Multiplex Reverse Transcription-Polymerase Chain Reaction as Diagnostic Molecular Screening of 4 Common Fusion Chimeric Genes in Taiwanese Children With Acute Lymphoblastic Leukemia

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Background: The classification of B-lineage acute lymphoblastic leukemia (ALL) by specific chromosomal translocations has prognostic implications for risk-directed therapy. Reverse transcription-polymerase chain reaction (RT-PCR) assay is a useful tool for detecting fusion transcripts from common chromosomal translocations of ALL cells.

Methods: Multiplex RT-PCR and nested-PCR assays were used to detect ALL-type *BCR-ABL1* transcripts of the t(9;22), *TCF-PBX1* transcripts of t(1;19), the *MLL-AF4* transcripts of t(4;11), and 2 variants of *ETV6-RUNX1* of the cryptic t(12;21) in 148 leukemic samples upon diagnosis. The patients received risk-directed protocols of the Taiwan Pediatric Oncology Group-ALL-2002 that consisted of multiple chemotherapeutic agents of different intensities. Event-free survival (EFS) and overall survival (OS) rates were analyzed for genetic abnormalities detected by multiplex PCR and conventional cytogenetic analysis by the Kaplan-Meier method, and compared with the Mantel-Haenszel test. The Cox proportional hazards model was implemented to identify independent prognostic factors for EFS and OS.

Results: In this cohort of Taiwanese children, the relative frequencies of the 4 translocations of B-lineage ALL were 8% with ALL-type t(9;22)/*BCR-ABL1*, 4% with (1;19)/*TCF-PBX1*, 2% with t(4;11)/*MLL-AF4*, and 17.6% with t(12;21)/*ETV6-RUNX1*. Patients with t(12;21)/*ETV6-RUNX1* fusion, hyperdiploidy, and t(1;19)/*TCF-PBX1* fusion had the most favorable outcomes, whereas those with the t(9;22)/*BCR-ABL1* fusion or t(4;11) and other *MLL* gene rearrangement had poor prognosis ($P < 0.001$ for EFS and OS). *BCR-ABL1*, *MLL* gene rearrangement, and very high-risk group were independent prognostic factors after Cox regression analysis.

Conclusions: The biological factors of leukemia cells are associated with treatment outcomes in childhood ALL. Multiplex RT-PCR assay is an efficient and sensitive diagnostic tool that may improve the ability to accurately and rapidly risk-stratify children with ALL.

Key Words: childhood acute lymphoblastic leukemia (ALL), multiplex PCR, *ETV6-RUNX1*

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Childhood acute lymphoblastic leukemia (ALL) is a highly heterogeneous disease with distinct biologic subgroups that have different event-free survival rate (EFS) ranging from 30% to 40% in cases with *BCR-ABL1* or *MLL-AF4* fusion genes to 90% in those with *ETV6-RUNX1* fusion.[1,2] In precursor-B (pre-B) ALL, such significant prognostic genetic abnormalities can be identified in more than 60% of cases.[3]

Even with advances in molecular biology and new molecular markers such as gene expression profiles,[4–10] recurrent chromosomal abnormalities are still the most important prognostic factors and risk factors for different protocol assignments.[2] Although conventional cytogenetics is the standard method for identifying chromosomal translocations, it requires considerable expertize. This results either in false negatives or in noninterpretable results in a large number of cases, especially in laboratories lacking experience in handling karyotyping of malignant cells. The most common *ETV6-RUNX1* cannot be identified by routine cytogenetic study.[11]

Therefore, identifying these recurrent chromosomal translocations rapidly and efficiently is extremely important.

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Molecular screening by reverse transcription-polymerase chain reaction (RT-PCR) is increasingly used to complement conventional karyotyping.[12,13] This study used RT-PCR to screen 4 translocations as diagnostic samples of childhood ALL and to investigate their prognostic importance.

METHODS

Patients and Treatment Protocols

Viable, diagnostic bone marrow or peripheral blood was taken from 146 children newly diagnosed with B-cell ALL from January 2002 to October 2009 at the National Taiwan University Hospital in northern Taiwan, at the China Medical University Hospital in central Taiwan, and at the National Cheng Kung University Hospital and Chang Gung Memorial Hospital, Kaohsiung Medical Center in southern Taiwan. Patients were prospectively assigned to 1 of 3 risk groups (ie, standard, high, and very high) based on their presenting clinical features and biologic features of their leukemic cells.

