, seven of 11 *CRLF2* OE patients with *CRLF2* gain had a HHD karyotype, suggesting the presence of an extra copy of the sex chromosome. A *P2RY8-CRLF2* fusion gene was detected in three (1.8%) of 167 patients, all of whom were in the *CRLF2* OE group. One of the three *P2RY8-CRLF2*-positive patients also carried an *IKZF1* deletion and another harbored an *MLL-AFF1* fusion gene (Table 1). Thus, 13 (43%) of 30 patients with *CRLF2* OE had either *CRLF2* gain

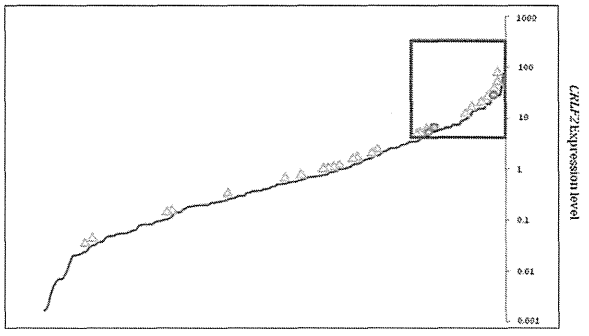

Figure 1. *CRLF2* expression levels measured by RQ-PCR. Red box, patients demonstrating *CRLF2* overexpression; yellow triangle, patients with *IKZF1* deletion.; green circle, patients with the *P2RY8-CRLF2* fusion.

or carried the *P2RY8-CRLF2* fusion. We also assayed for the *CRLF2* F232C point mutation in 14 of the 30 *CRLF2* OE patients with sufficient DNA sample available, no mutations were detected. To determine other genetic alterations leading to *CRLF2* OE, we performed mRNA-seq on seven of the 17 *CRLF2* OE-positive patients who did not have other genetic alterations of *CRLF2* and for whom sufficient RNA samples were available. Although we did not identify any known fusion transcripts, for example, *IgH-CRLF2*, we found that one patient had a novel fusion transcript, *CSF2RA-CRLF2*, which was associated with a 32 kb deletion, spanning from the region 5′ of *CRLF2* to its first intron, resulting in the absence of the *CRLF2* promoter region. This result was confirmed by RT-PCR and Sanger sequencing (Figs. 2A–2D, Supporting Information Table 1). Thus, we observed no genetic alteration of *CRLF2* in six of seven patients with *CRLF2* OE assayed by mRNA-seq. Of interest, mRNA-seq and RT-PCR identified three *EBF1-PDGFRB* positive cases among these six patients. Next, we performed 5′-RACE in nine of 10 patients without *CRLF2* gain, *P2RY8-CRLF2*, and *CSF2RA-CRLF2*. However, we could not find any *CRLF2* rearrangements in these patients (data not shown). We cannot exclude the possibility of *IgH-CRLF2* fusions in the remaining one patient with *CRLF2* OE, as there was insufficient material for 5′-RACE and FISH analysis. The summary of the genetic alterations related to *CRLF2* OE is presented (Fig. 3).

Clinical Features of Patients with *CRLF2* OE

The comparison of the characteristics of patients with or without *CRLF2* OE is provided in Supporting Information Table 2. Significant differences in age at onset, initial WBC count, and NCI risk, which are well-known prognostic factors for pediatric BCPALL, were not observed. There were also no significant differences in treatment responses, including initial prednisolone response, frequency of M3 at day 15 in induction phase, and remission–induction rate. In survival analysis, no significant differences in 5-year EFS or OS were observed between patients with and without *CRLF2* OE (70.7%, CI 49.7–84.2 vs. 75.4%, CI 67.2–81.9, log rank $P = 0.68$; 96.4%, CI 77.2–99.5 vs. 82.1%, CI 74.5–87.6, log rank $P = 0.11$; Table 2, Figs. 4A–4B).

Survival Analysis of BCPALL Patients According to *CRLF2* OE and *CRLF2* Genetic Alterations

To determine the prognostic significance of the genetic alterations observed in patients with *CRLF2* OE ($n = 30$), survival analyses were performed. Initially, we hypothesized that a particular type of *CRLF2* alteration was associated with poor prognosis. Thus, we focused on *CRLF2* gain and *P2RY8-CRLF2* fusion. However, neither of these features had prognostic impact on 5-year EFS or OS (Table 2). Next, we evaluated whether concomitant *IKZF1* deletion affected the prognostic significance of *CRLF2* OE. Interestingly, 5-year EFS and OS of the *CRLF2* OE patients with *IKZF1* deletions ($n = 9$) was inferior to that of those without *IKZF1* deletion ($n = 21$) (Figs. 5A–5B). None of the *CRLF2* OE patients with *CRLF2* gain also carried an *IKZF1* deletion. In univariate analyses of 167 patients, age at diagnosis and NCI risk were associated with inferior EFS and OS (Table 3). The M3 bone marrow status at day 15 was also a strong predictor for inferior EFS in univariate analysis. In terms of genetic alterations, univariate analysis revealed that neither *CRLF2* gain nor *P2RY8-CRLF2* fusion were associated

