Table I. Profiling of cell surface marker expression of ETP-ALL patients.

Score	CD1a	CD2	CD3	CD4	CD5	CD7	CD8	CD10	CD13	CD33	CD34	CD56	HLA-DR	Others
12	NT	3·4	12·7	1·3	1·9	68·0	1·2	1·0	67·7	61·9	61·9	0·7	52·1	$cyCD3^+$
10	0·4	2·5	1·6	0·5	26·2	99·0	2·2	1·4	92·4	3·4	0·8	0·3	4·4	
8	0·5	93·8	1·7	NT	4·1	97·6	NT	0·5	93·5	6·2	99·2	7·4	37·2	$CD11b^+$
7	0·8	11·7	2·7	18·1	71·5	96·7	1·9	66·2	17·5	0·3	16·8	NT	1·3	
7	0·2	78·8	0·3	0·6	1·1	95·7	2·3	72·0	81·9	12·6	2·4	NT	3·2	

NT, not tested.

of patients respectively) or not available at all (CD65 and CD117). Thus, we devised a scoring system based on the expression of six markers; CD5, CD8, CD13, CD33, CD34 and HLA-DR (Fig 1A) and applied it to the St Jude cohort, which included 17 ETP-ALL and 122 non-ETP T-ALL patients (Coustan-Smith *et al*, 2009). As shown in Fig 1B, the total score for all of the 122 typical T-ALL cases was a maximum of three, while it was four or more in 13 of the 17 ETP-ALL cases. Thus, the specificity was 100%, and the sensitivity 77%.

We next focused on the expression levels of the other antigens that were originally not included in the definition of ETP-ALL. In the St Jude cohort (Coustan-Smith *et al*, 2009), the expression levels of CD2 ($P < 0·01$, *t*-test), sCD3 ($P < 0·01$), CD4 ($P < 0·01$), and CD10 ($P = 0·035$) were significantly lower in ETP-ALL than in typical T-ALL, whereas the expression level of CD56 was significantly higher ($P = 0·018$) in ETP-ALL than in typical T-ALL. Thus, we established a second scoring system by the combination of these five additional markers (CD2, sCD3, CD4, CD10 and CD56) with the six used in the first analysis (CD5, CD8, CD13, CD33, CD34 and HLA-DR) (Fig 1C). When we applied this scoring system to the St Jude cohort, the total score in typical T-ALL patients was always six and lower, while it was seven or more in 16 of the 17 ETP-ALL patients (Fig 1D); specificity and sensitivity were 100% and 94%, respectively.

Application of scoring system to TCCSG L99-15 study

We applied the scoring system that included the 11 markers to the TCCSG L99-15 cohort (Fig 1E). In the TCCSG L99-15 study, median and mode of total score were -3 and -4 respectively, identical to those in the St Jude cohort. Among 91 T-ALL cases of the TCCSG L99-15 study, 5 (5·5%) had a score ≥7. The cell surface antigen expression profile of these five patients is summarized in Table I. Four patients showed typical ETP-ALL immunophenotype with negative CD1a expression; the remaining patient had no CD1a expression data but their cells had an immunophenotype with the highest total score of 12. The 86 patients (94·5%) whose total score were 6 and lower (Fig 1E) were considered as having non-ETP T-ALL. Among these 86 patients, however, there were 13 whose immunophenotype showed marginal patterns with a score of 3–6 (Table SI).

Clinical features and early treatment response of ETP-ALL

Table II summarizes the patient characteristics of ETP-ALL ($n = 5$) and T-ALL ($n = 86$). Distributions of gender, higher initial WBC count, age, National Cancer Institute (NCI) risk group, mediastinal mass, French-American-British

Table II. Demographic characteristics of the patients.

		T-ALL	ETP-ALL	χ^2-test
		$N = 86$	$N = 5$	P
Sex	Male	67	2	0·089
	Female	19	3	
WBC	≥100 × 10^9/l	42 (48·8%)	1 (20%)	0·36
Age	≥10 Years old	39 (45·3%)	3 (60%)	0·66
NCI risk group	Standard	14	1	1·0
	High	72	4	
Mediastinal mass	Yes	51 (59·3%)	3 (60%)	1·0
FAB classification	L1	59	2	0·32
	L2	25	3	
CNS involvement	Yes	3 (3·5%)	0 (0%)	1·0
Treatment subgroup	IR	22	1	
	HR	33	0	
	HR-SCT	31	4	
	HR-SCT%	36·0%	80%	0·070
Remission failure	Yes	4/85 (4·7%)	0/5 (0%)	1·0
Relapse	BM	15	4	
	CNS	2	0	
	Thymus	1	0	
	BM + thymus	2	0	
	Unknown	2	0	
SCT	Yes	42/85 (49·4%)	5/5 (100%)	0·057
Status at SCT	CR1	28	2	
	CR2	3	2	
	CR3	1	0	
	Failure	1	0	
	Rel1	3	1	
	Rel2	1	0	
	Unknown	5	0	

WBC, white blood cell count; NCI, National Cancer Institute; FAB, French-American-British; CNS, central nervous system; IR, intermediate risk; HR, high risk; SCT, allogeneic stem-cell transplantation; BM, bone marrow; CR1, first remission; CR2, second remission; CR3, third remission; Rel1, first relapse; Rel2, second relapse.

classification, and central nervous system involvement were not significantly different between the two groups. Clinical features at diagnosis of 13 borderline patients were similar to those of remaining 73 T-ALL patients (data not shown). Karyotypic analysis showed that two patients with ETP-ALL had +4 abnormality, which was not observed in the remaining patients (data not shown). Blast counts in peripheral blood (Fig 2A) and bone marrow (Fig 2B) at diagnosis were not significantly different between the two groups.

Although peripheral blast counts after 1 week monotherapy with prednisolone (Fig 2C) were similar between patients with ETP-ALL and T-ALL, bone marrow blast counts on day 14 remission induction therapy (Fig 2D) and blast counts in peripheral blood on day 15 (Fig 2E) were higher in patients with EPT-ALL ($P = 0{\cdot}057$ and $P = 0{\cdot}004$ by Mann–Whitney test respectively). Interestingly, among the 13 phenotypically borderline cases, blast counts in peripheral blood on day 8 and those in bone marrow on day 14 were significantly higher than those in remaining T-ALL patients (Fig S1).

Treatment outcome of ETP-ALL

Induction failures were observed in four of the patients with T-ALL but in none of those with ETP-ALL (Table II). Relapse occurred in 22 of the 82 (26·8%) patients with T-ALL who achieve remission and in four of the five patients with ETP-ALL. Due to HR-SCT classification and/or relapse, allo-SCT was performed in 41 patients (49·4%) with T-ALL and all five patients with ETP-ALL. With a median follow-up of 5·3 years, the estimated 4-year rate of event-free survival (Fig 3A) was 70·9% [95% confidence interval (CI), 61·1–80·7] for patients with T-ALL as compared to 40·0% (95% CI, 0–82·9) for those with ETP-ALL ($P = 0{\cdot}014$ by log-rank test). In a univariate analysis, ETP-ALL was a significant adverse risk factor for relapse ($P = 0{\cdot}048$). In a multivariate analysis including EPT-ALL, responses to prednisolone, NCI risk group, therapeutic subgroup, and gender as category terms, ETP-ALL was significant risk factor for relapse ($P = 0{\cdot}014$). Among the five patients with ETP-ALL (Table III), three patients who relapsed

