Fluorescence *in situ* hybridization comparison of the prognostic factors in adult and pediatric acute lymphoblastic leukemia: A retrospective analysis of 282 cases

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Abstract. Acute lymphoblastic leukemia (ALL) affects both children and adults. However, the prognosis of the two cohorts is quite different. The present aim was to review and evaluate one potential cause of why survival is poorer in adult ALL than pediatric ALL via fluorescence *in situ* hybridization (FISH). Clinical significant features were analyzed in 282 ALL cases. FISH was performed to study mixed lineage leukemia (*MLL)* translocation and the Philadelphia (Ph) chromosome in newly diagnosed patients, and was used to detect trisomy 4 or 10 and the translocation ETS leukemia-acute myeloid leukemia 1 (*TEL-AML1)* fusion gene. The overall survival/event-free survival (OS/EFS) outcome of adult ALL and pediatric ALL was analyzed using Kaplan-Meier analysis. Adult ALL had a higher median leukocyte count and lower hemoglobin level than pediatric ALL. FISH revealed that Ph positivity (Ph+) was associated with the high-risk feature of older age. In pediatric ALL, trisomy 4 or 10 was present in 71/207 cases (34.3%), while the *TEL-AML1* fusion gene was present in 16/207 cases (7.7%). By contrast, there were very few such positive cases in adult ALL. Survival analysis revealed that, in adult ALL, the 3-year OS and EFS rates were higher in the Ph-negative group than in the Ph+ group. Adult or pediatric ALL is an independent prognostic factor of OS. The present analysis of the clinical and biological features between adult and pediatric ALL indicates that adult ALL has a poorer prognosis than pediatric ALL based on Ph+ status and presence of trisomy 4 or 10. Ph+ ALL is an independent prognosis factor of ALL. FISH may serve an important role in the comparison of prognostic factors in adult and pediatric ALL.

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Abbreviations: ALL, acute lymphoblastic leukemia; FISH, fluorescence *in situ* hybridization; MLL, mixed lineage leukemia; Ph, Philadelphia; TEL-AML1, translocation ETS leukemia-acute myeloid leukemia 1; OS, overall survival; EFS, event-free survival; HR, hazard ratio; CI, confidence interval

Key words: adult acute lymphoblastic leukemia, pediatric acute lymphoblastic leukemia, fluorescence *in situ* hybridization, Philadelphia+ acute lymphoblastic leukemia, poor survival

Introduction

Acute lymphoblastic leukemia (ALL) affects both children and adults, with peak prevalence between the ages of 2-5 years and again after the age of 50 years (1). Overall age-adjusted incidence is 1.7 per 100,000 persons; roughly 60% of cases are diagnosed in patients younger than 20 years (2). ALL development includes genetic instability such as translocation and fusion genes. The relevance of the prognosis and cytogenetic abnormalities between adult ALL and pediatric ALL has been studied for decades (3-5). Retrospective studies (6,7) by diverse departments and various institutions worldwide have revealed that 20% of adult patients with ALL have the BCR-ABL fusion protein, while only ~5% of pediatric patients with ALL harbor the Philadelphia (Ph) chromosome at present. Due to the high frequency of unfavorable cytogenetic features such as t(9;22)(q34;q11) and t(1;19)(q23;p13), the outcome of treatment in ALL worsens with age (8).

During the last decade, the advances in our understanding of the clinical, immunobiological and genetic characteristics of ALL have led to improved risk stratification and to risk-adapted treatment strategies. In addition to conventional chemotherapy, stem cell (or bone marrow) transplant and chimeric antigen receptor T-cell therapy are used for certain unusual subtypes of ALL or refractory B-cell ALL. Monoclonal antibodies, new immunotherapy treatments and other targeted approaches are promising novel therapeutic approaches to ALL. With the current therapies, the vast majority of pediatric patients with ALL are now long-term survivors. In China, the 5-year and 10-year overall survival rates are 80.0±1.2 and 76.3±1.6%, respectively (9). In developed countries, the 5-year overall survival rate for ALL has increased to 90% in the last

30 years (10). Unfortunately, the same positive results have not been obtained for adult patients with ALL (11). In adult ALL, the cure rate is estimated to be 20-40% (12). Adults present with higher risk features at diagnosis, which predispose them to chemotherapy resistance and disease relapse after an initial achievement of complete remission (CR). The incorporation of targeted agents into adult ALL therapy has improved survival in several subsets (13,14). In the present study, the cryptic chromosomal alterations and genomic changes in a cohort of 75 adult ALL samples and 207 pediatric ALL samples were investigated. The cytogenetic abnormalities and prognosis of the two groups were analyzed using fluorescence *in situ* hybridization (FISH) in order to determine why survival is poorer in adult ALL than in pediatric ALL. The results indicate that adult ALL has a poorer prognosis compared with pediatric ALL based on Ph+ status and presence of trisomy 4 or 10. Ph+ is an independent prognosis factor of ALL.

Patients and methods

Patient characteristics. All ALL cases enrolled in the present study were diagnosed and treated at the Department of Hematology, Xiangya Hospital (Changsha, China) from January 2008-December 2012. Consecutive patients were enrolled, giving a total of 207 pediatric patients and 75 adult patients. Written informed consent was obtained from all patients. In order to maintain consistent treatment, patients who received HSCT or had never received chemotherapy were excluded from the study. All patients underwent blood routine examination, bone marrow (BM) aspiration, Wright's and peroxidase (POX) staining, cerebrospinal fluid, immunophenotyping and FISH examination. In addition, the pretreatment workup included a complete medical history, physical examination, chest X-ray, B-ultrasound scan of the abdomen and neck and routine laboratory analysis. Table I presents the characteristics of the whole cohort. The Xiangya Hospital Ethics Committee approved the present study (approval no. 201212478).

Immunophenotyping and FISH. Immunophenotype analysis was performed on BM specimens of 269 of 282 cases using a BD FACSCanto II flow cytometry instrument (BD Biosciences, Franklin Lakes, NJ, USA). The other 13 patients had no available immunophenotype analysis and were excluded from this assay. BM samples were stored at 4˚C prior to the assay and all samples were assessed within 24 h of collection. All antibody reagents were purchased from BD Biosciences. The ALL marker panel used was as follows: (CD) 45-PerCP (cat no. 347464), CD34-APC (cat no. 743533), CD19-PE Cy7 (cat no. 745907), CD10-FITC (cat no. 745553), CD20-APC (cat no. 743611), cytoplasmic CD22-PE (cat no. 562859), CD2-FITC (cat no. 745734), CD3-APC (cat no. 746776), cytoplasmic CD3-APC (cat no. 340440), CD5-APC (cat no. 742554), CD7-FITC (cat no. 562635), CD13-PE (cat no. 555394), CD33-APC (cat no. 551378), surface immunoglobulin M (IgM)-APC (cat no. 560575) and cytoplasmic IgM-APC (cat no. 560914). All the antibodies were diluted 1:100 and were detected by direct immunofluorescence using a flow cytometer. Data were analyzed by FACSDiva 6.0 (BD Biosciences).