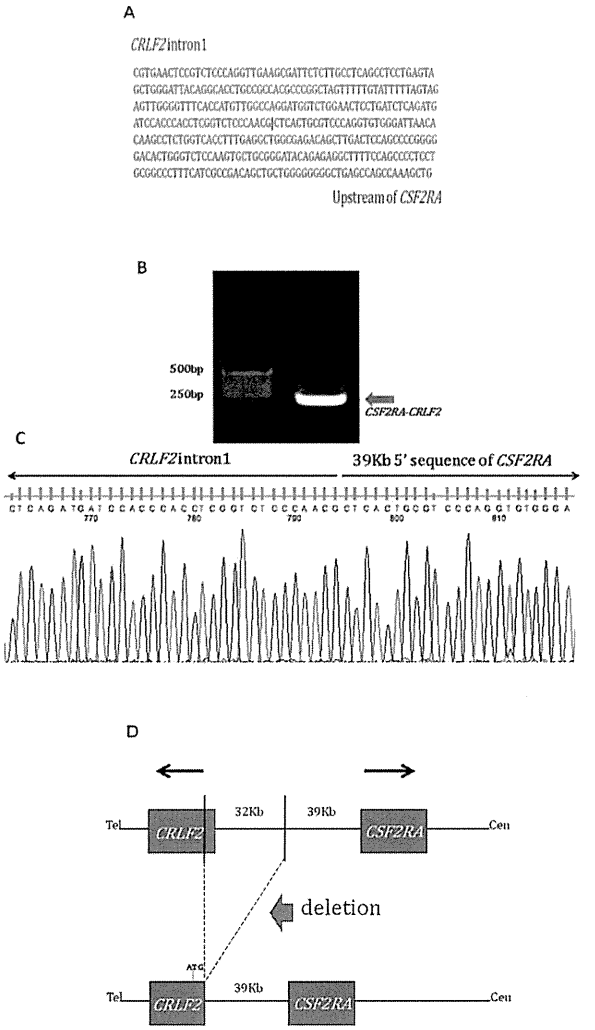

Figure 2. (A) Sequence of fusion junctions determined by mRNA-seq. (B) *CSF2RA-CRLF2* rearrangement detected by RT-PCR. (C) Sanger sequencing results of *CSF2RA-CRLF2* fusions. (D) Schematic representation of the *CSF2RA-CRLF2* genomic rearrangement.

with poor EFS or OS. By contrast, *IKZF1* deletion was strongly associated with poor EFS in univariate analysis (Table 3). In multivariate analysis of 167 patients, only *IKZF1* deletion was a significant predictor of inferior OS (hazard ratio 2.427, 95% CI 1.037–5.679, $P = 0.04$; Table 3). Collectively, the concomitant presence of both *IKZF1* deletion and *CRLF2* OE was associated with poor outcome.

DISCUSSION

In this study, we found *CRLF2* OE in 30 of 167 (18%) patients, only three of which (10%) carried the *P2RY8-CRLF2* fusion gene. We were unable to screen all samples for the *IgH-CRLF2* fusion due to lack of material for FISH analysis. However, mRNA-seq and 5′-RACE did not identify this fusion in 29 of 30 *CRLF2* OE patients. As part of this study, we identified a novel, rare chimeric fusion transcript, *CSF2RA-CRLF2* in the patient with the second highest *CRLF2* expression level, as measured by RQ-PCR (Fig. 2A). Sequence analysis indicated that the *CRLF2* promoter region was deleted, suggesting that an enhancer of *CSF2RA* may control the expression level of *CRLF2* in this patient (Figs. 2B–2D). However, the majority (16 of 20) of patients were found not to have *CRLF2* rearrangements by mRNA-seq and RT-PCR, which is a greater proportion than reported from the Children's Oncology Group (COG; 93/186) and the Berlin–Frankfurt–Műnster group (BFM) (2/36) studies (Cario et al., 2010;

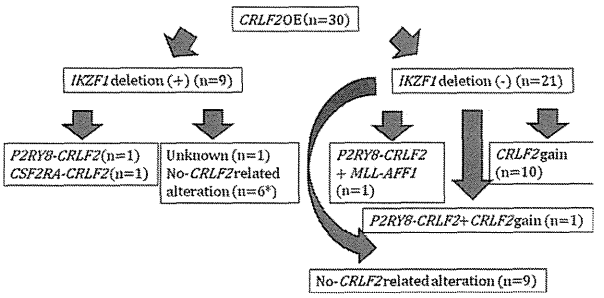

Figure 3. Summary of genetic alterations in 30 patients with *CRLF2* overexpression. OE, overexpression. * Including three patients with *EBF1-PDGFRβ* fusions. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE 2. Five-Year Event-Free Survival and Overall Survival of *CRLF2* OE Patients According to Genetic Alterations Related to *CRLF2* OE

		Survival, % (95% CI) at 5 years	
	N	Event-free	Overall
Total	30		
CRLF2			
Gain (+)	11	78.8 (38.1–94.3)	100
Gain (−)	19	66.7 (40.4–83.4)	94.4 (66.6–99.2)
P2RY8-CRLF2			
Positive	3	66.8 (54.1–94.5)	100
Negative	27	71.3 (48.8–85.2)	96 (74.8–99.4)
IKZF1			
Deletion (+)	9	44.4 (13.6–71.9)	88.9 (43.3–98.4)
Deletion (−)	21	83.1 (55.9–94.3)	100