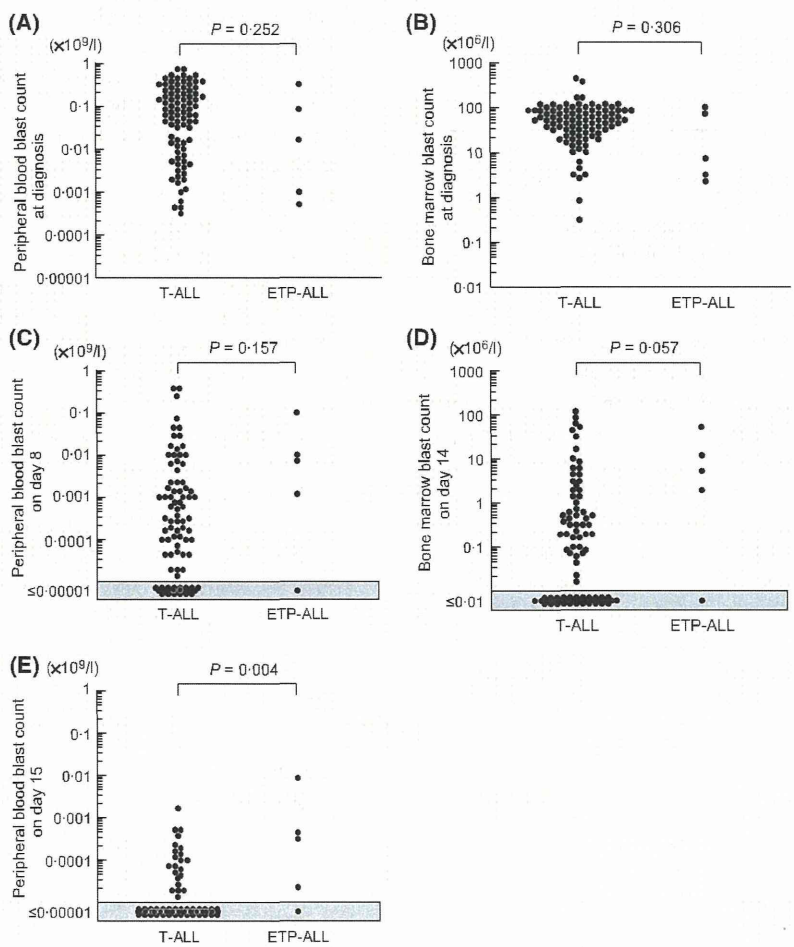

Fig 2. Comparison of blast counts in the patients having ETP-ALL with those in the patients having T-ALL. Blast counts in (A) peripheral blood at diagnosis, (B) bone marrow at diagnosis, (C) peripheral blood on day 8, (D) bone marrow on day 14, and (E) peripheral blood on day 15 were compared by Mann–Whitney analysis, and each *P* value is indicated at the top of figures.

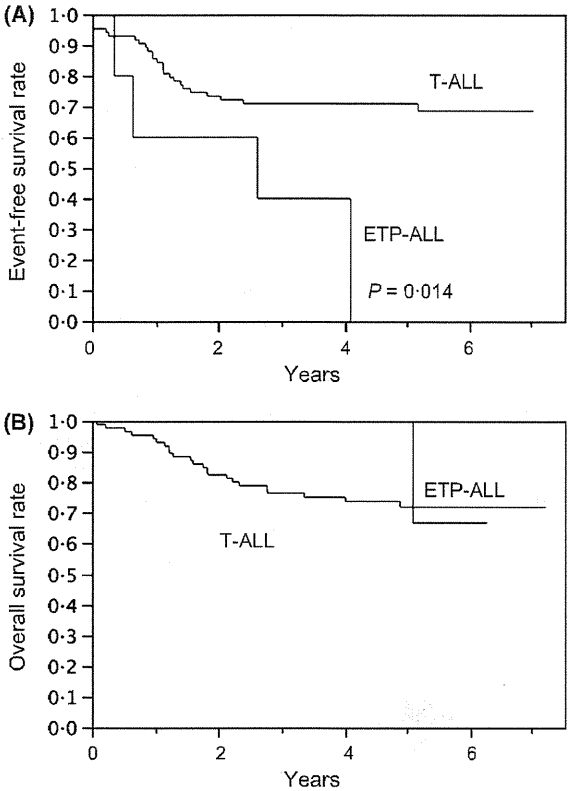

Fig 3. Kaplan–Meier plots of (A) event-free survival and (B) overall survival of the patients with ETP-ALL and T-ALL patients.

3, 7 and 48 months from diagnosis remained in second remission for 64, 67 and 8 months after allo-SCT. Thus, four of the five patients with ETP-ALL are alive in first (one patient) or second remission (three patients) (Fig 3B). Due to poor prednisolone response, 8 of the 13 borderline patients (62%) underwent allo-SCT in first remission at 6–9 months (median 7·5 months) after diagnosis, and all eight patients are alive in first remission (Table SII). As a result, the event-free survival of 13 borderline patients was similar (76·9%; 95% CI, 54·0–99·8) to that of the remaining T-ALL patients and significantly better than that of bona fide ETP-ALL patients ($P = 0·031$, log-rank test).

Discussion

As cure rates for children with ALL approach 90%, it has become ever more important to identify small subgroup of patients who are resistant to modern intensive chemotherapy. Among patients with T-ALL, reliable prognostic indicators have been lacking (Pui & Evans, 2006; Pui *et al*, 2008). Reportedly, however, patients with ETP-ALL have a particularly poor response to chemotherapy (Coustan-Smith *et al*, 2009) suggesting that alternative treatment approaches are needed for this leukaemia subtype. In the present study, we sought to determine the prevalence of ETP-ALL among patients with T-ALL enrolled in our TCCSG L99-15 study and assess their treatment outcome. Because of the limited panel of markers tested at diagnosis, we first devised a scoring system that allowed the identification of ETP-ALL among a previously reported cohort with 100% specificity and 94% sensitivity. The five patients with ETP-ALL identified among those enrolled in the TCCSG L99-15 study had a significantly poorer response to initial therapy as indicated by higher blast counts of peripheral blood at day 15, consistent with the previous findings of higher minimal residual disease levels observed among patients with ETP-ALL in both St Jude and AIEOP cohorts (Coustan-Smith *et al*, 2009). The event-free survival of ETP-ALL patients was significantly inferior in comparison with that of T-ALL patients. Four of the five patients with ETP-ALL enrolled in our study relapsed, confirming the dismal response to therapy of this T-ALL subtype. However, three of these four patients are alive in second remission after receiving allo-SCT, suggesting that allo-SCT should be considered as a frontline therapy for patients with ETP-ALL in first remission.

The prevalence of ETP-ALL in our study (5·5%) was lower than that determined in the St Jude's (12·2%) and AIEOP (13·0%) cohorts (Coustan-Smith *et al*, 2009). One possibility is that some cases with ETP-ALL may have been misclassified as typical T-ALL owing to the limited panel of markers used. Thus, we examined the borderline cases where patient immunophenotype showed marginal patterns, and found that

Table III. Clinical features of stem-cell transplantation in patients with ETP-ALL.

	SCT				Relapse		Final outcome		
Score	Status	Donor	Source	Time from diagnosis (months)	Site	Time from diagnosis (months)	Status	Survival	Time from diagnosis (months)
12	CR2	Unrelated	CB	6	BM	3	CR2	Alive	70
10	CR1	Sibling	BM	8	BM	31	Rel3	Dead	58
8	CR1	Unrelated	BM	8	No		CR1	Alive	31
7	Rel1	Sibling	PBSC	8	BM	7	CR2	Alive	75
7	CR2	Unrelated	BM	53	BM	48	CR2	Alive	61

SCT, allogeneic stem cell transplantation; CB, cord blood cell; BM, bone marrow; PBSC, peripheral blood stem cell; CR1, first remission; CR2, second remission; Rel1, first relapse; Rel3, third relapse.

almost two thirds of these borderline patients underwent allo-SCT early in first remission because of poor responses to prednisolone and early phase of induction therapy. Of note, all of these patients are alive in first remission, and the resultant event-free survival of the borderline patients was significantly better than that of bona fide ETP-ALL patients. These observations suggest that allo-SCT improves final outcome of the borderline subgroup even if some ETP-ALL patients are included. This seems to be consistent with the previous findings by the Berlin-Frankfürt-Münster group, which reported that allo-SCT was superior to chemotherapy alone in high-risk childhood T-ALL (Schrauder *et al*, 2006). Another possible explanation is that some cases may have been classified as acute myeloid leukaemia because of the expression of multiple myeloid markers and therefore were not enrolled in TCCSG L99-15. The possibility of differences in prevalence due to the different ancestry of the various cohorts should also be considered.