The multiprobe ALL panel was designed to detect eight FISH probes: *BCR/ABL* translocation, mixed lineage leukemia (*MLL*) rearrangement, translocation ETS leukemia-acute myeloid leukemia 1 (*TEL-AML1*) fusion gene and trisomy 4/10. The probes are located at 22q11.2 9q34, 11q23, 12p13 21q22, and 4p11.1-q11.1/10p11.1-q11.1, which represent the chromosome abnormalities t(9;22)(q34;qll), 11q23, t(12;21)(p13;q22) and trisomy 4/10, respectively. A total of 2 ml fresh BM was taken from each patient prior to treatment. The BM was centrifuged at 12,00 rpm for 10 min at room temperature to extract nucleated cells. Cells were subjected to hypotonic shock, fixed with methanol and acetic acid, then a cell suspension was prepared for FISH detection. FISH probes were purchased from Beijing GP Medical Technologies, Ltd., Beijing, China) and marked by nick translation. FISH was performed according to the manufacturer's instructions FISH probes are reversibly bound to the surface of a glass device, and dissolve upon contact with hybridization buffer, which is composed of formamide, SSC and glucan sulfate; probe and target DNA denaturation occurs simultaneously upon heating at 78˚C for 5 min. Hybridization conditions were 42˚C for 14-16 h. Probe cut-off values were established by testing 20 BM samples from healthy individuals (median age, 31 years; range, 18-50 years old; male to female ratio, 1.5:1; enrolled at Xiangya Hospital from May to December 2011, with informed consent). Data from 500 interphase cells were gathered from each individual in the control group to establish a normal database for the probes. The cut-off values for the Ph chromosome, trisomy 4, trisomy 10, *MLL* gene, and *TEL-AML1* gene were 4.08, 2.32, 2.88, 3.24 and 3.76%, respectively.

Diagnosis and subclassification. ALL diagnosis was mainly based on morphology of BM and immunotyping of flow cytometry (15,16): The proportion of primitive and juvenile lymphocytes in BM was >20%, which was the basic diagnosis requirement. Morphology diagnosis performed using routine procedures: Methanol fixing, Wright staining and POX staining, then cell morphology was examined with an optical microscope (Olympus CX21; Olympus, Japan, Tokyo). Flow cytometry served a crucial role in differential diagnosis and subclassification: Strong CD19 expression associated with weak expression of CD10, CD22, or CD79a or weak CD19 expression plus strong expression of two of the same markers were considered as B lineage; strong cytoplasmic or surface CD3 expression was considered as T lineage; CD5 or CD7 indicated pre-T-ALL and early pre-T-ALL; and ambiguous B-lineage or T-lineage markers, or 'bilineal' cases were considered as uncertain-lineage subtypes. All patients received the standard treatment strategies according to the National Comprehensive Cancer Network guideline (17). Remission-induction therapy, consolidation phase and maintenance chemotherapy comprise an integrity program for patients who achieved continued CR until the analysis of the present study. Adult patients received CVMD (cyclophosphamide, vincristine, mitoxantrone and dexamethasone), R-CVMDL (rituximab in combination with cyclophosphamide, vincristine, mitoxantrone, dexamethasone and L-asparaginase), or R-CHOP (rituximab in combination with cyclophosphamide, doxorubicin, vincristine and prednisone)

Table I. Characteristics of adult and pediatric patients with ALL.

Characteristic	Adult ALL (n=75) (%)	Pediatric ALL (n=207) (%)	P-value
Sex			0.865
Male	39 (52.0)	110 (53.1)	
Female	36 (48.0)	97 (46.9)	
WBC x10^9/l			0.462
<5	20 (26.7)	52 (25.1)	
5-50	30 (40.0)	99 (47.8)	
>50	25 (33.3)	56 (27.1)	
Median (range)	16.9 (1.2-462.0)	11.9 (1.5-910.0)	
Hemoglobin level, g/l			0.061
≥80	40 (53.3)	60 (29.0)	
<80	35 (46.7)	147 (71.0)	
LDH			0.551
High	49 (65.3)	143 (69.1)	
Normal/low	26 (34.7)	64 (30.9)	
CNS involvement			0.187
Yes	11 (14.7)	19 (9.2)	
No	64 (85.3)	188 (90.8)	
Minimal residual disease			0.009
Yes	16 (25.3)	21 (10.1)	
No	56 (74.7)	186 (89.9)	

Data are presented as n (%), unless otherwise stated. ALL, acute lymphoblastic leukemia; LDH, lactate dehydrogenase; CNS, central nervous system; WBC, white blood cell count.

treatment regimens, whereas pediatric patients were treated with VDLD (vincristine, daunorubicin, L-asparaginase and dexamethasone) or VDLP (vincristine, daunorubicin, L-asparaginase and prednisone) therapeutic regimens.

Follow-up. Follow-up was measured from the initial day of treatment to the final follow-up date (January 2015), or the day the patient succumbed. Following treatment, follow-up examinations were conducted every 3-6 months in the first 2 years, every month in the following 3 years and annually thereafter. The duration of overall survival (OS) was calculated from the day of treatment completion to the day of mortality or the final follow-up; the event-free survival (EFS) rate was calculated from the day of treatment completion to the day of tumor progression, the occurrence of fatal or intolerable side effects or mortality.

Statistical analysis. Statistical analyses were performed using SPSS 17.0 (SPSS, Inc., Chicago, IL, USA). The χ^2 test was used for comparing clinical and biological features between the 282 adults and pediatric ALL cases. Prognostic factors, including leukocyte count, hemoglobin level, *BCR-ABL* translocation, *MLL* rearrangement, and immunophenotype were analyzed. Multivariate analysis was performed to identify the independent prognostic factors. The OS and EFS rates were calculated using the Kaplan-Meier method and were compared using the log-rank test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Clinical characteristics of patients. The median follow-up time was 46 months (range, 3-68 months), with 96.8% of patients finishing a complete 3-year follow-up. The median age of the 207 pediatric ALL cases was 5.4 years (range, 0.5-14 years); that of the 75 adult ALL cases was 32.9 years (range, 15-68 years). Table I presents the other patient clinical characteristics. Only minimal residual disease was significantly different in the pediatric and adult ALL groups. Sex, leukocyte count, hemoglobin level and lactate dehydrogenase level were not significantly different between the two groups.