CI, confidence interval

Chen et al., 2012). Chen et al. (2012) reported that *CRLF2* OE was found in 186 of 1,061 (17%) pediatric BCPALL patients by RQ-PCR analysis, which is similar to our findings. Conversely, the frequency of *CRLF2* OE in our study was higher than that reported from the BFM study group (49/555, 8.8%; Cario et al., 2010). Although the cutoff was set between positivity and negativity for *P2RY8-CRLF2* and *IgH-CRLF2* in these two studies, the frequency of *CRLF2* OE was different. These findings suggest that *CRLF2* expression levels might vary among patients with the same fusion, due to the number of fusion-positive clones. In fact, *CRLF2* expression levels tend to be low in patients harboring a *CRLF2* rearrangement in a minor clone (Cario et al., 2010), which may account for the range of *CRLF2* high expression reported. Previously, we found that *CRLF2* rearrangements occur with a low frequency in a Japanese pediatric BCP-ALL cohort (Asai et al., 2013). Here, we confirmed a low frequency of *P2RY8-CRLF2* (3/30, 10%) fusion, considerably below those in reported by the COG (65/186, 34.9%) and BFM (21/49, 42.9%) study groups. Interestingly, Yamashita et al. (2012) also described a similar frequency of *P2RY8-CRLF2* positive patients with *CRLF2* OE (2/15; 13.3%) in another Japanese pediatric BCPALL cohort. In addition, Harvey et al. (2010a) also described an association of Hispanic ethnicity with *CRLF2* rearrangement. These findings suggest that the frequency of *CRLF2* rearrangement may be associated with ethnic differences. Previous reports have also described that *IgH-CRLF2* was much less frequent than *P2RY8-CRLF2* (0.7 vs. 3.8%, 1 vs. 5%, and 2.9 vs. 6.1% in BFM, the United Kingdom Medical Research Council (MRC), and COG studies, respectively; Cario et al., 2010; Ensor et al., 2011; Chen et al., 2012). Thus, it is unlikely that we would have found more patients with *IgH-CRLF2* than those with *P2RY8-CRLF2* in this study.

Interestingly, *CRLF2* gain was the most frequent genetic alteration identified in relation to *CRLF2* OE in this study (11/30, 37%; Table 1). Cario et al. (2010) reported that nine of 45 (20%) *CRLF2* OE patients had an extra copy of *CRLF2*, but none of these was among the 25 patients with the highest expression levels *CRLF2*. Chen et al. (2012) also found that 102 of 168 (61%) *CRLF2* OE patients had an extra copy of the sex chromosome or *CRLF2* itself. They determined that this copy number gain occurred with almost equal frequency in the *CRLF2* OE group, irrespective of the presence of *CRLF2* rearrangement. Thus, it is not entirely clear whether the presence of an extra copy affects the expression levels of *CRLF2*. However, in this study, only one of 11 patients carried a *P2RY8-CRLF2* fusion gene (Fig. 3). In addition, all of them had a higher *CRLF2* expression than the *CRLF2* expression level of

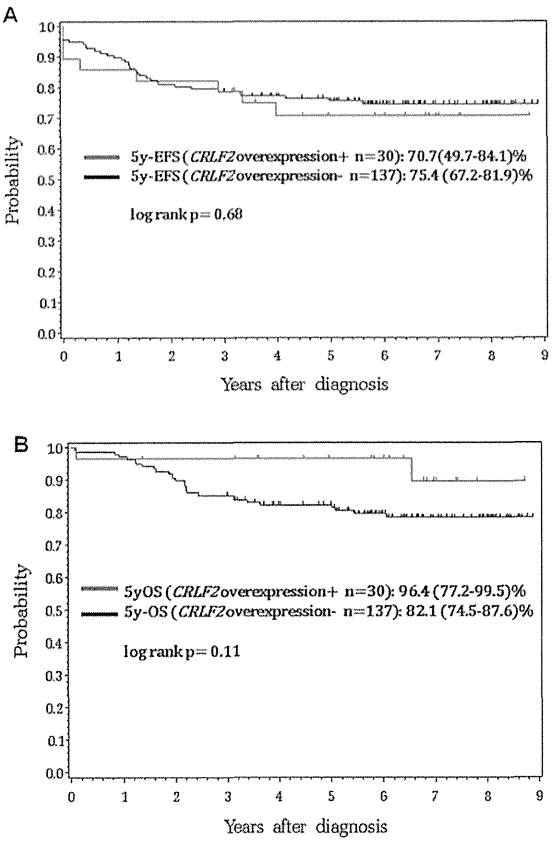

Figure 4. (A) Probability of EFS for patients with or without *CRLF2* overexpression. (B) Probability of OS for patients with or without *CRLF2* overexpression.

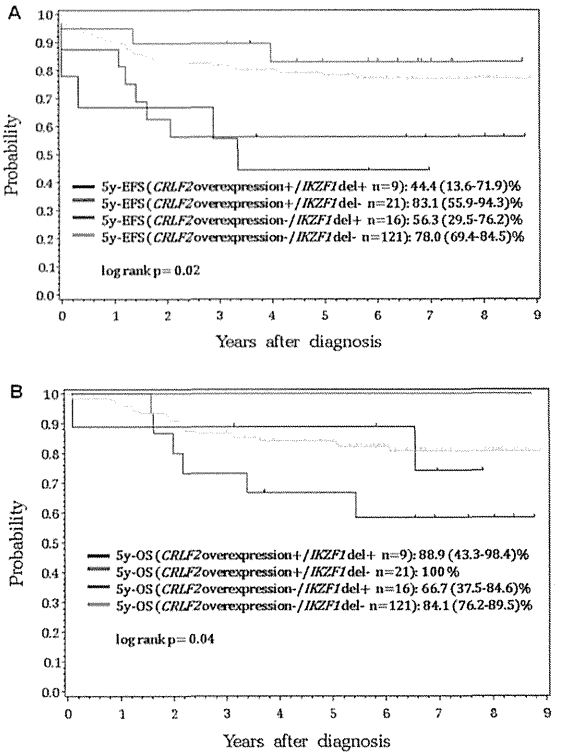

Figure 5. (A) Probability of EFS according to *CRLF2* expression level and *IKZF1* deletion. (B) Probability of OS according to *CRLF2* expression level and *IKZF1* deletion.

the *P2RY8-CRLF2* fusion-positive patient (data not shown). These findings suggest that *CRLF2* gain itself contributes to the high expression of *CRLF2* in this cohort. Collectively, *CRLF2* rearrangements (including *P2RY8-CRLF2*, *IgH-CRLF2*, and rare fusions) and *CRLF2* gain seem to be the major genetic alterations of *CRLF2* that induce *CRLF2* OE.