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Authorship

T.I., N.K. and D.C. analysed data and wrote the paper. E.C., A.K., M.K. and H.T. analysed data. K.K., A.M., M.K., K.I., Y.H., M.T. and K.S. designed the research study. A.O. designed the research study, analysed data, and wrote the paper.

Conflict of Interest

These authors declare no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig S1. Comparison of blast counts between borderline patients and remaining T-ALL patients.

Table SI. Profiling of cell surface marker expression of borderline patients.

Table SII. Clinical features of stem-cell transplantation in borderline patients.

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ORIGINAL ARTICLE

Flow cytometric analysis of de novo acute lymphoblastic leukemia in childhood: report from the Japanese Pediatric Leukemia/ Lymphoma Study Group

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Abstract Although the antigen expression patterns of childhood acute lymphoblastic leukemia (ALL) are well known, little attention has been given to standardizing the diagnostic and classification criteria. We retrospectively analyzed the flow cytometric data from a large study of antigen expression in 1,774 children with newly diagnosed ALL in JPLSG. T- and B-lineage ALL accounted for 13 and 87% of childhood ALL cases, respectively. Cytoplasmic CD3 and CD7 antigens were positive in all T-ALL cases. More than 80% of T-ALL cases expressed CD2, CD5 and TdT. In B-lineage ALL, the frequencies of early pre-B, pre-B, transitional pre-B and B-ALL were 81, 15.5, 0.6 and 2.9%, respectively. More than 90% of early pre-B ALL cases expressed CD19, CD79a, CD22, CD10 and TdT. CD34 was expressed in three-fourths of early pre-B ALL cases. The frequencies of TdT and CD34 expression were lower in pre-B ALL than in early pre-B ALL. B-ALL showed less frequent expression of CD22, CD10, CD34 and TdT than other B-lineage ALL cases. Expression of CD13 and CD33, aberrant myeloid antigens, was significantly more frequently associated with B-lineage ALL than with T-ALL. Based on this retrospective study of antigen expression in 1,774 de novo childhood ALL cases in JPLSG, we propose standardized clinical guidelines for the immunophenotypic criteria for diagnosis and classification of pediatric ALL.

Keywords Acute lymphoblastic leukemia · Childhood · Flow cytometry · Immunophenotype

1 Introduction

Flow cytometric immunophenotyping of childhood acute lymphoblastic leukemia (ALL) plays an important role not

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only in the diagnosis and classification of B and T cell lineages, but also in predicting the outcome [1–8].

Childhood ALL is a heterogeneous group of diseases. Therefore, leukemic cells from patients with ALL express a variety of differentiation antigens that are also found on normal lymphocyte precursors at discrete stages of maturation. With the development of monoclonal antibodies specific for relatively lineage-restricted or hematopoietic cell antigens, it has been possible to demonstrate considerable phenotypic heterogeneity in the vast majority of ALL cases by using panels of those antibodies [1, 2, 9–12].

The immunophenotypic patterns of acute leukemia, especially ALL, are well known, and classification into major immunologic categories is also accepted [1, 2, 9–12]. However, little attention has been given to standardizing the criteria for concluding which antigens are present on childhood leukemic cells, especially in Japan.

Herein, we report for the first time the results of a large, retrospective study of antigen expression in 1,774 children, older than 1 year and younger than 19 years of age, with newly diagnosed ALL, who had been enrolled between 1997 and 2007 at hospitals affiliated to the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG). Based on these results, we have formulated guidelines for use of immunologic markers and proper interpretation of the results. It should be noted that this study did not investigate possible associations of antigen expression with the clinical, hematological and biological features or their prognostic importance, because the present study included patients for whom a complete set of these information and the immunophenotypic characteristics based on flow cytometry were not available due to several limiting factors associated with the registration system.

2 Methods

2.1 Patient samples

This is a retrospective analysis of 1,774 pediatric patients with newly diagnosed and untreated ALL. It excluded acute undifferentiated leukemia and true mixed-lineage leukemia, defined as co-expression of golden markers of two different lineages, e.g., MPO^+ and $CD79a^+$, or MPO^+ and $CD3^+$ [10]. The analyzed patients had been enrolled between 1997 and 2007 at hospitals affiliated to the Japan Association of Childhood Leukemia Study (JACLS), the Tokyo Children's Cancer Study Group (TCCSG) and the Japanese Children's Cancer and Leukemia Study Group (JCCLSG). These three study groups, combined with the Kyushu Yamaguchi Children's Cancer Study Group (KY-CCSG), constitute the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG). All patients were diagnosed with ALL according to the French–American–British (FAB) morphology, enzyme cytochemical analysis and immunologic phenotype based on flow cytometric analysis. Samples obtained from bone marrow or peripheral blood of patients were immediately transported in sodium heparin tubes overnight to the central reference flow cytometry laboratories of the JPLSG. Informed consent for reference laboratory studies was obtained using forms approved by the local institutional review boards.

2.2 Flow cytometry

Ficoll–Hypaque-enriched blasts were stained by two-color immunofluorescence using various combinations of monoclonal antibodies, conjugated to phycoerythrin (PE) or fluorescein isothiocyanate (FITC), against the following antigens: CD1a, CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD13, CD14, CD15, CD19, CD20, CD22, CD33, CD34, CD38, CD41, CD42b, CD45, CD56, CD58, CD66c, CD117, glycophorin A, HLA-DR, immunoglobulin kappa (Igκ) and lambda (Igλ) light chains, T cell receptors ($\alpha\beta$ and $\gamma\delta$) on the surface of leukemic cells and cytoplasmic Igμ chain, CD3, CD22, CD79a and myeloperoxidase antigens, as well as nuclear TdT. For detection of cytoplasmic (cCD3, cCD22, CD79a and MPO) and nuclear TdT antigens, antibodies were added after permeabilization using an Intraprep Permeabilization reagent kit (Beckman Coulter Immunotech, Miami, FL, USA). Isotypical immunoglobulins were used as negative controls. Two-color flow cytometric immunophenotyping was performed on an FACScan (Becton–Dickinson, San Jose, CA, USA) or EPICS flow cytometer (Beckman Coulter, Fullerton, CA, USA) according to the manufacturer's directions. The analysis gate was set in the forward and side light-scattering positions with lymphoid morphology. Data were recorded by an observer blinded to the patient's clinical status and diagnostic features, except for the immunophenotype. An antigen was rated as "positive" if more than 20% of the gated cells showed specific labeling above that of controls, or if a positive subpopulation was distinctively identified even in less than 20% positive cases. In principle, the criteria recommended by the European Group for the Immunological Characterization of Leukemias and others [1, 9, 10] were used for immunophenotypic classification.

2.3 Statistical analysis

Statistical analysis was performed by taking into account gender, age and the presence or absence of myeloid antigens, i.e., CD13 and CD33. Differences in the distributions of variables between groups of patients were analyzed by Mann–Whitney's U test, Kruskal–Wallis test or the χ^2 test.