Patients were considered to have standard-risk (SR) ALL if they were between 1 and 9 years of age presenting with leukocyte count less than 10×10^9 (cells/L) or were between 2 and 7 years of age presenting with leukocyte count between 10×10^9 and 50×10^9 (cells/L). Patients were considered high-risk (HR) ALL if they were between 1 and 9 years of age presenting with leukocyte count between 50×10^9 and 100×10^9 (cells/L), or between 1 and 2 or 7 and 10 years of age presenting with leukocyte count between 10×10^9 and 50×10^9 (cells/L). HR also includes those with central nervous system leukemia (cerebrospinal fluid white blood cell (WBC) ≥ 5 with blasts), cranial nerve palsy, testicular leukemia, or pre-B ALL with *TCF-PBX1* fusion.

Patients with at least one of the following were assigned to the very high-risk (VHR) group: age below 1 year, initial leukocyte count greater than 100×10^9, T-cell ALL, lymphoblastic lymphoma with lymphoblasts greater than 25% in the bone marrow, hypodiploidy, HR patients with poor treatment response, and presence of *BCR-ABL1*, *MLL-AF4*, or other *MLL* rearrangements in pre-B ALL.

The risk-directed Taiwan Pediatric Oncology Group (TPOG) protocols consisted of multiple chemotherapeutic agents of different intensities. The treatment protocol was upgraded if complete remission was not achieved after initial induction therapy. Events were defined as any relapse or secondary malignancy. Patients or their parents provided informed consent before sample collection. The TPOG-2002-ALL protocols were described in detail elsewhere.[14]

Laboratory Methods

Mononuclear cells were collected from the bone marrow or peripheral blood by density centrifugation using Ficoll-Hypaque according to standard methods. Cells were re-suspended in Roswell Park Memorial Institute 1640 medium containing 10% heat-inactivated fetal calf serum, 100 U/mL penicillin, 100 μg/mL streptomycin, 2 mM L-glutamine, and 10% dimethyl sulfoxide, and frozen in liquid nitrogen for long-term storage. Total RNA was extracted with Trizol reagent (Invitrogen, Paisley, UK) according to the manufacturer's instructions, and was then subjected to DNase I treatment.

After RNA extraction, multiplex RT reaction for patients' samples was performed by the method of Scurto et al[12] with slight modifications. For each sample, 1 μg RNA, diethyl pyrocarbonate-treated water, and specific 3′ oligonucleotide primers for *BCR-ABL1*, *MLL-AF4*, *TCF-PBX1*, and *ETV6-RUNX1* with final concentrations of 0.2 μM were heated at 65°C for 5 minutes and then placed in ice bath. RNA was then reverse transcribed in a final volume of 10.1 μL containing reverse transcriptase, buffer, deoxyribonucleotide triphosphates (dNTPs), dithiothreitol, and RNAsin. Tubes were incubated for 42°C for 1.5 hours.

Multiplex PCR was performed in a final volume of 25 μL containing 5 μL cDNA (5 μg), 0.8 mM dNTPs, 1 × PCR buffer, 0.1 U Tag, and a combination of 0.2 μM 3′ and 0.2 μM 5′ oligonucleotide primers sets for *BCR-ABL1*, *MLL-AF4*, *TCF-PBX1*, and *ETV6-RUNX1* on a thermal cycler (ABI 9600) using a touchdown program. The sequences of primers were according to those of Scurto et al.[12] After an initial denaturing step at 94°C for 5 minutes, a 3-step PCR (94°C for 1 min, 60°C for 2 min, followed by 72°C for 2 min) was performed for 40 cycles. After amplification, 10 μL of PCR product was run on 3% agarose gel stained with ethidium bromide and visualized