Previous studies demonstrating the prognostic significance of *CRLF2* OE have been controversial (Mullighan et al., 2009a; Cario et al., 2010; Harvey et al., 2010a; Ensor et al., 2011; Chen et al., 2012; Palmi et al., 2012). Recently, van der Veer et al. (2013) reported that a *BCR-ABL1*-like gene expression signature and *IKZF1* deletion were independent adverse prognostic factors for EFS in pediatric BCPALL (HR 3.1, 95% CI 1.8–5.2, $P < 0.001$ and HR 2.5, 95% CI 1.5–4.2, $P = 0.001$, respectively). They also found that *CRLF2* OE was not an adverse prognostic factor for EFS. In terms of the prognostic impact of *CRLF2* OE, we did not find a significant effect in this study, consistent with the results of the Dutch group (Fig. 4). Multivariate analysis also determined that *IKZF1* deletion, but not *CRLF2 OE*, was an adverse prognostic predictor for OS (HR 2.427, 95% CI 1.037–5.679, $P = 0.04$; Table 3), which was consistent with our previous result (Asai et al., 2013).

Interestingly, we found that three *CRLF2* OE-positive patients carried an *EBF1-PDGFRβ*fusion gene (Roberts et al., 2012) using mRNA-seq (data not shown). As *BCR-ABL1*-positive patients show *CRLF2* OE despite *CRLF2* rearrangement (Cario et al., 2010), some patients with *BCR-ABL1*-like signatures might also show *CRLF2* OE, resulting in poor prognosis. Conversely, we identified 11 patients with *CRLF2* gain, none of whom had *IKZF1* deletions, suggesting that these two genetic alterations are mutually exclusive. The good prognosis of patients with *CRLF2* gain in this study may be a consequence of their lack of *IKZF1* deletion. In conclusion, this study showed

TABLE 3. Univariate and Multivariate Cox Models of Event-free and Overall Survival for 167 Patients

	Univariate			Multivariate		
Variable	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value
Event-free survival						
CRLF2 overexpression (yes vs. no)	1.175	0.544–2.539	0.68	0.746	0.319–1.741	0.50
Age (yrs) at diagnosis (10–18 vs.1–9)	2.051	1.116–3.768	0.02	1.343	0.608–2.969	0.47
Sex (male vs. female)	1.458	0.782–2.718	0.24			
WBC count (×1,000 cells /μl) (≥ 100 vs. < 100)	2.117	1.126–3.982	0.02	1.589	0.736–3.430	0.24
NCI risk (HR vs. SR)	2.193	1.102–4.364	0.03	1.317	0.507–3.424	0.57
PPR vs. PGR	0.416	0.101–1.721	0.23			
Day 15 BMA (M3 vs. M1/M2)	2.695	1.353–5.369	0.005	2.015	0.948–4.284	0.07
IKZF1 deletion (yes vs. no)	2.681	1.371–5.242	0.004	2.006	0.942–4.272	0.07
CRLF2 gain (yes vs. no)	0.644	0.199–2.082	0.46			
P2RY8-CRLF2 (yes vs. no)	1.161	0.160–8.439	0.88			
Overall survival						
CRLF2 overexpression (yes vs. no)	0.331	0.079–1.388	0.13	0.181	0.039–0.827	0.03
Age (yrs) at diagnosis (10–18 vs.1–9)	2.101	1.025–4.306	0.04	1.360	0.537–3.441	0.52
Sex (male vs. female)	1.584	0.754–3.330	0.22			
WBC count (x1,000 cells /μl) (≥ 100 vs.< 100)	1.740	0.814–3.718	0.15	1.387	0.551–3.491	0.49
NCI risk (HR vs. SR)	2.433	1.044–5.672	0.04	1.652	0.525–5.196	0.39
PPR vs. PGR	0.619	0.147–2.598	0.51			
Day 15 BMA (M3 vs. M1/M2)	2.135	0.916–4.977	0.08	1.860	0.736–4.698	0.19
IKZF1 deletion (yes vs. no)	2.192	0.975–4.925	0.06	2.427	1.037–5.679	0.04
CRLF2 gain (yes vs. no)	0.274	0.037–2.010	0.20			

CI, confidence interval; WBC, white blood cell; NCI, National Cancer Institute; SR, standard risk; HR, high risk; PPR, prednisolone poor response; BMA, bone marrow aspiration; SCT, stem cell transplantation; CR, complete remission.

that *CRLF2* OE is not an independent prognostic factor in pediatric BCPALL.

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話　題

小児Ph-like急性リンパ芽球性白血病の臨床的および細胞遺伝学的特徴*

清　河　信　敬**

Key Words：B-cell precursor acute lymphoblastic leukemia, *BCR-ABL1*, tyrosine kinase, tyrosine kinase inhibitor, fusion genes

はじめに

Ph-like急性リンパ芽球性白血病(Ph-like ALL)あるいは*BCR-ABL1*-like(*BA*-like)ALLは，最近提唱されているB前駆細胞性(BCP-)ALLの予後不良亜群であり，チロシンキナーゼ抑制剤(tyrosine kinase inhibitor, TKI)が有効な症例が含まれるため，治療層別化の対象として注目されている．概念としては，“*BCR-ABL1*陰性であるにもかかわらず，*BCR-ABL1*陽性のPh1-陽性ALL(Ph1-ALL)に類似した遺伝子発現プロファイルを示すBCP-ALLの症例群”とまとめることができるが，その実態には不確定な部分が多い．本稿では，Ph-like/*BA*-like ALLの臨床的および細胞遺伝学的特徴に関する最新の知見を紹介し，今後の治療上の位置づけについて考察する．