Immunophenotyping and FISH results. The expression of lineage markers in the two groups did not differ significantly. Table II shows that 82.6 and 14.7% of the adult ALL cases had B-lineage markers and T-lineage markers, respectively. In pediatric ALL cases, 81.2% had B-lineage markers and 12.1% had T-lineage markers.

Cytogenetically, genetic rearrangement, including the *BCR-ABL* fusion gene and *MLL* rearrangement, were detected in all adult patients. In adult patients, the rate of Ph chromosome positivity (Ph+) and *MLL* rearrangement was 38.6% (29/75) and 16.0% (12/75), respectively, and 1.3% (1/75) and 0% of patients were trisomy 4/10 and had the *TEL-AML1* fusion gene, respectively. In pediatric ALL, 24.2% of patients (50/207) had *MLL* rearrangement and only 9.2% of patients (19/207) were Ph+, while 34.3% (71/207) and 7.7% (16/207) of

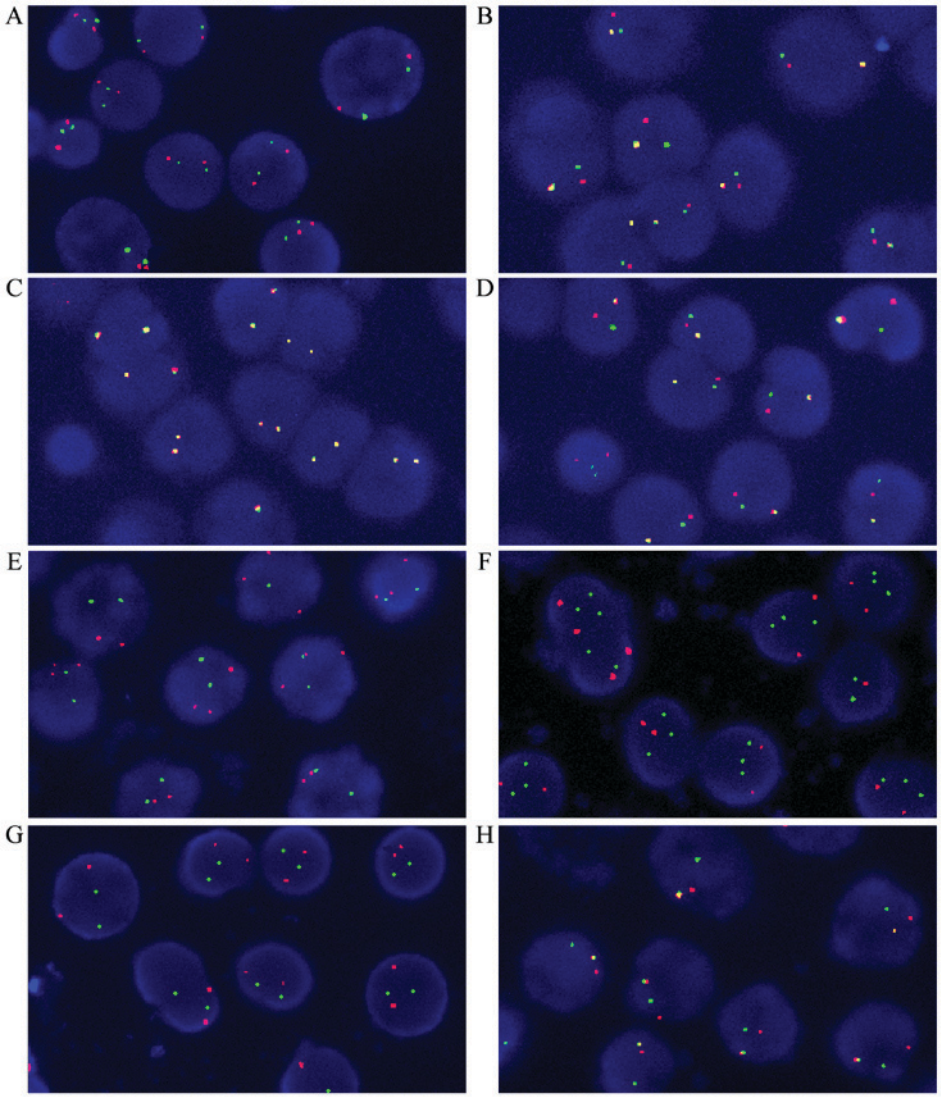

Figure 1. Fluorescence *in situ* hybridization results (original magnification, x1,000). (A) Normal signal of Ph-negative and chromosomes 4/10 (two red, two green particles). (B) Ph-positive sample (one red, one green, one yellow particle). (C) *MLL*-negative sample (two yellow particles). (D) *MLL*-positive sample (one red, one green, one yellow particle). (E) Chromosome 4 amplification (three red, two green particles). (F) Chromosome 10 amplification (two red, three green particles). (G) *TEL-AML1*-negative sample (two red, two green particles). (H) *TEL-AML1*-positive sample (one red, one green, one yellow particle). Ph, Philadelphia; MLL, mixed lineage leukemia; TEL-AML1, translocation ETS leukemia-acute myeloid leukemia 1.

patients were trisomy 4/10 or had the *TEL-AML1* fusion gene, respectively. Fig. 1 depicts the FISH results. In Ph-negative and trisomy 4/10 negative patients, two red and two green particles were observed in the nucleus (Fig. 1A). In Ph-positive patients, there was one red, one green and one yellow particle in the nucleus (Fig. 1B). In MLL-negative patients, two yellow particles were observed in the nucleus (Fig. 1C). One red, one green and one yellow particle were observed in MLL-positive patients (Fig. 1D). Chromosome 4 amplification was observed as three red and two green particles (Fig. 1E); Chromosome 10 amplification resulted in two red and three green particles (Fig. 1F). In the TEL-AML1-negative sample, there are two red and two green particles (Fig. 1G); The TEL-AML1-positive sample usually presented with one red, one green and one yellow particle (Fig. 1H).

Prognosis analysis of cases with different chromosomes or immunophenotypes. Survival rates were calculated using the Kaplan-Meier method and compared with the log-rank test. The mean 3-year OS rate for the entire cohort was 59.5% (range, 56.3-62.7%), 74% (range, 70.7-77.3%) in the pediatric ALL group, and 18.8% (range, 13.6-24%) in the adult ALL group [hazard ratio (HR) =5.28; 95% confidence interval (CI), 3.59-7.78; P<0.001]. The 3-year EFS rate for the entire cohort was 50.6% (range, 47.4-53.8%), 62.8% (range, 59.1-66.5%) in the pediatric ALL group, and 16.5% (range, 12.0-21.0%) in the adult ALL group (HR=4.58; 95% CI, 3.22-6.52; P<0.001).