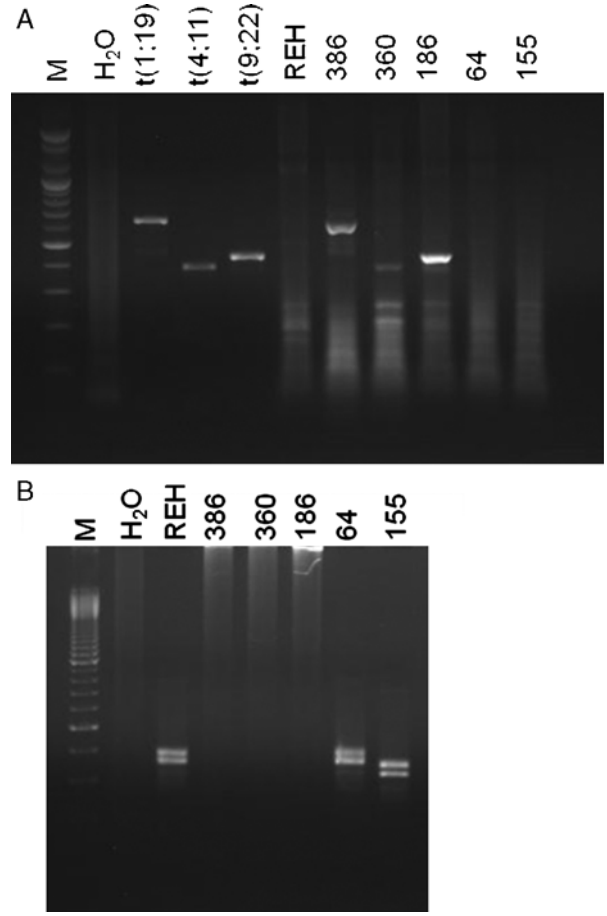

FIGURE 1. A, Agarose gel analysis of different PCR products from patients and control cell lines or positive control plasmid cDNA by multiplex RT-PCR. Patients with t(1;19), t(4;11), and t(9;22) can be identified in the first multiplex RT-PCR. Number 386, 160, and 186 are representative patients' samples positive for a fusion transcript in multiplex reaction. B, The nested PCR can identify patients with t(12;21) (number 64 and 165). Lane M indicates molecular weight marker; RT-PCR, reverse transcription-polymerase chain reaction; REH, positive control cell lines for *ETV6-RUNX1*.

under ultraviolet lamp (Fig. 1A). *BCR-ABL1*, *MLL-AF4*, and *TCF-PBX1* were visualized after this PCR reaction.

Another nested PCR was performed to amplify the *ETV6-RUNX1*. PCR was performed in a final volume of 25 μL containing 2.5 μL of the first PCR product, 0.8 mM dNTPs, 0.2 μM t(12;21)3′, 0.2 μM t(12;21)5′A, 1× PCR buffer, and 0.1 U Tag. Samples were amplified at 94°C for 5 minutes; 1 cycle at 94°C for 1 minute, 55°C for 2 minutes, and 72°C for 2 minutes for 35 cycles. After amplification, 10 μL of PCR product was run on 3% agarose gel stained with ethidium bromide and visualized under ultraviolet lamp (Fig. 1B).

Cytogenetics

Bone marrow cells were harvested directly or after 1 to 3 days of unstimulated culture as described earlier.[15] Metaphase chromosomes were banded by trypsin-Giemsa technique and karyotyped according to the International System for Human Cytogenetic Nomenclature.

Statistical Methods

Comparison of baseline clinical variables across groups was made using Fisher exact test for categorical data. A *P* value less than 0.05 (2-sided) was considered significant.

Patients analyzed for chromosomal abnormalities were initially divided into 6 groups according to their cytogenetic changes. These were t(12;21), t(1;19), t(9;22), *MLL* gene rearrangement, hyperdiploidy, and others. Overall survival (OS) was calculated using the Kaplan-Meier method, whereas the log-rank test was used to compare differences between survival curves. OS was measured from the protocol commencement date until the date of death regardless of cause, excluding patients who were alive on their last follow-up. EFS was measured from the date of attaining complete remission until the date of event. Patients who did not achieve complete remission were considered to have an EFS of 0. Patients with no report of relapse by the end of follow-up were included in a census based on the date of last follow-up. EFS and OS were estimated by the Kaplan-Meier method and compared with the Mantel-Haenszel test.

Cox proportional hazard models were constructed for EFS and OS. The covariates included in the full model of OS and EFS were sex, WBC (< 10,000, 10,000 to 50,000, 50,000 to 100,000, and > 100,000/μL), age (< 1, between 1 and 10, and > 10 y), hospital, and genetic subtypes. Stepwise backward selection was performed. All calculations were carried out using the SAS software package, version 9 (SAS Inc).