Ph-like ALLと*BA*-like ALLの概念と臨床的特徴

BCP-ALLは，特定の染色体転座に基づくキメラ遺伝子の存在や，染色体数の多寡など，さまざまな細胞遺伝学的異常を有する亜群に分類され(図1)，それぞれの異常が，予後を含む臨床的特徴と密接に関連する[1]．なかでも，Ph1-ALLは，特に予後不良である．従来，BCP-ALL全体の約1/3の症例では，上記の既知の異常が検出されず，その細胞遺伝学背景が明らかではなかった．これらの症例はB-othersと呼ばれ，臨床的特徴においては多様な集団であることから，さまざまな未知の異常を有する症例の集合体であることが予想されるが，近年の遺伝子解析技術の著しい進歩に伴い，その細胞遺伝学的異常について詳細な解析が可能となり，全貌が明らかになりつつある．その中で，2つのグループの独立した研究により，B-othersの一部の症例は，*BCR-ABL1*陰性であるにもかかわらずPh1-ALL症例に類似した遺伝子発現プロファイルや臨床的特徴を示すことが報告された．

まず，エラスムス大学ロッテルダム(ソフィア小児病院)のDen Boerらは，遺伝子発現パターンによるALLの予後予測分類法開発を目的に，小児ALLの標準的な集団に対してマイクロアレイ解析を行い，各症例を遺伝子発現プロファイルに基づいてBCP-ALLのそれぞれの亜群(*TEL-AML1*-陽性，*E2A-PBX1*陽性，Ph1-陽性，*MLL*-再構成，hyperdiploid)とT-ALLの6つのグループに正確に予測・分類可能な，110(遺伝子)プローブセットによるアルゴリズムを確立した[2]．ところが，この方法によりB-otherを解析したところ，その68%(BCP-ALL全体の19.5%)がPh1-ALL症例と同じグループに分類され，予後も，再発率37%，5年無病生存率59.5%(対照症例ではそれぞれ16%，84.4%)と，Ph1-ALL症例と同等に不良であることが明らかになった．さらに，他

* Ph-like and *BCR-ABL1*-like acute lymphoblastic leukemia.

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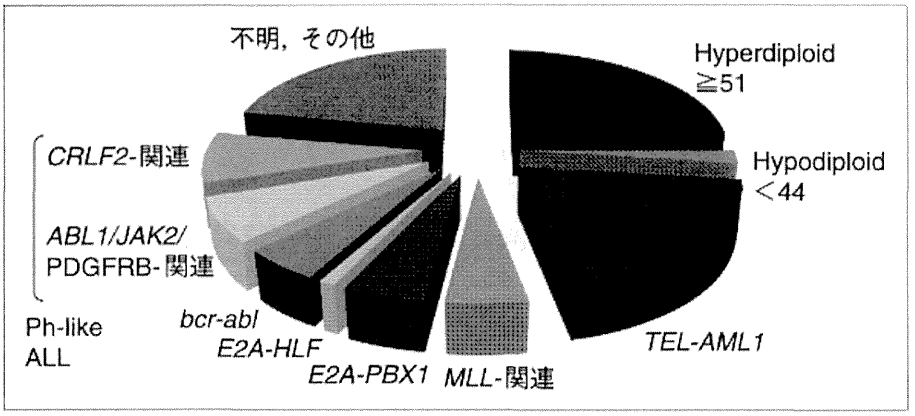

図1　小児B前駆細胞性急性リンパ芽球性白血病の細胞遺伝学的分類

の症例集団(validation cohort)の解析によって同様の傾向が再現された．彼女らはこの手法によって同定される症例群を*BA*-like ALLと呼んでいる．

一方，St. Jude小児病院のMullighanらは，既知の異常が認められなかった高危険群のBCP-ALLについて網羅的な細胞遺伝学的解析を行い，20遺伝子のゲノムコピー数異常に基づいて予後不良な症例を予測するスコア法を示したが，これによって同定された予後不良群は，B細胞分化関連転写因子IKAROSをコードする遺伝子*IZKF1*の変異と強く相関し，Ph1-ALLに類似した遺伝子発現プロファイルを示していた[3]．彼らはこの予後不良な症例群をPh-like ALLと呼び，Ph1-ALLとPh-like ALLを他のBCP-ALLから鑑別するための257遺伝子プローブセットによるアルゴリズムを確立している[4]．彼らの解析では，Ph-like ALLは小児BCP-ALLの約15%を占め，他の*BCR-ABL1*-陰性症例に比較して高い再発率を示し，5年無病生存率63%(対照症例86%)であると報告されている．

国内の症例についても，筆者らが東京小児がん研究グループ(TCCSG)の登録ALL症例約300例について，マイクロアレイ解析を行っており，欧米からの報告と同様に，BCP-ALLの1割程度にPh1-ALLに類似した遺伝子プロファイルを示す症例が認められ，その予後は著しく不良であることを確認している(未発表データ)．一方，米国の解析では，Ph-like ALLは小児よりもadolescents & young adults(15～39歳，AYA世代)での頻度が高く，BCP-ALLの約1/4を占めるとされ，小児科のみでなく内科領域でも重要性が報告されている．

以上のように解説してくると，*BA*-like ALLとPh-like ALLとは一見，同一の症例グループであるような印象を受けるが，実は両者は大きく異なる．確かに，双方とも"*BCR-ABL1*-陰性であるにもかかわらずPh1-ALLに類似した遺伝子プロファイルを示す予後不良なB-others症例群"という疾患概念では一致しているものの，"Ph1-ALLに類似した遺伝子プロファイル"の判定に用いるプローブセットやアルゴリズムはそれぞれ独自のものである．実際に同一の症例コホートのマイクロアレイデータを双方の方法によって比較解析した結果，共通して選択されたのは3割程度であったという．筆者らも，自検例のALLのマイクロアレイデータをDr. Den Boerのグループに解析を依頼し，独自の解析結果と比較したところ，同じプローブセットを用いた解析にもかかわらず，選択された"*BA*-like ALL"症例は一部しか一致していなかった．つまり，同じ概念であっても，判定する方法によって，*BA*-likeあるいはPh-like ALLとして診断されてくる症例は異なる集団であり，共通する症例は一部にすぎないと考えられる．その違いについては，事項で述べるそれぞれの細胞遺伝学的特徴を比較すると，さらに明確に理解できる．