The multivariate analysis suggested that age, i.e., pediatric vs. adult patients, was most associated with OS (HR=4.44; P<0.001); Ph chromosome status (HR=2.71; P<0.001), *MLL* status (HR=1.65; P=0.020), *TEL-AML1* fusion gene (HR=0.76; P=0.067) and white blood cell (WBC) level (HR=1.001; P=0.045) were independent prognostic factors of OS (Table III).

In pediatric ALL cases, Ph chromosome (HR=3.56; P<0.001) and *MLL* status (HR=2.27; P=0.007) were independent prognostic factors of OS (Table IV). In adult ALL cases,

Table II. Immunophenotype and cytogenetic features in adult and pediatric ALL.

Feature	Adult ALL (n=75) (%)	Pediatric ALL (n=207) (%)	χ^2	P-value
Immuno-phenotype			1.933	0.380
B-lineage	62 (82.6)	168 (81.2)		
T-lineage	11 (14.7)	25 (12.1)		
Uncertain-lineage	2 (2.7)	14 (6.7)		
Ph			33.893	<0.001
Positive	29 (38.6)	19 (9.2)		
Negative	46 (61.4)	188 (90.8)		
+4/+10			29.553	<0.001
Positive	1 (1.3)	71 (34.3)		
Negative	74 (98.7)	146 (65.7)		
TEL-AML1			6.146	0.013
Positive	0 (0)	16 (7.7)		
Negative	75 (100)	191 (92.3)		
MLL			2.134	0.144
Positive	12 (16.0)	50 (24.2)		
Negative	63 (84.0)	157 (75.8)		

Data are presented as n (%). ALL, acute lymphoblastic leukemia; Ph, Philadelphia; +4/+10, trisomy 4/10 positive; TEL-AML1, translocation ETS leukemia-acute myeloid leukemia 1; MLL, mixed lineage leukemia.

Table III. Multivariate analysis of prognostic factors of overall survival in acute lymphoblastic leukemia.

					95% CI	
Variable	Regression coefficient	Standard error	P-value	HR	Lower	Upper
Sex	-0.131	0.206	0.524	0.877	0.586	1.313
Age (adult or pediatric)	1.491	0.221	<0.001	4.441	2.878	6.852
MLL	0.503	0.217	0.020	1.653	1.081	2.529
TEL-AML/4-10	-0.275	0.150	0.067	0.759	0.566	1.019
Ph	0.997	0.218	<0.001	2.711	1.767	4.159
B/T/uncertain-lineage	0.246	0.186	0.187	1.278	0.888	1.841
WBC	0.001	0.001	0.045	1.001	1.000	1.003
Hb	0.002	0.004	0.599	1.002	0.994	1.010
Pt	0.000	0.001	0.847	1.000	0.997	1.002

HR, hazard ratio; CI, confidence interval; MLL, mixed lineage leukemia; TEL-AML or 4/10 positive; Ph, Philadelphia; WBC, white blood cell count; Hb, hemoglobin count; Pt, platelet count.

Ph chromosome status (HR=2.21; P=0.016) and WBC level (HR=1.003; P=0.012) were independent prognostic factors of OS (Table V).

Tables III-V demonstrate that Ph chromosome, *MLL* status and WBC level were independent prognostic factors of OS. Tables I and II indicate that *MLL* status and WBC level were not significantly different in the pediatric and adult ALL cases. This suggests that Ph+ is the primary reason for the worse prognosis in adult ALL than in pediatric ALL.

The prognosis of the two groups in the present study may differ so greatly as the pediatric and adult patients with ALL have different biological features. Prognostic analysis of all patients was stratified by the following biological features: i) In Ph-negative (Ph-) and Ph+ patients the 3-year OS rate was 68.5 vs. 16.9% (HR=4.21; P<0.001) and the 3-year EFS rate was 60.0 vs. 6.7% (HR=4.33; P<0.001); ii) in *MLL*-negative and -positive patients the 3-year OS rate was 62.5 vs. 51.8% (HR=1.34; P=0.112) and the 3-year EFS rate was 56.5 vs. 34.5% (HR=1.59; P=0.011); iii) in patients negative for trisomy 4/10 and *TEL-AML1*, trisomy 4-positive, trisomy 10-positive, *TEL-AML1*-positive patients, the 3-year OS rate was 52.6 vs. 60.6 vs. 82.6 vs. 87.2% (HR=0.587, P=0.004) and the 3-year EFS rate was 42.6 vs. 65.2 vs. 78.3 vs. 71.3% (HR=0.646; P=0.003); iv) in B lineage, T lineage or uncertain-lineage

Table IV. Multivariate analysis of prognostic factors of overall survival in pediatric acute lymphoblastic leukemia.

Variable	Regression coefficient	Standard error	P-value	HR	95% CI	
					Lower	Upper
Sex	-.0117	0.301	0.698	0.890	0.493	1.605
Age	0.034	0.039	0.386	1.035	0.958	1.118
MLL	0.818	0.305	0.007	2.266	1.247	4.118
TEL-AML/4-10	-0.317	0.184	0.085	0.729	0.508	1.045
Ph	1.269	0.350	0.000	3.558	1.790	7.070
B/T/uncertain-lineage	0.349	0.263	0.185	1.417	0.846	2.372
WBC	0.001	0.001	0.497	1.001	0.999	1.002
Hb	0.004	0.007	0.530	1.004	0.991	1.018
Pt	-0.002	0.002	0.355	0.998	0.995	1.002

HR, hazard ratio; CI, confidence interval; MLL, mixed lineage leukemia; TEL-AML or 4/10 positive; Ph, Philadelphia; WBC, white blood cell count; Hb, hemoglobin count; Pt, platelet count.

Table V. Multivariate analysis of prognostic factors of overall survival in adult acute lymphoblastic leukemia.