3 Results

3.1 Clinical features and FAB morphology

The clinical presenting features, which include gender and age, and the FAB morphology, are summarized in Table 1. The boys-to-girls ratio of the incidence and the median age in cases of T-lineage ALL were significantly higher than in cases of B-lineage ALL ($p < 0.001$). Among patients with B-lineage ALL, these clinical characteristics were statistically more frequent in cases of mature B-ALL than in other types of B-lineage ALL ($p < 0.05$). In FAB morphology,

Table 1 Characteristics and immunophenotypic profile of 1,774 de novo cases of acute lymphoblastic leukemia

	T-ALL	B-lineage ALL		
		Early pre-B	Pre-B[a]	Mature B
Number of cases	231	1250	248	45
Frequency (%)	13.0	70.5	14.0	2.5
Clinical features				
Gender (boy/girl) (%)	74/26	55/45	51/49	74/26
Median age (range)	8 (1–16)	4 (1–18)	5 (1–15)	10 (1–15)
FAB morphology				
L1/L2/L3 (%)	72/28/0	82/17.5/0.5	84/16/0	0/0/100
T-lineage markers				
CD1a	53.7	0.3	1.5	0.0
CD2	83.5	4.1	4.0	2.2
cCD3	100	0.0	0.0	0.0
sCD3	49.3	0.0	0.0	0.0
CD4	54.8	0.8	0.0	0.0
CD5	94.2	0.5	10.1	0.0
CD7	100	3.2	6.9	2.2
CD8	68.3	1.1	0.0	0.0
TCR$\alpha\beta$	29.4	6.3	8.5	0.0
TCR$\gamma\delta$	10.9	0.0	0.0	0.0
B-lineage markers				
CD19	0.0	99.6	98.8	100
CD20	0.0	19.2	23.6	88.9
cCD22	2.9	90.1	97.3	77.8
sCD22	1.8	70.3	87.6	60.5
CD79a	21.8	99.2	100	100
cIgμ	0.0	0.0	100	88.9
sIgμ	0.0	2.1	9.0	83.3
sIgκ or λ	0.0	0.0	0.0	100
Non-lineage specific markers				
TdT	84.4	97.0	83.8	13.0
CD10	31.6	91.2	93.5	77.8
CD34	37.3	74.6	44.5	7.0
HLA-DR	16.7	99.3	94.7	97.7
Myeloid markers				
MPO	0.0	0.0	0.0	0.0
CD13	20.7	36.0	22.7	14.3
CD14	0.0	0.6	0.0	0.0
CD33	15.2	31.6	15.0	2.2
CD41	0.0	0.8	3.3	0.0
CD66c	0.5	43.5	25.9	0.0
CD117	15.6	10.1	13.4	11.5
GlyA	0.0	0.0	0.0	0.0

Values indicate the proportion of positive cases (%)

c cytoplasmic, *s* surface

[a] Pre-B cases include transitional pre-B cases

the L3 subtype was detected in all cases of mature B-ALL and only in five cases of early pre-B ALL without $t(8;14)$ or its variants. The present study did not evaluate any further possible associations of immunophenotypic characteristics with other clinical, hematological or biological features or their prognostic importance because of several limiting factors associated with the registration system.

3.2 T-lineage ALL

T-lineage ALL accounted for 13% (231/1,774) of de novo childhood ALL (Table 1). Cytoplasmic CD3 and CD7 antigens were expressed in all T-ALL cases, which we were able to analyze. More than 80% of this subset expressed CD2, CD5 and the nuclear antigen, terminal deoxynucleotidyl transferase (TdT). Surface CD1a, CD3, CD4 and CD8 were detected in 49.3–68.3% of 231 cases of T-ALL. The HLA-DR antigen was not commonly expressed, and about 30% of the T-lineage ALL cases were $CD10^+$ and/or $CD34^+$. T cell receptor (TCR) proteins were heterogeneously expressed in T-lineage ALL. About 30% of the T-lineage cases expressing surface TCR chains expressed the $\alpha\beta$ form of TCR, whereas a minority, less than 15% of the T-lineage cases, expressed TCR$\gamma\delta$ proteins. Cytoplasmic CD79a and CD22, reliable markers for B-lineage ALL, were expressed in 21.8 and 2.9% of the T-lineage ALL cases, respectively. None of the T-ALL cases expressed CD19, CD20 or immunoglobulin molecules. Myeloid-associated antigen expression analysis found that CD13 and CD33 were expressed in 20.7 and 15.2% of the T-lineage ALL cases, respectively (Fig. 1). None of the T-ALL cases in this study expressed MPO or CD14. Early T cell precursor-ALL, a poor prognosis subgroup defined by its associated distinctive immunophenotype ($CD1a^-$, $CD8^-$, CD5 weak with stem-cell/myeloid markers) [13], was found in 3.7% of de novo T-ALL cases.

3.3 Early pre-B ALL

In this study, early pre-B ALL was found in 70.5% (1,250/1,774) of our de novo ALL cases (Table 1). Almost all of the early pre-B ALL cases were positive for CD19, cytoplasmic CD79a and cytoplasmic or surface CD22, but immunoglobulins were not detected. CD20, known to be a specific marker for early pre-B ALL, was detected in just 20% of the early pre-B ALL cases. More than 90% of the early pre-B ALL cases expressed CD10, TdT and HLA-DR, which are non-lineage specific antigens for B-lineage ALL. Moreover, CD34, a progenitor cell antigen, was expressed in 74.6% of the early pre-B ALL cases. CD66c, a member of the carcinoembryonic antigen family, was detected in nearly half of the early pre-B ALL cases. CD13 and CD33 antigens were expressed in 36.0 and 31.6% of the early pre-B ALL cases, respectively (Fig. 1). It is of note that neither cytoplasmic nor surface CD3 antigens were expressed in any B-lineage ALL (early pre-B, pre-B and B cell ALL) case in this series.

3.4 Pre-B ALL

According to the general consensus [1, 10, 14, 15], pre-B ALL blasts express cytoplasmic immunoglobulin μ heavy chains, but have no detectable surface immunoglobulins in B-lineage ALL. On the other hand, lymphoblasts of transitional pre-B ALL have both cytoplasmic and surface immunoglobulin μ heavy chains, without κ or λ light chains [1, 10, 15]. Since transitional pre-B ALL cases represented only 0.5% (9/1,774) of our de novo ALL cases, we analyzed these cases together with the pre-B ALL cases. This immunophenotype accounted for 14.0% (248/1,774) of our cases of newly diagnosed childhood ALL (Table 1) and expressed CD19, cCD22 and CD79a. Surface CD20 was detected in about a quarter of these pre-B

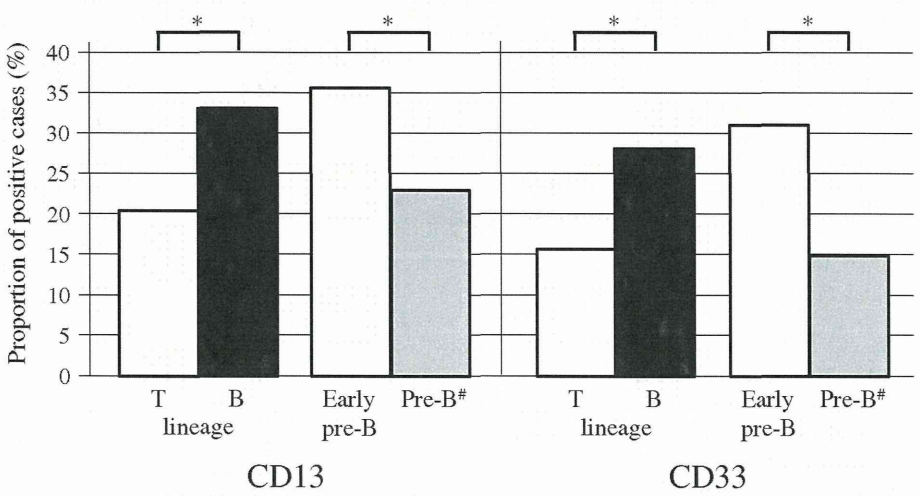

Fig. 1 Distribution of myeloid antigen (CD13 and CD33) expression. Acute lymphoblastic leukemia immunophenotypes: T-lineage ALL, B-lineage ALL, early pre-B ALL, pre-B ALL and B-ALL. *Values* indicate proportion of positive cases (%). #Pre-B cases include transitional pre-B cases. Expression was observed in all cases. *$p < 0.001$

ALL cases, and more than 90% expressed CD10 and HLA-DR. However, the frequencies of TdT and CD34 expression were 83.8 and 44.5%, respectively, which are lower than for early pre-B ALL cells. The expression frequencies of CD13 and CD33 were also lower than in the early pre-B ALL cases, at 22.7 and 15.0% ($p < 0.001$) (Fig. 1).