Ph-like ALLの細胞遺伝学的特徴

Ph1-ALLでは，チロシンキナーゼ(TK)をコードする*ABL1*が*BCR*と融合することによって，ABL1のキナーゼ部分が恒常的に活性化した融合

タンパクBCR-ABL1が形成され，異所性に持続的な刺激伝達を起こすことが白血病の発症の重要な要因となっており，臨床特性にも関与している．Ph1-ALLとよく似た特徴を有するPh-like ALLでも類似した細胞遺伝学的異常の存在が容易に予測される．そこで，Robertsらは15例のPh-like ALL症例に対して次世代シーケンシングによるRNA解析と全ゲノム解析を行い，全例にTK関連の異常を明らかにした[4]．

このうち10例では，*ABL1*（*BCR*以外の融合パートナー），*JAK2*，*PDGFRB*，*EPOR*などのTKが関与した9種類の融合遺伝子が同定され，完全に新規のものやこれまでBCP-ALLでは検出されていなかったものが含まれていた（表1）．残りの症例のうち，3例では既知の*CRLF2*関連の融合遺伝子*IgH@CRLF2*が検出され，このうち2例は*JAK2*の，他の1例は*FLT3*の点変異異常を伴っていた．CRLF2は非TK型のサイトカイン受容体で，*CRLF2*関連の融合遺伝子では*CRLF2*の過剰発現が起り，またその下流に位置するTK JAK2の活性型変異を伴う場合が多く，双方が相乗的に作用してALL発症に関与すると考えられている．また，上記のような融合遺伝子が同定されなかった2例では*FLT3*あるいは*IL7R*の点変異が確認された．

一方，前述のように，もともとPh-like ALLは*IKZF1*の変異が特徴的な集団であるため，彼らが解析した15例中10例でその欠失が認められ，残りの症例のうち2例ではやはりB細胞分化に関与する*PAX5*の変異が検出された．以上の解析結果から，Ph-like ALLでは*IKZF1*などのB細胞分化に関連した転写因子の変異の存在に加えて，いずれかのTKの過剰な刺激伝達を誘導する変異の存在が，その細胞遺伝学的な特徴であると考えられる．彼らは，さらに多数例で検証を行い，Ph-like ALL症例の約1/2に*CRLF2*の異常が，8%に*EBF1-PDGFRB*がそれぞれ認められること，*ABL1*，*JAK2*，*PDGFRB*の異常がPh-like ALLに特徴的なものであること，その他の症例では*IL7R*や*FLT3*の活性型の変異や*JAK2*の抑制因子をコードする*SH2B3*の異常が認められることを示した．

さらに，Yangらは，genome-wide association study（GWAS）によってPh-like ALLに有意な相関を示す遺伝子領域として*GATA3*（rs3824662）を同定した[5]．この相関には人種差が認められ，特にヒスパニック系で高かった．GATA3の危険対立遺伝子変異はPh-like ALLに特徴的な*CRLF2*遺伝子再構成，*JAK*異常，*IKZF1*欠損および*GATA3*の発現の多様性と相関しており，ALLの早期治療反応性や再発の危険性とも関連していたという．

表1　Ph-like関連融合遺伝子

SNX2	-	*ABL1*	13)
ZMIZ1	-	*ABL1*	14)
FOXP1	-	*ABL1*	13)
NUP214	-	*ABL1*	4)
ETV6	-	*ABL1*	4)
RANBP2	-	*ABL1*	4)
RCSD1	-	*ABL1*	4)
PAX5	-	*JAK2*	4)
BCR	-	*JAK2*	4)
STRN3	-	*JAK2*	4)
EBF1	-	*PDGFRB*	4)
ATF7IP	-	*PDGFRB*	8)
IGH@	-	*EPOR*	4)
P2RY8	-	*CRLF2*	4)
IGH@	-	*CRLF2*	4)

BA-like ALLの細胞遺伝学的特徴とPh-like ALLとの違い

一方，*BA*-like ALL症例における*IKZF1*の欠失の頻度は40%と報告されており，*BA*-like ALLと*IKZF1*の欠失とは必ずしも一致しない[6]．また，*BA*-like ALL症例で*CRLF2*のmRNA過剰発現を認めるのは16%であったという．さらに，2014年4月末に開催されたInternational BFM study groupの第25回Annual Meetingにおいて*BA*-like ALLの25%はdic(9;20)，14%はiAMP21であり，*JAK2*関連の異常が6%，*EBF1-PDGFRB*が5%，*ABL1*関連の異常が3%と報告されていた．したがって，Ph-likeと*BA*-like ALLとは細胞遺伝学的特徴においても，一部のTK関連融合遺伝子を含む点では共通するものの，それぞれ異なる集団であることがわかる．両者の違いについては，Ph-like ALLでは解析の対象にヒスパニック系の患者が多いのに対し，*BA*-like ALLでは主にコーカソイド系の患者であることから，人種差の影響も考えられるが，もともと両者の判定法が細

胞遺伝学的に異なる特徴を有する集団を選択するためのアルゴリズムであるとも考えられる．

筆者らも，NGSによるRNA解析で国内の症例におけるTK関連融合遺伝子の探索を行っており，新規の*ATF7IP-PDGFRB*を含め，稀少で多様な融合遺伝子の存在を確認している[7][8]．