RESULTS

Multiplex PCR Assay

In the 146 cases with childhood B-lineage ALL, 26 (17.6%) had *ETV6-RUNX1* fusion transcript, 6 (4%) *TCF-PBX1* fusion transcript, 2 (1%) MLL-AF4 fusion transcript, and 12 (8%) ALL-type BCR-ABL1 fusion transcript. Representative cases analyzed by the multiplex assay and detected by gel electrophoresis were shown in Figure 1.

As expected, none of the 26 cases with *ETV6-RUNX1* fusion transcript was identified cytogenetically. However, 5 of the 8 with *BCR-ABL* studied cytogenetically had the t(9;22). Both cases with *MLL-AF4* studied by cytogenetics had detectable t(4;11), whereas all 6 with *TCF-PBX1* had the t(1;19).

Patient Characteristics

The clinical characteristics of patients at the time of diagnosis are presented in Table 1. The median age among the 146 patients (78 boys and 70 girls) was 6.66 years (range: 0 to 17 y) and their median leukocyte count 10^9 (cells/L) was 20.0 (range: 0.2 to 1826). The numbers of SR, HR, and VHR patients were 55, 52, and 41, respectively. The clinical presenting features including age, sex, and initial WBC counts were not statistically different from a larger cohort reported from TPOG recently.[14] However, the numbers of VHR patients of this smaller cohort are larger than that of the original cohort. In addition, more patients with t(9;22) were detected in this cohort and this might be because of the smaller case numbers or lack of full screenings of fusion transcripts for the original cohort.

Biologic Factors of Leukemic Cells Associated With EFS and OS

Patients with *ETV6-RUNX1* fusion, hyperdiploidy, and t(1;19)/*TCF-PBX1* fusion had the most favorable outcomes, whereas those with the t(9;22)/*BCR-ABL1*

TABLE 1. Clinical Features of the Study Patients

	No.	%	*P**
Sex			0.236†
Male	78	52.7	
Female	70	47.3	
Age at diagnosis (y)			0.527‡
Mean ± SD	6.66 ± 4.63		
< 1	7	4.7	
1-9	106	71.7	
> 10	35	23.6	
WBC count × k/μL			0.174‡
< 10	59	39.9	
10-49	44	29.7	
50-99	17	11.5	
> 100	28	18.9	
Risk groups			0.016‡
Standard risk	55	37.1	
High risk	52	35.1	
Very high risk	41	27.8	
Chromosomal abnormalities			
Hyperdiploid	16	10.8	0.351†
T(12;21)	26	17.6	0.349†
T(1;19)	6	4.0	0.832†
T(9;22)	12	8.1	0.002†
T(4;11) and Other *MLL* gene	7	4.7	1.0†
Others	81	54.8	
Hospital			
NTUH	93	62.9	
CGMH	24	16.2	
CMUH	22	14.9	
NCKUH	9	6.0	

*In comparison with the larger cohort of reference 14 in Taiwan.
†Fisher exact test.
‡Chi-square test.
CGMH indicates Chang Gung Memorial Hospital, Kaohsiung Medical Center; CMUH, China Medical University Hospital; NCKUH, National Cheng Kung University Hospital; NTUH, National Taiwan University Hospital; WBC, white blood cell.