3.5 B cell ALL

B-ALL cells are characterized by L3 morphology, as defined in the FAB classification, and by surface membrane expression of immunoglobulin μ heavy chains (sIg) plus monotypic light chain [1, 9, 10]. In our present study, B-ALL cases accounted for 2.5% (45/1,774) of our de novo ALL cases (Table 1). The blasts of the B-ALL cases also expressed CD19, cCD79a, CD20 and HLA-DR. Both CD22 and CD10 were less frequently expressed in these cases than in other B-lineage ALL cases, including early pre-B and pre-B ALL. Although B-ALL cells are generally negative for expression of TdT and CD34, a few B-ALL cases with blasts that expressed TdT and/or CD34 have been reported [10, 16–19]. Moreover, Gluck et al. [20] diagnosed a B-ALL case that was L3 in the FAB classification with typical Burkitt's type translocation, but lacking sIg. In fact, we also identified a few cases with expression of TdT and/or CD34 and one case without sIg expression (positive for monotypic light chain) in this series. CD13 and CD33 antigens were expressed in some cases: 14.3 and 2.2%, respectively (Fig. 1).

4 Discussion

Immunophenotypic analysis of acute leukemia by flow cytometry has been used clinically as an indispensable tool for identification of the lineage association of leukemic cells and evaluation of the response to treatment [1, 2, 10–12, 21]. Recently, panels of monoclonal antibodies specific for lineage-associated antigens have been expanded. As a result, immunophenotyping of ALL has been applied to distinguish it from acute myeloid leukemia (AML) and to achieve more accurate phenotyping within ALL.

We retrospectively analyzed the flow cytometric data from a large study of antigen expression in 1,774 children with newly diagnosed ALL who were enrolled at hospitals affiliated to the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG) between 1997 and 2007. Each central reference flow cytometry laboratory of the JPLSG made immunophenotypic diagnoses based on the criteria recommended by the European Group for the Immunological Characterization of Leukemias and others for childhood acute leukemia [1, 9, 10]. Although these criteria are actually similar to each other and standardized, they advocate some different subclasses in T- or B-lineage ALL. Additionally, ALL with myeloid antigen expression might be observed frequently in cases with mixed-lineage leukemia. However, the criteria for myeloid marker-positive childhood ALL and the clinical significance of these antigens also vary. We then formulated guidelines for the use of immunomarkers and proper interpretation of the results in childhood ALL, as summarized in Table 2.

T-lineage ALL, according to our analytical findings, is characterized by cytoplasmic or surface membrane expression of CD3 together with CD2, CD5, CD7 or CD8 (Table 2). Some of our T-ALL cells expressed CD79a or CD22 as a marker for B-lineage ALL. Although such T-ALL cases have been reported by other investigators [22, 23], none of our T-ALL cases satisfied the diagnostic criteria for B-lineage ALL described below. Recently, Campana et al. [13] reported diagnosis of early T cell precursor (ETP)-ALL, as a subgroup with a poor prognosis,

Table 2 Proposed immunophenotypic criteria for de novo cases of acute lymphoblastic leukemia

T-lineage ALL
1. $CD3^+$
2. Express CD2, CD5, CD7 or CD8
B-lineage ALL
Early pre-B ALL
Express at least two B-lineage markers (CD19, CD20, CD22 or CD79a)
Pre-B ALL[a]
1. Express at least two B-lineage markers (CD19, CD20, CD22 or CD79a)
2. Negative for surface membrane immunoglobulin κ or λ light chains
3. Express cytoplasmic and/or surface immunoglobulin μ heavy chains
B-ALL
1. Express at least two B-lineage markers (CD19, CD20, CD22 or CD79a)
2. Express surface membrane immunoglobulin κ or λ light chains
ALL with aberrant myeloid-associated antigen expression
My Ag^+ T-lineage ALL
1. $CD3^+$ and express CD2, CD5, CD7 or CD8
2. $CD79a^-$
3. MPO^- and express myeloid-associated markers (CD13, CD15, CD33 or CD65)
My Ag^+ B-lineage ALL
1. Express at least two B-lineage markers (CD19, CD20, CD22 or CD79a)
2. $CD3^-$
3. MPO^- and express myeloid-associated markers (CD13, CD15, CD33 or CD65)

[a] Pre-B ALL cases include transitional pre-B cases

characterized by absence of CD1a and CD8 expression and weak CD5 expression. At least 25% of ETP-ALL cells also express one or more of the following myeloid or stem-cell markers: CD117, CD34, HLA-DR, CD13, CD33, CD11b and CD65. Interestingly, they also pointed out that for patients with T-ALL, a diagnosis of ETP-ALL should be a stronger predictor of the outcome than is flow cytometric-based minimal residual disease [13]. We also found some ETP-ALL cases in our present study. The exact number of these immunophenotypic cases could not be indicated because not all of the myeloid or stem-cell markers reviewed above were used to diagnose our de novo ALL cases. However, six of 164 cases diagnosed using all these markers met the criteria for ETP-ALL. This frequency, 3.7%, was much less than the 12.6% reported by Campana et al. [13]. The difference in its frequency and correlation with the outcome should be ascertained in a future study.

Next, we classified B-lineage ALL into three categories, i.e., early pre-B ALL, pre-B ALL and mature B-ALL, according to the degree of B lymphoid differentiation of leukemic cells. Most cases of early pre-B ALL were positive for the common ALL antigen (CD10), CD34, HLA-DR and TdT. However, these antigens are not lineage specific. Although the immunoglobulin heavy chains are usually rearranged in these leukemic blasts, immunoglobulins were not detected. Early pre-B ALL can be conclusively defined as expression of at least two of the following four early B cell markers: CD19, CD20, CD22 and CD79a (Table 2). Pre-B ALL can be generally distinguished from transitional pre-B ALL based on their respective immunophenotypic characteristics [1, 10, 15]. However, in this study, we combined these two phenotypes as pre-B ALL, because discrimination of them might not be so important in the clinic [15, 21]. Pre-B ALL, including transitional pre-B ALL, can be defined as expression of cytoplasmic immunoglobulin μ heavy chains without κ or λ light chains and the presence of at least two of the following markers: CD19, CD20, CD22 and CD79a (Table 2). Additionally, B-ALL can be defined as expression of surface membrane immunoglobulin κ or λ light chains and at least two of the following markers: CD19, CD20, CD22 and CD79a (Table 2). Since, in rare instances, surface immunoglobulin μ heavy chains are absent in B-ALL cases, these markers are excluded from the definition of this immunophenotype [20].

Aberrant expression of one or more immunologic markers of another lineage might be observed in cases with mixed-lineage leukemia, which include myeloid antigen-positive ALL (B-lineage or T-lineage), lymphoid antigen-positive AML and true mixed-lineage leukemia [10]. Although our study included myeloid antigen-positive ALL, we did not find either biclonal or oligoclonal leukemias, which consist of two or more morphologically or immunophenotypically distinct leukemic cell populations. Expression of aberrant myeloid antigens (MyAgs) reportedly occurs in 5–22% of pediatric patients with de novo ALL [24–29]. We chose CD13 and CD33 as MyAgs, because they have been the most common antigens in MyAg-positive ALL. In our study, CD13 and CD33 were expressed in 31.7 and 26.5%, respectively, of de novo childhood ALL cases. Moreover, the frequency of CD13 expression was 33.3% in B-lineage ALL compared with 20.7% in T-ALL, while CD33 expression was 28.1% in B-lineage ALL versus 15.2% in T-ALL. These MyAgs were significantly more frequently associated with B-lineage ALL than with T-ALL ($p < 0.001$). In addition, the expression of these MyAgs was more frequent in early pre-B ALL cases than in pre-B ALL cases ($p < 0.001$). These incidences of MyAg expression in our study are in line with the data reported in the literature [24–29].