Variable	Regression coefficient	Standard error	P-value	HR	95% CI	
					Lower	Upper
Sex	-0.003	0.302	0.992	0.997	0.551	1.803
Age	0.001	0.010	0.934	1.001	0.982	1.020
MLL	-0.221	0.411	0.591	0.802	0.358	1.795
TEL-AML/4-10	-0.197	0.254	0.438	0.821	0.499	1.351
Ph	0.795	0.330	0.016	2.214	1.159	4.229
B/T/uncertain-lineage	0.361	0.304	0.234	1.435	0.791	2.601
WBC	0.003	0.001	0.012	1.003	1.001	1.006
Hb	-0.002	0.006	0.737	0.998	0.987	1.009
Pt	0.001	0.003	0.774	1.001	0.995	1.006

HR, hazard ratio; CI, confidence interval; MLL, mixed lineage leukemia; TEL-AML or 4/10 positive; Ph, Philadelphia; WBC, white blood cell count; Hb, hemoglobin count; Pt, platelet count.

patients, the 3-year OS rate was 48.8 vs. 61.8 vs. 47.6% (HR=1.24; P=0.423) and the 3-year EFS rate was 35.8 vs. 53.7 vs. 40.0% (HR=1.29; P=0.131). Fig. 2 depicts the corresponding survival curves. Fig. 3 depicts the representative FACS results and morphological/histological images of B cell ALL, T cell ALL and atypical B-ALL patients. Patient 1 is a typical B-ALL patient. Immunologic cell markers of B-ALL show CD19, CD22, CD34 and HLA-DR are positive; CD3, CD7 and CD33 are negative. Wright's staining demonstrates the nuclei of B-ALL are regular and not indented or twisted. The lymphoblast has a high nuclear/cytoplasmic ratio. The POX staining is negative in all lymphoblasts of B-ALL. Patient 2 is a typical T-ALL patient. Immunologic cell markers of T-ALL indicate CD5, CD7, CD34 and CD38 are positive; CD19, CD20 and CD22 are negative. Wright's staining of T-ALL demonstrates that the cells are mostly larger than in B-ALL. There are more variations in cytologic features of the lymphoblast. The POX staining is negative in all lymphoblasts of T-ALL. Patient 3 is an atypical B-ALL patient accompanied by myelogenous markers. Immunologic cell markers of atypical ALL indicate CD10, CD19, CD22 and CD34 are positive; CD13 and CD33 are partially positive; CD3, CD7 and CD56 are negative. Wright's staining reveals that this cell type is larger than typical B-ALL. These lymphoblasts have also a high nuclear/cytoplasmic ratio. The POX staining is negative in almost all lymphoblasts.

Discussion

An estimated 6,000 new ALL cases (3,400 male and 2,600 female) are diagnosed annually in the USA (2). In China, this number is almost four times higher due to the larger population (18). ALL occurs in both children and adults but its incidence peaks between 2 and 5 years of age (3). The survival rate of childhood ALL is ~90%, but improvement is required for treatment in infants and adults (8).

ALL affects infants, children, adolescents, and adults. With current therapies, the vast majority of children with ALL are now long-term survivors (19). However, the same positive