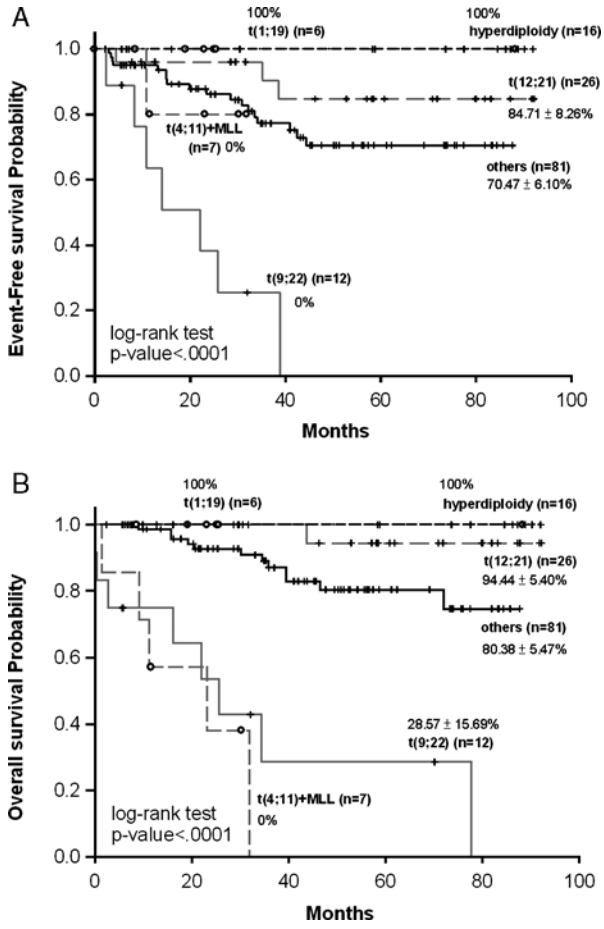

FIGURE 2. Kaplan-Meier analysis of (A) 5-year event-free survival and (B) 5-year overall survival according to biologic subtype of acute lymphoblastic leukemia.

fusion or t(4;11) and other *MLL* gene rearrangement had poor prognosis ($P < 0.001$ for EFS and OS) (Fig. 2).

In multivariable regression analyses examining the association of EFS with cytogenetic changes, other known prognostic factors and treatment groups (Table 1) such as *BCR-ABL1* status, WBC counts greater than 100,000/μL, and the VHR group were significantly associated with EFS. *BCR-ABL1* status, *MLL* gene rearrangement, and the VHR group were significantly associated with OS (Table 2).

TABLE 2. Multivariate Analysis of EFS and OS

Step Selection Variables	HR	95% HR CI	*P*
Multivariate Cox regression analysis for EFS			
BCR-ABL	3.51	1.37-8.99	0.009
WBC count > 100,000/μL	2.17	1.02-7.26	0.0452
Very high risk	5.30	1.63-17.24	0.006
Multivariate Cox regression analysis for OS			
BCR-ABL	4.39	1.65-11.67	0.003
MLL gene rearrangement	5.81	1.74-19.35	0.004
Very high risk	10.52	3.39-32.68	< 0.0001

CI indicates confidence interval; EFS, event-free-survival; HR, hazard ratio; OS, overall survival; WBC, white blood cell.

TABLE 3. Clinical Features of Patients With and Those Without *ETV6-RUNX1*

	ETV6-RUNX1 Status				
	Negative		Positive		
	No.	%	No.	%	*P**
Sex					0.283
Male	67	54.9	11	42.3	
Female	55	45.1	15	57.7	
Age at diagnosis (y)					
Mean ± SD	6.98 ± 4.93		5.18 ± 2.38		0.007†
< 1	7	5.5	0	0	0.008
1-10	88	72.1	25	96.2	
> 10	34	27.9	1	3.8	
WBC count × k/μL					0.139
< 10	47	38.5	12	46.1	
10-49	34	27.9	10	38.5	
50-99	14	11.5	3	11.5	
> 100	27	22.1	1	3.9	
Risk groups					0.019
Standard risk	41	33.6	14	53.9	
High risk	42	34.4	10	38.4	
Very high risk	39	32.0	2	7.7	

*Fisher exact test.
†Student *t* test.
WBC indicates white blood cell.

ETV6-RUNX1 had Better EFS and OS but not Independent Prognostic Factor

The EFS and OS were analyzed for patients with or without *ETV6-RUNX1*. Patients' characteristics are listed in Table 3. Patients with *ETV6-RUNX1* had trend of young age and received HR or SR protocols. Patients with *ETV6-RUNX1* had better EFS than those without (84.71 ± 8.26 vs. 70.14 ± 5.13) and better OS (94.44 ± 5.40 vs. 74.69 ± 4.73) ($P = 0.163$ and 0.028, respectively) (Fig. 3).

In multivariable regression analyses examining the association of EFS with *ETV6-RUNX1* status and other known prognostic factors and treatment groups (Table 1), none of the following were statistically significant predictors of outcome: *ETV6-RUNX1* status, sex, age, and WBC counts. When controlling for other significant predictors of EFS in the final model, the VHR group ($P < 0.001$), but not *ETV6-RUNX1* status ($P = 0.174$), was significantly associated with EFS.

BCR-ABL1 was Associated With Very Poor EFS and OS

Patients with *BCR-ABL1* (n = 12) had inferior EFS (25.40 ± 15.47 vs. 83.03 ± 3.73) (n = 136) and OS (28.57 ± 15.69 vs. 87.75 ± 3.23) than patients without *BCR-ABL1* (all $P < 0.001$). In multivariable regression analyses examining the association of EFS with *BCR-ABL1* status and the other known prognostic factors and treatment groups (Table 1), *BCR-ABL1* status, WBC counts more than 100,000/μL, and VHR group were significantly associated with EFS. Cox regression revealed that t(9;22) was the independent prognostic factor.