Recently, several notable studies investigated differences of race and ethnicity in the immunophenotypic subsets of childhood ALL [30–32]. Bhatia et al. [30] analyzed 8,762 children with de novo ALL who were categorized according to five groups: white, black, Hispanic, Asian and others. They showed that there was a significantly greater incidence of black children (25%) with T-ALL compared with Asian (19%), white (15%) and Hispanic (13%) children. In comparison, the frequency of T-ALL in our present report (the largest scale report in Japan to date), as representative data of East Asian children with ALL, was 13% of all cases, which is less than the 19% reported by Bhatia et al. [30]. This disparity cannot be readily explained. However, Kandan-Lottick et al. [32] pointed out that the reason might be that the Asian children analyzed by Bhatia et al. [30] were not Japanese, but from the Indian subcontinent and South Asia because they had been enrolled in the Children's Cancer Group Study.

In conclusion, based on the results of our large, retrospective study of antigen expression in 1,774 children with newly diagnosed ALL enrolled between 1997 and 2007, we have formulated clinically useful guidelines for flow cytometric immunophenotypic criteria for the diagnosis and classification of pediatric ALL in the JPLSG. The JPLSG was established in 2003 to create a research base for multi-center clinical trials for promotion of evidence-based medicine in pediatric hematologic malignancies. The JPLSG unifies several pediatric leukemia study groups, including the Japan Association of Childhood Leukemia Study (JACLS), the Tokyo Children's Cancer Study Group (TCCSG), the Japanese Children's Cancer and Leukemia Study Group (JCCLSG) and the Kyushu Yamaguchi Children's Cancer Study Group (KYCCSG), which had been functioning in Japan since the 1970s. The patients analyzed in this study have been treated according to different clinical protocols in each study group, and some of

them have not been clinically observed long enough. In addition, the central reference flow cytometry laboratories of the JPLSG received samples and made immunophenotypic diagnoses even during the intervals between clinical studies. Therefore, in this study we did not concern ourselves with possible associations of antigen expression with the clinical, hematological or biological features, or attempt to determine the prognostic importance of antigen expression for the decision of treatments. Nevertheless, flow cytometric data generated by extensive use of our newly proposed immunological criteria together with common diagnostic panels developed according to the present analysis may be valuable for achieving more precise characterization of the leukemic blasts in each individual patient. This information, combined with the molecular and clinical features presented in the next standard clinical protocol for childhood ALL that will be issued by the JPLSG, will also contribute to the development of personalized medicine, the so-called tailor-made therapy, for each patient.

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bjh research paper

Chromosome abnormalities in advanced stage T-cell lymphoblastic lymphoma of children and adolescents: a report from Japanese Paediatric Leukaemia/Lymphoma Study Group (JPLSG) and review of the literature

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Summary

T-cell acute lymphoblastic leukaemia (T-ALL) and T-cell lymphoblastic lymphoma (T-LBL) are combined into one category as T lymphoblastic leukaemia/lymphoma in the current World Health Organization (WHO) classification. However, there is still ongoing discussion on whether T-ALL and T-LBL are two separate entities or represent two variant phenotypes of the same disease. Cytogenetic analysis has been used to identify the molecular background of haematological malignancies. To compare the distribution of chromosomal abnormalities of T-ALL and T-LBL, large series of cytogenetic data are required, but are absent in T-LBL in contrast to the abundant data in T-ALL. Among 111 T-LBL cases in our clinical trial, we obtained complete cytogenetic data from 56 patients. The comparison between our cytogenetic findings and those from three published T-LBL studies revealed no significant difference. However, meta-analysis showed that translocations involving chromosome region 9q34 were significantly more common in T-LBL than in T-ALL. In particular, four out of the 92 T-LBL cases, but none of the 523 paediatric T-ALL cases, showed translocation t(9;17)(q34;q22–23) ($P = 0{\cdot}0004$). Further studies are needed for the possible linkage between abnormal expression of genes located at 9q34 and/or 17q22–23 and the unique 'lymphoma phenotype' of T-LBL.

Keywords: T-cell lymphoma, child, non-Hodgkin lymphoma, cancer cytogenetics, leukaemia.

In children and adolescents, precursor T lymphoblastic neoplasms have been classified into two diseases: T-cell acute lymphoblastic leukaemia (T-ALL) and T-cell lymphoblastic lymphoma (T-LBL). Although the current World Health Organization (WHO) classification designates both malignancies as T lymphoblastic leukaemia/lymphoma (Borowitz & Chan, 2008), there is continuing discussion on whether T-ALL and T-LBL are two separate entities or whether they represent two different clinical presentations of the same disease. They show overlapping clinical, pathological and immunophenotypic features. In general, the word 'lymphoma' is used if there is a bulky mass in the mediastinum or elsewhere, with less peripheral blood and bone marrow (BM) involvement. Most study groups distinguish between leukaemia and lymphoma on the basis of the extent of BM involvement: patients with <25% lymphoblasts in the BM are diagnosed with lymphoblastic lymphoma; in cases

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of 25% or more BM blasts, the diagnosis is leukaemia. While this distinction may appear somewhat arbitrary, a notable observation is that T-LBL patients with large mediastinal masses frequently exhibit little, if any, evidence of tumour dissemination and BM involvement, but the molecular background for this difference is unknown.

Chromosomal analysis has been widely used as a primary step that is required to narrow down the responsible genes that define a disease entity. For instance, discovery of Ph chromosome led to the identification of the chimeric *BCR/ABL1* gene, which is responsible for and defines chronic myeloid leukaemia. Compared with T-ALL, chromosomal abnormalities in T-LBL are not well defined. Reports in the literature and current textbooks claim that the typical chromosomal aberrations reported in T-ALL can also be found in T-LBL (Borowitz & Chan, 2008). However, there are no large series of cytogenetic data on T-LBL (Burkhardt, 2010).

This study aimed to fill the gap regarding cytogenetic data in T-LBL and compare the cytogenetic findings of T-ALL and T-LBL, which may lead to identification of the molecular background behind phenotypical differences between the two disease entities.

Study patients

From November 2004 to October 2010, 154 eligible children (aged 1–18 years) with newly diagnosed advanced stage LBL (Murphy stages III and IV) (Murphy, 1980) were entered in the Japanese Paediatric Leukaemia/Lymphoma Study Group (JPLSG) ALB-NHL03 study (UMIN000002212, http://www.umin.ac.jp/ctr/index-j.htm). Patients with primary immunodeficiencies, Down syndrome and T-cell diseases as second malignancies were excluded. The ethics committee of each participating institute approved the study protocol.

Cytogenetic analysis

Cytogenetic analysis was performed on cell suspensions obtained from 31 tumour/lymph nodes, 19 pleural effusions and six bone marrow samples. The methods of chromosome preparation for cytogenetic analysis are described elsewhere (Sanger *et al*, 1987; Horsman *et al*, 2001). Karyotypes are described according to the International System for Human Cytogenetic Nomenclature (ISCN) (Shaffer & Tommerup, 2005). Only those cases with abnormal cytogenetic study results, defined as two or more cells with the same structural abnormality or the same numerical gain, three or more cells with the same numerical loss or isolated cells with disease-associated abnormalities, were eligible for inclusion in this study.

Statistical methods

Two-tailed Fisher's exact test was used to analyse the patients' characteristics and the frequency of each chromosome abnormality. Significant differences in the analysis of he frequency of each chromosome abnormality were determined by the two-tailed Fisher's exact test with Bonferroni correction comparison. The P value threshold for inclusion of a new variable was chosen to be $P < 0{\cdot}003$ in this analysis (0·05/17, after Bonferroni correction). A review of T-LBL and T-ALL karyotypes reported in the literature was obtained from a PubMed search and information on chromosome abnormalities and gene fusions was obtained from Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer (http://cgap.nci.nih.gov/Chromosomes/Mitelman).