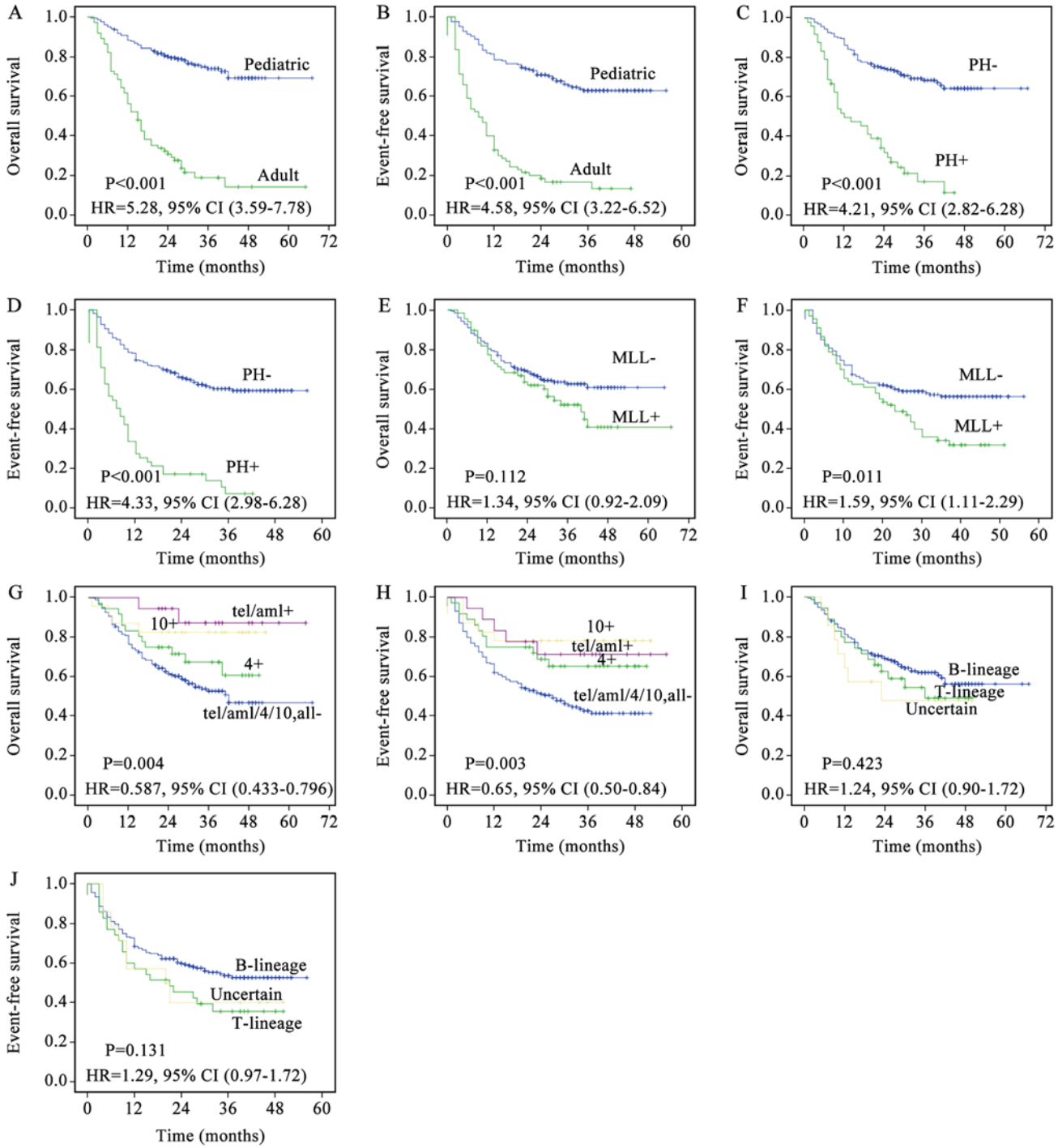

Figure 2. Kaplan-Meier survival curves of 277 patients with ALL. (A) OS of pediatric and adult ALL. (B) EFS of pediatric and adult ALL. (C) OS of Ph- and Ph+ ALL. (D) EFS of Ph- and Ph+ ALL. (E) OS of MLL- and MLL+ ALL. (F) EFS of MLL- and MLL+ ALL. (G) OS of patients negative for trisomy 4/10 and *TEL-AML*, and trisomy 4-positive, trisomy 10-positive, and *TEL-AML*-positive patients. (H) EFS of patients negative for trisomy 4/10 and *TEL-AML*, and trisomy 4-positive, trisomy 10-positive, and *TEL-AML*-positive patients. (I) OS of T-lineage, B-lineage, and uncertain-lineage patients. (J) EFS of T-lineage, B-lineage, and uncertain-lineage patients. P-values were calculated using the unadjusted log-rank test; HR were calculated using the unadjusted Cox proportional hazards model. ALL, acute lymphoblastic leukemia; OS, overall survival; EFS, event-free survival; Ph-, Philadelphia-negative; Ph+, Philadelphia-positive; MLL-, mixed lineage leukemia-negative; MLL+, mixed lineage leukemia-positive; TEL-AML1, translocation ETS leukemia-acute myeloid leukemia 1; HR, hazard ratio; CI, confidence interval.

results have not been reported for adults with ALL (11). The present study confirms these results.

The cause of the differing prognosis of ALL is multifactorial, and largely includes genomic alterations, exogenous or endogenous exposure to environmental toxins and chance. The inferior prognosis of adult ALL is not fully understood but could be attributed, in part, to genetic susceptibility as compared to pediatric ALL (20,21). Significant differences were also detected in immunophenotype, i.e., Ph chromosome, trisomy 4 and 10, and the *TEL-AML1* fusion gene, between pediatric and adult patients. The differences in Ph chromosome status may be a leading cause for the worse prognosis.

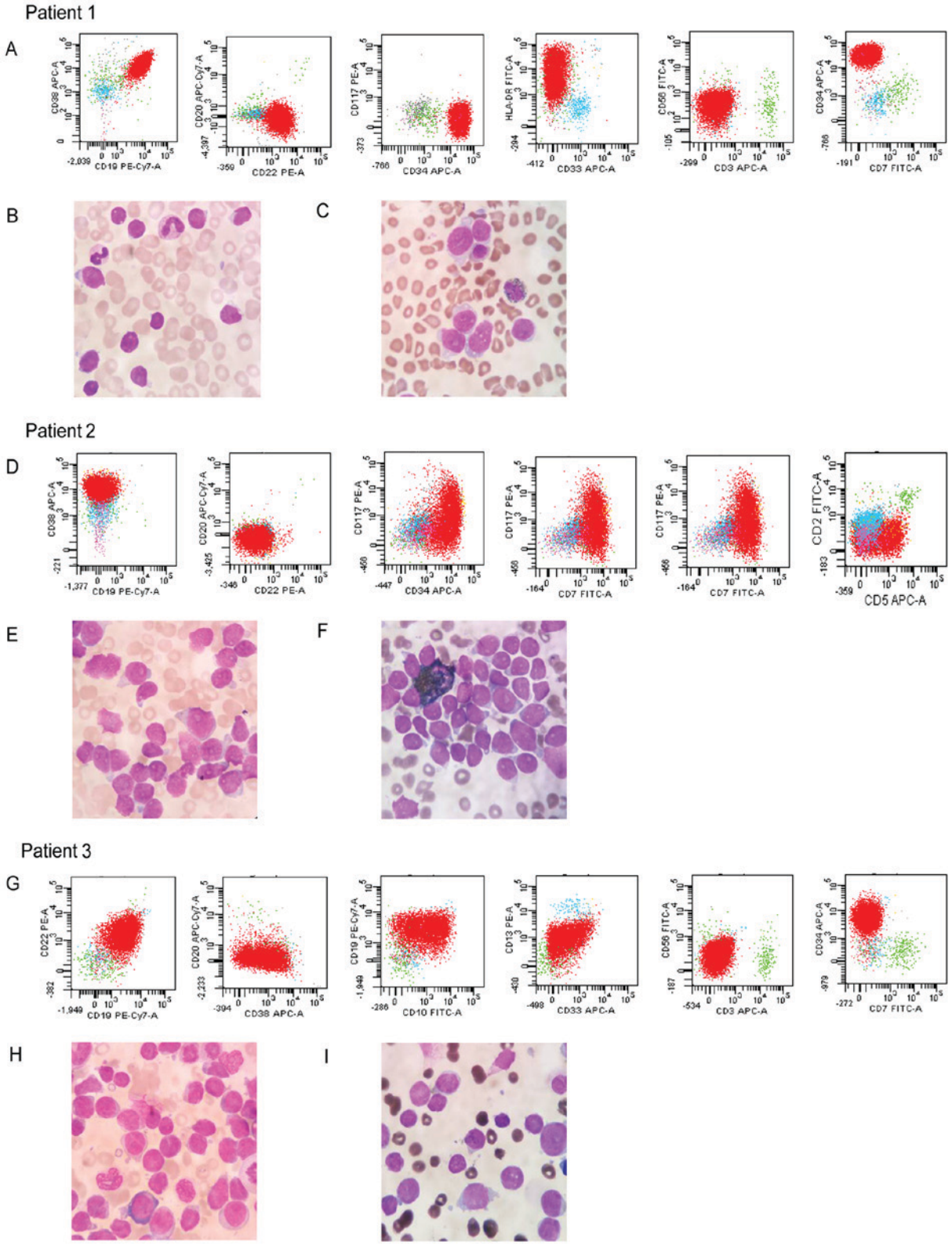

Figure 3. Representative flow cytometry results and morphological/histological images. Patient 1 (a B-ALL patient). (A) Immunologic cell markers of B-ALL (CD19, CD22, CD34 and HLA-DR are positive; CD3, CD7 and CD33 are negative). (B) Wright's staining of B-ALL. Original magnification, x1,000. The nuclei are regular and not indented or twisted. The lymphoblast has a high nuclear/cytoplasmic ratio. (C) POX staining of B-ALL. Original magnification, x1,000. The POX staining is negative in all lymphoblasts of B-ALL. Patient 2 (a T-ALL patient). (D) Immunologic cell markers of T-ALL (CD5, CD7, CD34 and CD38 are positive; CD19, CD20 and CD22 are negative). (E) Wright's Staining of T-ALL. Original magnification, x1,000. The cell type prevails are larger than B-ALL. There are more variations in cytologic feature of the lymphoblast. (F) POX staining of B-ALL. Original magnification, x1,000. The POX staining is negative in all lymphoblasts of this type of ALL. Patient 3 (an atypical B-ALL patient). (G) Immunologic cell markers of atypical ALL (CD10, CD19, CD22 and CD34 are positive; CD13 and CD33 are partially positive; CD3, CD7 and CD56 are negative). (H) Wright's Staining of B-ALL. Original magnification, x1,000. The cell type prevails are larger and the lymphoblast has also a high nuclear/cytoplasmic ratio. (I) POX staining of atypical B-ALL. Original magnification, x1,000. The peroxidase staining is negative in almost all lymphoblasts of this atypical B-ALL. ALL, acute lymphoblastic leukemia; B-ALL, B cell ALL; CD, cluster of differentiation; POX, peroxidase; T-ALL, T cell ALL; PE, phycoerythrin; Cy7, cyanine7; APC, allophycocyanin; HLA-DR, human leukocyte antigen-antigen D related; FITC, fluorescein isothiocyanate.