MLL-AF4 and Other *MLL* Gene Rearrangement Were Associated With Very Poor OS

Patients with *MLL-AF4* or other *MLL* had inferior EFS and OS than patients without *MLL-AF4* or other

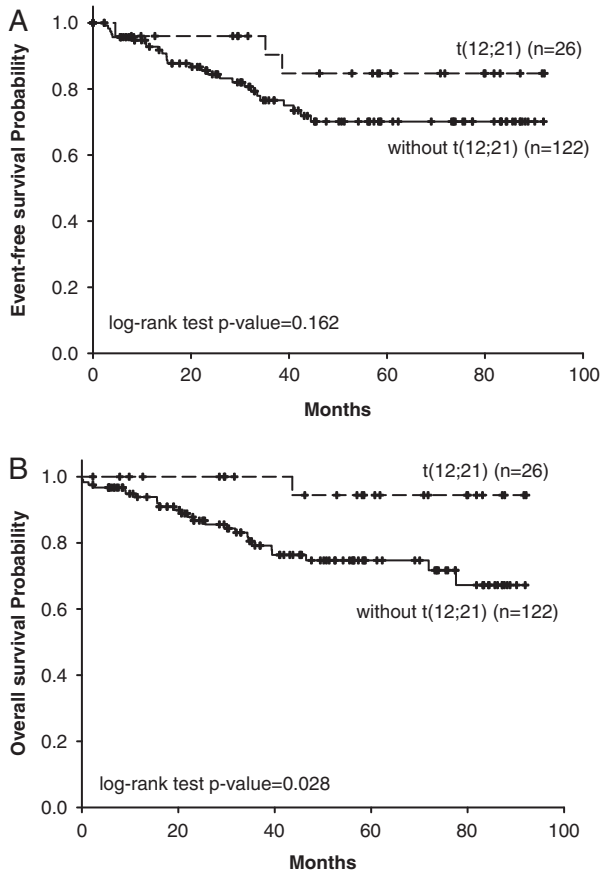

FIGURE 3. A, Event-free survival and (B) overall survival of *ETV6-RUNX1*-positive patients compared with *ETV6-RUNX1*-negative patients.

MLL (38.10 ± 19.33 vs. 91.85 ± 2.48, $P < 0.001$). In multivariate regression analyses examining the association of OS with *MLL* status and the other known prognostic factors and treatment groups (Table 1), *MLL* status and VHR groups were significantly associated with EFS. Cox regression revealed that *MLL* gene rearrangement was an independent prognostic factor.

Treatment Outcomes by Genotypes of Other International Groups

In this February issue of Leukemia, an international representation of study groups reported and compared the long-term results of patients treated between 1985 and 2007.[14,16–29] We listed the treatment results of these major childhood ALL study groups and one St Jude report in 2009 according to the genotypes if data were available (Table 4).[30] As expected, patients with *ETV6-RUNX1* have good EFS and OS. *BCR-ABL1*, *MLL-AF4*, or other *MLL* gene rearrangements were poor prognostic genotypes. The prognosis for patients with *TCF3-PBX1* improved gradually. In addition, the frequency of *ETV6-RUNX1* fusion in our study is lower than that (~25%) in western countries. Although our sample size was relatively smaller, our treatment results for patients with *ETV6-RUNX1* and *TCF3-PBX1* were quite good in comparison with other groups.

DISCUSSION

This study shows the feasibility of multiplex PCR to screen 4 common fusion transcripts in B-cell ALL. As expected, the RT-PCR can detect t(12;21), which is not detected during routine cytogenetic study. However, some patients with t(9;22) cannot be diagnosed by the first cytogenetic study. Thus, the RT-PCR can add diagnostic accuracy to the cytogenetic study, partly because some patients have no mitotic cells to be analyzed. The biologic factors of leukemic cells are associated with EFS and OS. Patients with *ETV6-RUNX1* fusion, hyperdiploidy, and *TCF-PBX1* fusion have the most favorable outcomes, whereas those with the *BCR-ABL* fusion and *MLL* gene rearrangement have poor prognosis.