Ph-like ALLあるいは*BA*-like ALL症例に対するTKIの有効性

近年，Ph1-ALLでは新たな治療薬としてのTKIの有効性が報告され，化学療法への併用によって骨髄移植することなく治癒させられる可能性が報告されている[9]．また*PDGFRB*関連の融合遺伝子は骨髄系腫瘍に認められる場合があり[10]，imatinibがすでに治療に用いられている．したがって，機能的に類似した*ABL1*あるいは*PDGFRB*関連融合遺伝子を発現する一部のPh-like ALLでもTKIの有効性が期待される．実際に，*EBF1-PDGFRB*を発現するBCP-ALLの再発例に対してimatinibが有効であった事例が2件相次いで報告された[11][12]．ただし，*EBF1-PDGFRB*症例では，通常の化学療法でも初回寛解維持を継続している場合も確認されており，治療初期からTKI投与の適応とするかどうかは検討を要する．また，筆者らも，*ABL1*関連の融合遺伝子*SNX2-ABL1*陽性のBCP-ALL症例に対してimatinibが部分的に有効であったことを報告している[7]．しかし，Ph-like ALLに対するTKI投与の経験に関する報告はまだ限られている．

Ph-like ALLと*BA*-like ALLの臨床における意義

Ph-like ALL，*BA*-like ALLは遺伝子発現プロファイルに基づく概念で，診断にはマイクロアレイ解析が必須であり，現状で前向き研究に応用することは現実的ではない．仮に，治療初期に診断できても，予後不良の予測は可能だが，もともと高危険群に分類される症例が多いと考えられる．したがって，どのように診断して，どう取り扱うべきかについては今後十分な検討や評価が必要である．臨床的には，Ph-like ALLか*BA*-like ALLかはあまり問題ではなく，むしろ，それぞれの解析によって明らかにされた予後不良な亜群を形成する細胞遺伝学的異常の同定の方が重要である．特に，一部のTKを含む融合遺伝子を有する症例に関しては，TKIの有効性が期待されるため，治療初期の診断が可能であれば有用である．これらの症例に対するTKIの有用性について科学的根拠はいまだ乏しいため，ただちにTKI投与の適応にすべきかどうかはさらに議論が必要であるが，万一治療抵抗性であったり，早期に再発した際には，サルベージ治療の選択肢の1つとしてTKIを考慮することができる．

臨床研究の中でのPh-like ALL，*BA*-like ALLの診断や，治療上の取り扱いについては，各国・治療グループにより対応が異なるのが現状である．米国が最も進んでおり，米国内のすべての症例を対象として，新たに開発されたLow Density Array Cardを用いた診断と，既知の融合遺伝子に対するPCR，必要に応じてNGSによるRNA解析や全ゲノムシーケンスを駆使し，すべてのPh-like ALLを診断し，遺伝子異常を同定し，適応となる異常にはTKIを併用する試みが開始されつつある．一方，国内では，現時点で使用可能なTKIはimatinibとdasatinibだけなので，対象となるのは*ABL1*と*PDGFRB*関連の融合遺伝子を有する症例のみで，両者を早期に診断する方法の確立と，TKI併用の可否について，国内の統一グループである日本小児白血病リンパ腫研究グループ(JPLSG)内で検討が進められている．

おわりに

Ph-like ALLと*BA*-like ALLは，概念としては明確で，共通しているものの，それぞれ独自の診断法・基準に基づいた，異なる細胞遺伝学的特徴を持つ別々の疾患集団であり，今後，その定義や診断基準について国際的な統一，整理が必要である．具体的な診断方法や，臨床研究の中での取り扱いについても，今後の検討が必要である．しかし，Ph-like ALLや*BA*-like ALLの疾患亜群の存在が提起され，その細胞遺伝学的な背景が明らかにされてきたことによって，従来不明であったB-othersの中の一部の予後不良な集団の病態が明らかにされた．いずれも，さまざまな異常に起因する多様な症例の集合体であるが，今後，それぞれの細胞遺伝学的異常に対して，治

療上の対応が確立され，特にTKIの適応がある症例が適切に治療層別化されることにより，小児のみでなく，AYA世代も含めたBCP-ALL全体の治療成績がさらに改善されることが期待される．

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CASE REPORT

Identification of a homozygous *JAK3* V674A mutation caused by acquired uniparental disomy in a relapsed early T-cell precursor ALL patient

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Abstract Investigation of genetic alterations associated with relapse in acute lymphoblastic leukemia (ALL) may help to identify druggable targets for specific therapies. Early T-cell precursor ALL (ETP-ALL) is a subtype of T-ALL with poor prognosis. Although the genetic landscape of ETP-ALL has been determined, genetic alterations related to the relapse of ETP-ALL have not been fully investigated. Here, we report the first patient with relapsed pediatric ETP-ALL to exhibit a homozygous JAK3 activating mutation, V674A, caused by acquired uniparental disomy (UPD). Single nucleotide polymorphism array analysis revealed acquired UPD (aUPD) at the 19p13.3-p12 locus only in leukemic cells at relapse. Sanger sequence of the *JAK3* gene, which was located at 19p13.1 and frequently mutated in ETP-ALL, was performed in paired leukemic samples to determine homozygous JAK3 V674A mutation only in relapsed leukemic cells. In contrast, leukemic cells at initial diagnosis harbored hemizygous JAK3 V674A mutation. Further, whole-exome sequencing revealed mutations in 18 genes only in relapsed samples, although none of these was recurrent in T-ALL. These findings suggest that aUPD at 19p13.1 is partly associated with relapse in this patient. Pharmacological inhibition of JAK3 may be therapeutic in such cases.