It was also demonstrated that the OS or EFS of Ph+ patients were lower than that of Ph- patients. The same conclusion can be drawn from the multivariate analysis, in that Ph+ is an independent poor prognostic factor in ALL overall.

FISH is one of the most sensitive molecular methods for detecting genetic abnormalities such as chromosome translocation and submicroscopic chromosomal abnormalities with specific DNA probes (22). Even in non-mitotic cells or when cytogenetic studies are insufficient, FISH may detect cryptic or rare chromosomal rearrangement (23). However, conventional cytogenetic analysis cannot accurately detect non-dividing (interphase) cells, which represent the most important fraction of bone marrow cells (16). Therefore, FISH is more sensitive and time-efficient than traditional chromosomal tests, which was also confirmed previously (24). The inferior prognosis in adult ALL is attributed, in part, to the higher rate of Ph+ detected by FISH in adult patients as compared with pediatric patients.

The present retrospective study was designed predominantly to elucidate the relevance of the prognosis in pediatric and adult ALL and to define why the prognosis of these two groups differs, and to clarify whether it can be explained through the differing biological features detected by FISH. Significant differences were identified between the biological features and prognostic associations in adult and pediatric patients with ALL.

According to the present findings, the incidence of ALL decreases with age. A previous study of pediatric and adult patients with ALL identified no significant differences between sex, race/ethnic group and mean presenting WBC count (1).

Studies (25,26) have indicated that Ph+ ALL presents a dismal prognosis, representing an independent prognostic factor not only in pediatric patients, but also in adult patients. In the majority of patients with chronic myeloid leukemia, the *ABL* gene moves from chromosome 9 to the major breakpoint cluster region on chromosome 22. This translocation results in a 210-kDa fusion protein (p210). However, the *ABL1* gene can also translocate to the minor breakpoint cluster region on chromosome 22, resulting in a 190-kDa fusion protein (p190) that occurs exclusively in ALL (27).

Ph+ ALL is characterized by poor response to the majority of chemotherapy combinations, short remission durations and poor survival rates (28). The findings suggested that the *BCR-ABL* fusion gene is an independent unfavorable prognostic factor for adult patients with ALL. Nevertheless, the development of allogeneic bone marrow transplantation and specific tyrosine kinase inhibitors for Ph+ ALL has potentially changed this (28).

In ALL, acute myeloid leukemia and therapy-associated leukemia, the *MLL* gene is rearranged with >70 partner genes and is located on the long arm of chromosome 11 (29). Reverse transcriptase-polymerase chain reaction previously revealed that this translocation was present in not only 40-50% of infants, but also in 2-3% of children and ~10% of adults with ALL (25). In the present study, 24.2% of pediatric patients and 16.0% of adult patients with ALL had *MLL* translocation, suggesting the high incidence rate of this location in China. Furthermore, the follow-up results indicate that the *MLL* gene is not associated with the poor prognosis of adult ALL, but that it is associated with poor prognosis of pediatric ALL.

The *TEL-AML1* fusion gene, generated by the t(12;21) (p13;q22) chromosomal translocation, occurs in ~25% of cases of B cell precursor ALL. It is one of the most common forms of acute leukemia in children (30). The *TEL* gene is an important regulator in hematopoietic cell development, and the *AML1* gene serves an important role in definitive embryonic hematopoiesis (25). The presence of the TEL-AML1 fusion protein in B-cell progenitors seems to be a hallmark of leukemic lymphoblasts, and leads to disordered early B-lineage lymphocyte development (26). The present findings indicated that the frequency of *TEL-AML1* fusion was much higher in children than in adults, and is a favorable prognostic factor in patients with ALL. The EFS in *TEL-AML1*-positive patients was markedly longer than that of *TEL-AML1*-negative patients. A previous Pediatric Oncology Group study (31) revealed that trisomy 4 and 10 are strongly indicative of favorable prognosis, particularly in standard-risk B-precursor ALL. Although a number of genetic abnormalities are associated with clinical outcome, only a few are routinely used for treatment stratification (32,33). In the present study, pediatric patients with combined chromosome 4 and 10 trisomies appeared to have more prognostically favorable clinical features.

A limitation of the present study is that more sophisticated techniques were not used for comparing the disadvantages of FISH. Using second-generation sequencing technology, Zhao *et al* (34) previously revealed that 2,825 genes were upregulated and 1,952 were downregulated in the ALL group compared with the normal control group. Based on the digital gene expression profiling data, they investigated a further seven genes (WT1, RPS26, MSX1, CD70, HOXC4, HOXA5, OXC6) predominantly associated with immune cell differentiation, metabolic processes and programmed cell death. Although FISH is widely used in diagnosis and prognosis prediction of hematological malignancies, minimal residual disease (MRD) diagnostics has proven to be the strongest prognostic factor that may be used to guide treatment decisions. MRD techniques are required to be sensitive, accurate, reliable and fast. Recently developed high-throughput sequencing and next-generation (multidimensional) flow cytometry have been demonstrated to have greater potential means (35).

In conclusion, adult ALL has poorer prognosis than pediatric ALL. Ph+ status is associated with the high-risk features of increased age and is frequently observed and associated with unfavorable prognosis. Trisomies 4 and 10 are also associated with favorable prognosis but are not independent prognostic factors of ALL. Ph+ ALL is an independent prognostic factor of ALL that is frequently present in patients.

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Availability of data and materials

All data generated or analyzed during this study are included in this published. No any other data available for supplementary materials.

Authors' contributions

The study presented here was performed as a collaboration between all authors. PC, YY, WW and HX performed the majority of the experiments. YH and PC made contributions to the design, data analysis and interpretation and drafting of the manuscript. PC, YY and WW collected and assembled the data. All authors gave their final approval for publication of the manuscript.