Results

Patient characteristics

A total of 154 children were enrolled on JPLSG ALB-NHL03 protocols; 111 cases were T-LBL. Among 111 T-LBL cases, the study population for the current analysis included 56 patients for whom complete cytogenetic data were obtained. With respect to presenting features, patients with reviewed and accepted cytogenetic data were similar to both those without accepted cytogenetic data and the entire cohort of concurrently enrolled T-lineage LBL patients (Table S1).

Frequency of chromosomal abnormalities

Multiple chromosome abnormalities were identified in 31 patients (45%). Structural chromosome abnormalities were identified in 29 patients (52%), and numerical chromosome abnormalities were identified in 18 patients (32%). Ploidy results included pseudodiploid in 14 patients (25%), hypodiploid in three patients (5%), hyperdiploid with 47–50 chromosomes in 10 patients (18%), hyperdiploid with more than 50 chromosomes in four patients (7%) and diploid in 25 patients (45%) (Table S2).

All of the hypodiploid cases had 43–45 chromosomes; none had a near-haploid karyotype. Of the four cases with more than 50 chromosomes, two had near-tetraploid karyotypes. The frequencies of ploidy groups in this series are compared with those reported in other series of karyotyped T-LBL patients and paediatric T-ALL (Table S2). Structural chromosome abnormalities were identified in 29 patients (52%). In the current study, seven patients (13% of those with abnormal karyotypes) exhibited a rearrangement at one or more of the chromosome bands (7p15, 7q32–36 and/or 14q11–13) that are the locations of T-cell receptor chain genes. Rearrangements in the 14q11–13 region, in which the T-cell receptor α/δ chain genes are located, were present in three patients (5%) of the karyotypically abnormal cases in this series (Table S2). Structural abnormalities involving chromosome region 9q34 were identified in nine patients (16%). Translocations involving chromosome region 9q34 were identified in three patients (5%) (t(9;17)(q34;q22), t(7;9)(q34;q34) and t(2;9)(q23;q34)). In comparison between cytogenetic findings in the current data and combined data of three published reports (Burkhardt

et al, 2006; Lones *et al*, 2007; Uyttebroeck *et al*, 2007; Table S1), the frequencies of numerical and structural cytogenetic abnormalities in T-LBL and T-ALL had no significant difference (Table S2).

We compared the cytogenetic findings in the current study with the published reports from the three largest-scale studies on T-LBL (Burkhardt *et al*, 2006; Lones *et al*, 2007; Uyttebroeck *et al*, 2007; Table S3) and those from the two largest-scale studies on T-ALL combined (Heerema *et al*, 1998; Schneider *et al*, 2000; Table S3) (Table I). The frequencies of almost all of the cytogenetic abnormalities in T-LBL and T-ALL had no significant difference, but translocation involving chromosome region 9q34 was significantly more common in T-LBL than in T-ALL ($P = 0{\cdot}0004$, Table S3) and translocation t(9;17) was also more common in T-LBL (4%, 4/92) than in T-ALL (0%, 0/523, $P = 0{\cdot}0004$) (Table I).

The current study included a patient with translocation t(9;17)(q34;q22). As far as we could tell from the consulted published reports, all T-LBL patients with translocation t(9;17) presented with a mediastinal mass and without any bone marrow involvement (Kaneko *et al*, 1988; Shikano *et al*, 1992) (Table II).

Table I. Comparison of cytogenetic findings between T-LBL and T-ALL.

	T-LBL		T-ALL		
	n	%	*n*	%	*P* value
Total	92		523		
Normal karyotype†	36	39	219	42	0·6478
Abnormal karyotype	56	61	304	58	0·6478
Hypodiploid	4	4	20	4	0·9999
Pseudodiploid	30	33	204	39	0·2000
Hyperdiploid(47–50)	18	20	64	12	0·0328
Hyperdiploid(>50)	4	4	16	3	0·5217
Any translocation	26	28	177	34	0·3367
Any del chrome.	19	21	160	31	0·0328
Any der chrome.	4	4	58	11	0·0583
del(6q)	6	7	69	13	0·0833
Loss of 9p	10	11	44	8	0·5487
Any 14q11–13 abnormality	10	11	72	14	0·5100
Any 7q32–36 abnormality	7	8	35	7	0·8220
Any translocation including 9q34	8	9	7	1	0·0004*
t(7;10)	1	1	2	0	0·3855
t(10;11)	1	1	8	2	0·9999
t(9;17)	4	4	0	0	0·0004*

†Includes one Klinefelter syndrome, and one inv(9) without other abnormality in current report.
The *P* value threshold for inclusion of a new variable was chosen to be 0·003 (0·05/17, after Bonferroni correction). *$P < 0{\cdot}003$.
T-LBL: current study (JPLSG ALB-NHL03) combined with three published reports(Burkhardt *et al*, 2006; Lones *et al*, 2007; Uyttebroeck *et al*, 2007).
T-ALL: combined two published reports (Heerema *et al*, 1998; Schneider *et al*, 2000).

Discussion

This is the largest study involving cytogenetic analysis of T-LBL and the first study to directly compare cytogenetic findings of T-LBL and T-ALL. The frequencies of almost all of the cytogenetic abnormalities in both entities were found to have no significant difference, but translocation involving chromosome region 9q34 was significantly more common in T-LBL than in T-ALL. The current study included a patient with unique translocation t(9;17)(q34;q22). Interestingly, four out of the 92 T-LBL cases, but none of the 523 paediatric T-ALL cases, showed this translocation ($P = 0{\cdot}0004$) (Table I). Translocation t(9;17) has been reported in several haematological diseases, such as precursor B-cell ALL (Coyaud *et al*, 2010), acute myeloid leukaemia (Mrózek *et al*, 2001), chronic myeloid leukaemia (DeAngelo *et al*, 2004), chronic lymphocytic leukaemia (Michaux *et al*, 2005), diffuse large B-cell lymphoma (Hammond *et al*, 1992) and follicular lymphoma (Aamot *et al*, 2007), but these breakpoints, 9q34 and 17q22–23, are limited in the cases of T-LBL (http://cgap.nci.nih.gov/Chromosomes/Mitelman). These results imply a linkage between abnormal expression of genes located at 9q34 and/or 17q22–23 and the unique phenotypes of the T-LBL mentioned above.

Cytogenetic analysis has been used to identify the molecular background of haematological malignancies. To compare the distribution of chromosomal abnormalities of T-ALL and T-LBL, large series of cytogenetic data are required, but are absent in T-LBL in contrast to the abundant data in T-ALL. Three recent series of cytogenetic data on paediatric T-LBL have been published, reporting the cytogenetic findings in 13, 11 and 12 paediatric T-LBL cases (Burkhardt *et al*, 2006; Lones *et al*, 2007; Uyttebroeck *et al*, 2007). Thus, this study can play a role to fill the gap of cytogenetic data on T-LBL.

Translocation involving chromosome region 9q34 was found to be significantly more common in T-LBL than in T-ALL (Table I). Among genes located in the 9q34 region, *SET, PKN3, ABL1, NUP214* and *NOTCH1* have previously been implicated in malignancy, with *SET, ABL1, NUP214* and *NOTCH1* being implicated in leukemogenesis (Ellisen *et al*, 1991; van Vlierberghe *et al*, 2008; Hagemeijer & Graux, 2010).

An oncogenic *SET-NUP214* fusion gene has been reported in a case of acute undifferentiated leukaemia with a reciprocal translocation t(9;9)(q34; q34) (von Lindern *et al*, 1992) and NK adult acute myeloid leukaemia as a result of a cryptic deletion of 9q34 (Rosati *et al*, 2007). van Vlierberghe *et al* (2008) identified the *SET-NUP214* fusion gene in three patient samples out of 92 paediatric cases of T-cell leukaemia. *SET-NUP214* may contribute to T-ALL pathogenesis by inhibition of T-cell maturation through the transcriptional activation of the *HOXA* genes (van Vlierberghe *et al*, 2008). However, the frequency of this mutation in T-LBL is unknown.