The t(12;21) is a submicroscopic rearrangement that cannot be detected during routine cytogenetic study.[11] This abnormality is very important in terms of prognosis.[11,31–34] Patients with *ETV6-RUNX1* have good long-term treatment outcomes, although some studies show late-relapse in this group of patients or fail to identify this as an independent prognostic factor when considering onset age and initial WBC count.[32,35–38] Patients with *ETV6-RUNX1* have better EFS ($P = 0.163$) and OS ($P = 0.028$). However, multivariable regression analyses fail to show the *ETV6-RUNX1* status as an independent prognostic factor. With improved EFS for B-lineage cases, the favorable prognostic impact of *ETV6-RUNX1* fusion is less noticeable. In addition, the frequency of *ETV6-RUNX1* fusion in Taiwan, which is 18% to 19% in B-lineage ALL and in some Asian countries, is consistently lower than that (~25%) in western countries.[39–42] Careful epidemiologic studies are needed to determine these genetic differences of leukemic genetics.

The other important prognostic chromosomal translocation is *BCR-ABL1*. Although the overall outcome for ALL children has improved, the cure rate for specific VHR subgroups, including those with *BCR-ABL1* ALL, are disappointing. Several patients have not been detected to have this translocation upon diagnosis. Clinically, some patients are noted to have this translocation because of relapses despite normal initial chromosomal screening.

Recently, Schultz et al[43] highlighted that imatinib ($340\,mg/m^2/d$) plus intensive chemotherapy improved 3-year EFS in children and adolescents with *BCR-ABL1* ALL, with no appreciable increase in toxicity. Stem cell transplantation plus imatinib offered no advantage over transplantation alone. Although additional follow-up is required to determine the impact of this treatment on long-term EFS and to determine whether chemotherapy plus imatinib can replace bone marrow transplant, this study further highlights the importance of fusion transcript screening through molecular methods so that patients with *BCR-ABL1*-positive ALL can benefit from intensified or alternative therapies with imatinib as early as possible.

The *MLL* gene is located at 11q23.[44] MLL is found in 73 different translocations and 54 partner genes have been cloned.[45] The *MLL-AF4* ALL is the most common subtype, accounting for 50% of ALL cases in infants and 2% in children. It is also associated with steroid resistance and poor outcome, even with stem cell transplantation.[1,44] This assay cannot detect each of the HR-associated *MLL* fusion transcripts that result from translocations affecting 11q23. As most of these rearrangements occur in infants, and in this group of patients the t(4;11) accounts for greater than 80% of lesions, this assay should serve as a useful screening

TABLE 4. Overview of Study Groups, Incidence of Major Cytogenetic Changes, and Outcomes