Keywords ETP-ALL · JAK3 · Acquired UPD · SNP array · Whole-exome sequencing

Introduction

Early T-cell precursor acute lymphoblastic leukemia (ETP-ALL) is a subtype of pediatric T-cell acute lymphoblastic leukemia (T-ALL) with a poor prognosis [1]. Although a

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recent comprehensive genetic analysis revealed the landscape of genetic alterations in ETP-ALL [2], little is known about additional genetic alterations related to relapse. Here, the present study identified the first case of relapsed ETP-ALL with a homozygous *JAK3* V674A activating mutation [3] caused by uniparental disomy (UPD) of the 19p region.

Case report

The patient is 15-year-old male who developed ETP-ALL at the age of 13. Peripheral blood at the onset showed a white blood cell count of 230,800/μL (with 95 % lymphoblasts). By bone marrow (BM) aspiration, abnormal lymphoblasts accounted for 97.6 % of the nucleated cell count. The blasts were positive for cytoplasmic CD3 (cCD3), CD7, CD13, CD38, CD45, and CD58, and negative for CD1a, CD5, and CD8 by flow cytometric analysis (FCM), suggesting a diagnosis of ETP-ALL.

G-banding analysis of the BM cells revealed the karyotype to be 46,XY (2/4), 46,XY,add(1)(q32),add(9)(p24), -12, +mar (2/4). Fluorescence in situ hybridization (FISH) analysis revealed a hemizygous deletion of *ETV6*. The patient received induction chemotherapy consisting of prednisolone, dexamethasone, vincristine, daunorubicin, cyclophosphamide, and L-asparaginase, according to the Japan Association of Childhood Leukemia Study (JACLS) ALL02 protocol [4]. However, complete remission (CR) was not achieved and thus the patient was treated with salvage regimens, including arranon-G monotherapy (650 mg/m^2/day for 5 days) and combination chemotherapy consisting of high-dose cytarabine (AraC) (2 g/m^2/day for 5 days), fludarabine (Flu) (30 mg/m^2/day for 5 days), and idarubicin (Ida) (8 mg/m^2/day for 2 days). However, CR was not achieved. The patient then received allogenic hematopoietic stem cell transplantation (allo-HSCT) from his mother, a 6/8 HLA match. The pretransplantation-conditioning regimen consisted of cyclophosphamide (60 mg/kg/day on days −3 to −2) and total body irradiation (TBI) (4 Gy on days −8 to −6). Graft vs. host disease (GVHD) prophylaxis consisted of tacrolimus (FK), mycophenolate mofetil (MMF), methylprednisolone, and short-term methotrexate (MTX). Desired neutrophil counts (>500 per μL) were obtained by day 12, reticulocyte counts (>1.0 %) by day 18, and platelet counts (>5.0 × 10^4 per μL) by day 22. Genotyping using XY-FISH analysis of a BM sample taken at day 14 revealed that 97.2 % of total nucleated cells were of donor origin. There was no evidence of acute GVHD. Chronic GVHD developed 7 months after allo-HSCT, and was successfully treated with FK and prednisolone.

At 22 months after allo-HSCT, the patient experienced severe constipation. Positron emission tomography–computed tomography (PET–CT) scanning demonstrated that uptake of 18F-fluorodeoxyglucose (FDG) was increased in mediastinum lymph nodes, abdominal paraaortic lymph nodes, and mesenteric lymph nodes, which were enlarged. Peripheral blood tests showed a white blood cell count of 3,900/μL (with no lymphoblasts). BM aspiration showed no abnormal lymphoblasts. Histological examination of mesenteric lymph nodes revealed the proliferation of lymphoblasts, which were positive for cCD3, CD7, CD10, CD13, CD19, cCD79a, and CD117, and negative for CD2, CD4, CD5, CD8, CD20 and CD33, HLA-DR, myeloperoxidase (MPO), terminal deoxynucleotidyl transferase (TdT), and κ and λ immunoglobulin by FCM. FISH analysis revealed that the relapsed lymphoblasts harbored a hemizygous deletion of *ETV6*; thus we diagnosed extramedullary relapse of ETP-ALL and treated the patient with reinduction chemotherapy consisting of etoposide, AraC, and mitoxantrone. After one cycle of reinduction chemotherapy, the abnormal uptake previously seen in the mediastinum lymph nodes, abdominal paraaortic lymph nodes, and mesenteric lymph nodes by PET-CT was not observed, suggesting CR was achieved. The patient then received a second allo-HSCT from a 4/8 HLA-matched elder brother. The pretransplantation-conditioning regimen comprised Flu (30 mg/m^2 on days −5 to −2), melphalan (L-PAM) (70 mg/m^2 on days −4 to −3), antithymocyte globulin (ATG) (1.25 mg/kg on days −2 to −1), and TBI (2 Gy on day -6). GVHD prophylaxis consisted of FK, methylprednisolone, dexamethasone palmitate (0.1 mg/kg/day on days

Table 1 Genomic abnormalities at initial diagnosis and at relapse, determined by single nucleotide polymorphism analysis

Initial diagnosis (bone marrow)	Relapse (lymph node)
UPD 1q25.2–q25.3	UPD 1q25.2–q25.3
	del 2q14.1–q14.3
UPD 4q28	del 4q28
UPD 5p13.1–q12	UPD 5p13.1–p12
	del 5q31.1
del 9p22.1–p21.3	del 9p22.1–p21.3
UPD 10p15.3–p12.3	UPD 10p15.3–p12.3
UPD 10q23.1–q23.2	UPD 10q23.1–q23.2
UPD 11p11	UPD 11p11
del 12p13.3–p12.3	del 12p13.3–p12.3
	del 13q13.3–q14.1
	del 13q14.2–q21.1
	del 13q21.1–q21.3
del 18q22.1–q22.3	del 18q22.1–q22.3
	UPD 19p13.3–p12
UPD 20p11.2–p11.1	UPD 20p11.2–p11.1
UPD 20p11.1	UPD 20p11.1
UPD 20q11.1–q12.1	UPD 20q11.1–q12.1

UPD uniparental disomy, *del* deletion