NOTCH1, previously termed *TAN1*, was discovered as a partner gene in T-ALL with a translocation t(7;9)(q34;q34.3), and was found in <1% of T-ALLs (Ellisen *et al*, 1991). Several

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Table II. Clinical characteristics and detailed karyotype data in T-LBL patients with t(9;17).

	Age (years)	Sex	Tumour site	Stage	BM blast %	Karyotype
Kaneko *et al* (1988)	14	F	Mediastinum	III	0	46,XX,t(9;17)(q34;q23)
	15	M	Mediastinum	III	0	46,XY,-9,del(6)(q13q21),t(9;17)(q34;q23),+der(9)t(9;17)(q34;q23)
	10	M	Mediastinum	III	0	47,XY,+19,t(9;17)(q34;q23)
Shikano *et al* (1992)	14	F	Mediastinum	III	0	46,XX,t(9;17)(q34;q23)
	7	M	Mediastinum	III	0	49,XY-l,+der(l)t(l;?)(p36;?),t(9;17)(q34;q23),+14,+marl,+mar2
	5	F	Mediastinum	III	0	47,XX,t(9;17)(q34;q23),+der(17)t(9;17)(q34;q23)
Burkhardt *et al* (2006)	ND	ND	ND	ND	ND	46,XX,del(6)(q1?2q1?6),t(9;17)(q34;q22)
	ND	ND	ND	ND	ND	47,XX,t(9;17)(q34;q22),+20
Lones *et al* (2007)	8	M	Mediastinum	III	0	47,XY,t(9;17)(q3?4;q2?3),+20
Current study	7	M	Mediastinum	III	0	46,XY,t(9;17)(q34;q22)

ND, no data available.

study groups reported *NOTCH1* mutations in 31–62% of T-ALL patients (Weng *et al*, 2004; Breit *et al*, 2006; van Grotel *et al*, 2006; Zhu *et al*, 2006; Malyukova *et al*, 2007; Asnafi *et al*, 2009; Gedman *et al*, 2009; Park *et al*, 2009). In contrast, only two studies reported *NOTCH1* mutation analyses in T-LBL: Park *et al* (2009) reported *NOTCH1* mutations in six out of 14 paediatric T-LBL patients (43%), and Baleydier *et al* (2008) reported mutations in six out of nine paediatric T-LBL (66%), with 32 adult patients with *NOTCH1* mutations in 16 cases (54% in all patients) (Baleydier *et al*, 2008). According to these reports, the frequencies of *NOTCH1* mutation were not significantly different between T-LBL and T-ALL.

ABL1 fusion genes have been identified that provide proliferation and survival advantage to lymphoblasts. *NUP214-ABL1, EML1-ABL1, BCR-ABL1* and *ETV6-ABL1* chimeric genes have been reported. The most frequent one in T-ALL is the *NUP214-ABL*1 fusion gene, which has been identified in 6% of cases, in both children and adults (Graux *et al*, 2009). In addition, using an oligonucleotide microarray, *ABL1* overexpression was identified in 8% of cases in T-ALL (Chiaretti *et al*, 2007). Our review of these published reports indicated that the frequency of ABL1 mutation in T-LBL is unknown.

Raetz *et al* (2006) analysed the gene expression profiles of ten T-ALL BM samples and nine T-LBL samples using a microarray. They identified 133 genes for which the expression levels differed between T-LBL and T-ALL. *ZNF79* (encoding zinc finger protein 79) and *ABL1*, both located in chromosome region 9q34, were included in these genes and showed at least twofold higher overexpression in T-LBL than that in T-ALL. Additionally, *MED13* (previously termed *THRAP1*), which is located in 17q22-q23, also showed at least twofold higher overexpression in T-LBL than that in T-ALL (Raetz *et al*, 2006). Taking these findings together, it is possible that *ZNF79, ABL1* or *THRAP1* as well as other genes at 9q34 and 17q22–23 are involved in the 'lymphoma phenotype' such as a bulky mass in the mediastinum and minimal BM involvement. These findings need further study to determine if this linkage constitutes a unique 'lymphoma phenotype'.

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Authorship

MS designed the study, prepared the data file, performed the analysis, interpreted data and wrote the manuscript. SS is a lead principal investigator for the JPLSG ALB-NHL03 study. AN contributed to pathological diagnosis. YH contributed to chromosome analysis. YO is a principal investigator contributing a patient to this study. AMS contributed to statistical analysis. KH received a research grant from the Ministry of Health, Labour and Welfare of Japan. MT is a chairperson of JPLSG. TM is a chairperson of JPLSG lymphoma committee. SS, KH, MT and TM were primarily responsible for the study design, data analysis and interpretation of the data. All authors approved the final manuscript.

Disclosure

The authors declare no competing financial interests.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Respective clinical characteristics with and without karyotype data in 111 T-LBL patients in the current study.

Table S2. Comparison of cytogenetic findings in T-LBL between current study and combined data of three published reports.

Table S3. Published data of cytogenetic findings in T-LBL and T-ALL.

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Distinct Impact of Imatinib on Growth at Prepubertal and Pubertal Ages of Children with Chronic Myeloid Leukemia

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Objective To determine the extent of growth impairment resulting from imatinib treatment in children with chronic myeloid leukemia (CML).

Study design Clinical records of 48 chronic-phase CML children administered imatinib as the first-line therapy between 2001 and 2006 were analyzed retrospectively. Cumulative change in height was assessed using the height height-SDS and converted height data from age- and sex-adjusted Japanese norms.

Results A decrease in height-SDS was observed in 72.9% of children, with a median maximum reduction in height-SDS of 0.61 during imatinib treatment. Median follow-up time was 34 months (range, 10-88 months). Growth impairment was seen predominantly in children who started imatinib at a prepubertal age compared with those who started at pubertal age. Growth velocity tended to recuperate in prepubertal children with growth impairment, as they reached pubertal age, suggesting that imatinib had little impact on growth during puberty.

Conclusions Growth impairment was a major adverse effect of long-term imatinib treatment in children with CML. We report the distinct inhibitory effect of imatinib on growth in prepubertal and pubertal children with CML. We should be aware of growth deceleration in children, especially in young children given imatinib before puberty and subjected to prolonged exposure. *(J Pediatr 2011;159:676-81).*

Since the introduction of imatinib, the treatment of chronic myeloid leukemia (CML) has changed from cure by allogeneic stem cell transplantation to maintenance of the best achievable treatment response (hematologic, cytogenetic, and molecular responses). Various side effects, including nausea, vomiting, diarrhea, skin rash, edema, elevated liver enzyme values, and cytopenia, are known to be common during imatinib treatment, but generally are mild to moderate.[1] However, the long-term side effects of imatinib therapy remain unknown, and its effects on growth are a major concern when treating children. Growth deceleration has been reported in 3 children as well as in a cohort given imatinib.[2-5] The present study was conducted to evaluate the effect of imatinib on growth in children and adolescents with CML.

Methods

In Japan, imatinib was approved and became available for treatment of CML in December 2001. The Japanese Pediatric Leukemia/Lymphoma Study Group's CML Committee reviewed records of 99 Japanese children under age 18 years diagnosed with chronic-phase CML between 2001 and 2006. Among these children, 76 who received imatinib as first-line therapy were eligible for the study. Concurrent hydroxyurea administration was permitted. Exclusion criteria were as follows: (1) reached final height at the time of diagnosis (n = 3); (2) afflicted by a chronic disease (eg, schistorrhachis) or on any treatment that could affect growth (n = 4); and (3) a follow-up period of <10 months while receiving imatinib (n = 21). Forty-eight children (21 girls, 27 boys) met these criteria and were enrolled in the study. The study design was approved by the Keio University School of Medicine's Ethics Committee.

BSA	Body surface area
CML	Chronic myeloid leukemia
GH	Growth hormone
PDGF	Platelet-derived growth factor

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