Genotype	Study Group	Incidence (%)	5-year EFS†	5-year OS†
ETV6-RUNX1	AIEOP	22.4	85.1 ± 2.5	94.5 ± 1.6
	BFM	21.5	91.7 ± 1.9	97.5 ± 1.0
	CCG	*	*	*
	COALL	23.9	87.9 ± 3.1	96.4 ± 1.8
	CPH	22.4	81.2 ± 4.2	94.1 ± 4.6
	DCOG	15.0	94.3 ± 2.0	94.8 ± 2.1
	DFCI	25.8	88.2 ± 3.7	97.4 ± 1.8
	INS	13.7	89.0 ± 5.2	97.3 ± 2.7
	JCCLSG	*	*	*
	NOPHO	22.8	80.0 ± 3.0	94.0 ± 2.0
	POG	26.3	86.2 ± 2.3	96.2 ± 1.3
	SJCRH	15.8	84.5 ± 5.9	86.8 ± 5.5
	SJCRH XV	19.3	97.6 ± 3.2	98.9 ± 3.0
	TCCSG	*	*	*
	TPOG 2002	13.1	82.7 ± 5.4	94.2 ± 3.3
	UKALL	*	*	*
TCF3-PBX1	AIEOP	*	*	*
	BFM 95	*	*	*
	CCG	3.0	77.6 ± 5.5	85.0 ± 4.8
	COALL	*	*	*
	CPH	*	*	*
	DCOG	2.3	85.0 ± 8.0	85.0 ± 8.0
	DFCI	*	*	*
	INS	0.3	100 ± 0.0	100 ± 0.0
	JCCLSG	*	*	*
	NOPHO	2.2	81.0 ± 9.0	95.0 ± 5.0
	POG	4.8	74.5 ± 2.8	83.3 ± 2.4
	SJCRH	4.0	80.0 ± 11.9	90.0 ± 9.0
	SJCRH XV	5.6	85.9 ± 11.4	100 ± 0.0
	TCCSG	4.4	70.2 ± 9.5	73.0 ± 8.7
	TPOG 2002	4.4	81.0 ± .7.9	92.2 ± 5.4
	UKALL	3.1	80.4 ± 5.6	*
BCR-ABL1	AIEOP	2.3	28.3 ± 7.4	41.8 ± 8.1
	BFM 95	2.2	27.9 ± 6.8	39.5 ± 7.5
	CCG	2.8	36.7 ± 6.7	45.5 ± 7.2
	COALL	1.9	43.6 ± 15.5	53.0 ± 15.5
	CPH	2.4	11.1 ± 10.5	33.3 ± 15.7
	DCOG	1.6	28.6 ± 12.1	34.3 ± 12.1
	DFCI	0.2	0.0 ± 0.0	0.0 ± 0.0
	INS	3.3	55.6 ± 16.6	55.6 ± 16.6
	JCCLSG	*	*	*
	NOPHO	3.3	44.0 ± 9.0	59.0 ± 9.0
	POG	2.4	24.9 ± 3.9	34.8 ± 4.3
	SJCRH	2.8	28.6 ± 13.9	42.9 ± 16.2
	SJCRH XV	2.0	40.0 ± 21.9	48.0 ± 20.0
	TCCSG	4.0	26.4 ± 9.7	41.7 ± 10.1
	TPOG 2002	2.1	35.5 ± 17.8	71.1 ± 12.8
	UKALL	2.0	43.9 ± 7.6	*
MLL-AF4 or other *MLL* rearrangement‡	AIEOP	1.1	61.1 ± 11.5	61.1 ± 11.5
	BFM 95	2.2	42.3 ± 9.7	53.8 ± 9.8
	CCG	2.3	44.1 ± 7.6	47.9 ± 7.6
	COALL	0.9	0 ± 0	0 ± 0
	CPH	*	*	*
	DCOG	2.0	88.2 ± 7.8	88.2 ± 7.8
	DFCI	*	*	*
	INS	5.9	56.3 ± 12.4	68.8 ± 11.6
	JCCLSG	*	*	*
	NOPHO	0.4	25.0 ± 22.0	25.0 ± 22.0
	POG	1.0	47.8 ± 6.1	65.7 ± 5.8
	SJCRH	2.8	42.9 ± 16.2	42.9 ± 16.2
	SJCRH XV	0.6	100.0 ± 0.0	100.0 ± 0.0
	TCCSG	0.8	75.0 ± 21.5	80.0 ± 17.9

TABLE 4. (*continued*)

Genotype	Study Group	Incidence (%)	5-year EFS†	5-year OS†
	TPOG 2002	1.9	34.3 ± 13.1	35.7 ± 12.8
	UKALL	1.4	64.7 ± 11.6	*

*Not mentioned in this study.
†5-year EFS and OS for the latest studies in each group.
‡*MLL-AF4* or other *MLL* gene rearrangement depend upon groups.
AIEOP indicates Associazione Italiana di Ematologia ed Oncologia Pediatrica; BFM, Berlin–Frankfurt–Munster ALL Study Group; CCG, Children's Cancer Group; COALL, Cooperative ALL Study Group; CPH, Working Group for Pediatric Hematology in the Czech Republic; DCOG, Dutch Childhood Oncology Group; DFCI, Dana-Farber Cancer Institute ALL Consortium; EFS, event-free survival; INS, Israeli National Studies of childhood ALL; JCCLSG, Japanese Childhood Cancer and Leukemia Study Group; NOPHO, Nordic Society of Pediatric Hematology and Oncology; OS, overall survival; POG, Pediatric Oncology Group; SJCRH, St Jude Children's Research Hospital; TCCSG, Tokyo Children's Cancer Study Group; TPOG, Taiwan Pediatric Oncology Group; UKALL, UK Medical Research Council Working Party on Childhood Leukaemia.

for prognostic markers. Currently available drugs will not enable cure for all infants with ALL, particularly those with *MLL* rearrangements. Therefore, future innovative strategies are warranted to direct against novel therapeutic targets.

In conclusion, multiplex PCR is a good method to screen the 4 important fusion transcripts. This can complement cytogenetic study and guide risk-directed therapy in childhood ALL.

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