Ethics approval and consent to participate

The Xiangya Hospital Ethics Committee approved the present study (approval no. 201212478).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Pui CH, Robison LL and Look AT: Acute lymphoblastic leukaemia. Lancet 371: 1030-1043, 2008.
2. Siegel R, Naishadham D and Jemal A: Cancer statistics, 2012. CA Cancer J Clin 62: 10-29, 2012.
3. Inaba H, Greaves M and Mullighan CG: Acute lymphoblastic leukaemia. Lancet 381: 1943-1955, 2013.
4. Lafage-Pochitaloff M, Baranger L, Hunault M, Cuccuini W, Lefebvre C, Bidet A, Tigaud I, Eclache V, Delabesse E, Bilhou-Nabéra C, *et al*: Impact of cytogenetic abnormalities in adults with Ph-negative B-cell precursor acute lymphoblastic leukemia. Blood 130: 1832-1844, 2017.
5. Pui CH, Crist WM and Look AT: Biology and clinical significance of cytogenetic abnormalities in childhood acute lymphoblastic leukemia. Blood 76: 1449-1463, 1990.
6. Faderl S, O'Brien S, Pui CH, Stock W, Wetzler M, Hoelzer D and Kantarjian HM: Adult acute lymphoblastic leukemia: Concepts and strategies. Cancer 116: 1165-1176, 2010.
7. Aricò M, Schrappe M, Hunger SP, Carroll WL, Conter V, Galimberti S, Manabe A, Saha V, Baruchel A, Vettenranta K, *et al*: Clinical outcome of children with newly diagnosed philadelphia chromosome-positive acute lymphoblastic leukemia treated between 1995 and 2005. J Clin Oncol 28: 4755-4761, 2010.
8. Bassan R and Hoelzer D: Modern therapy of acute lymphoblastic leukemia. J Clin Oncol 29: 532-543, 2011.
9. Shen S, Cai J, Chen J, Xue H, Pan C, Gao Y, Tang Y, Wang J, Li B, Wang X, *et al*: Long-term results of the risk-stratified treatment of childhood acute lymphoblastic leukemia in China. Hematol Oncol, 2018 (Epub ahead of print).
10. Möricke A, Reiter A, Zimmermann M, Gadner H, Stanulla M, Dördelmann M, Löning L, Beier R, Ludwig WD, Ratei R, *et al*: Risk-adjusted therapy of acute lymphoblastic leukemia can decrease treatment burden and improve survival: Treatment results of 2169 unselected pediatric and adolescent patients enrolled in the trial ALL-BFM 95. Blood 111: 4477-4489, 2008.
11. Gökbuget N, Kneba M, Raff T, Trautmann H, Bartram CR, Arnold R, Fietkau R, Freund M, Ganser A, Ludwig WD, *et al*: Adult patients with acute lymphoblastic leukemia and molecular failure display a poor prognosis and are candidates for stem cell transplantation and targeted therapies. Blood 120: 1868-1876, 2012.
12. Sive JI, Buck G, Fielding A, Lazarus HM, Litzow MR, Luger S, Marks DI, McMillan A, Moorman AV, Richards SM, *et al*: Outcomes in older adults with acute lymphoblastic leukaemia (ALL): Results from the international MRC UKALL XII/ECOG2993 trial. Br J Haematol 157: 463-471, 2012.
13. Hoelzer D, Walewski J, Dohner H, Viardot A, Hiddemann W, Spiekermann K, Serve H, Dührsen U, Hüttmann A, Thiel E, *et al*: Improved outcome of adult Burkitt lymphoma/leukemia with rituximab and chemotherapy: Report of a large prospective multicenter trial. Blood 124: 3870-3879, 2014.
14. Thomas DA, O'Brien S, Faderl S, Garcia-Manero G, Ferrajoli A, Wierda W, Ravandi F, Verstovsek S, Jorgensen JL, Bueso-Ramos C, *et al*: Chemoimmunotherapy with a modified hyper-CVAD and rituximab regimen improves outcome in de novo Philadelphia chromosome-negative precursor B-lineage acute lymphoblastic leukemia. J Clin Oncol 28: 3880-3889, 2010.
15. Hoelzer D, Bassan R, Dombret H, Fielding A, Ribera JM and Buske C; ESMO Guidelines Committee: Acute lymphoblastic leukaemia in adult patients: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol 27 (Suppl 5): v69-v82, 2016.
16. Chiaretti S, Zini G and Bassan R: Diagnosis and subclassification of acute lymphoblastic leukemia. Mediterr J Hematol Infect Dis 6: e2014073, 2014.
17. Brown PA, Shah B, Fathi A, Wieduwilt M, Advani A, Aoun P, Barta SK, Boyer MW, Bryan T, Burke PW, *et al*: NCCN guidelines insights: Acute lymphoblastic leukemia, version 1.2017. J Natl Compr Canc Netw 15: 1091-1102, 2017.
18. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ and He J: Cancer statistics in China, 2015. CA Cancer J Clin 66: 115-132, 2016.
19. Hunger SP and Mullighan CG: Acute lymphoblastic leukemia in children. N Engl J Med 373: 1541-1552, 2015.
20. Szczepański T, Harrison CJ and van Dongen JJ: Genetic aberrations in paediatric acute leukaemias and implications for management of patients. Lancet Oncol 11: 880-889, 2010.
21. Chilton L, Buck G, Harrison C J, Ketterling RP, Rowe JM, Tallman MS, Goldstone AH, Fielding AK and Moorman AV: High hyperdiploidy among adolescents and adults with acute lymphoblastic leukaemia (ALL): Cytogenetic features, clinical characteristics and outcome. Leukemia 28: 1511-1518, 2014.
22. Mazloumi SH, Madhumathi DS, Appaji L and Prasannakumari: Combined study of cytogenetics and fluorescence *in situ* hybridization (FISH) analysis in childhood acute lymphoblastic leukemia (ALL) in a tertiary cancer centre in South India. Asian Pac J Cancer Prev 13: 3825-3827, 2012.
23. Moorman AV: The clinical relevance of chromosomal and genomic abnormalities in B-cell precursor acute lymphoblastic leukaemia. Blood Rev 26: 123-135, 2012.
24. Cao P, Li Y, Li X, Zhang G and Chen F: Detecting chromosomal aberrations in myelodysplastic syndrome with fluorescence in situ hybridization and conventional cytogenetic analysis. Zhong Nan Da Xue Xue Bao Yi Xue Ban 39: 605-611, 2014 (In Chinese).
25. Moorman AV, Harrison CJ, Buck GA, Richards SM, Secker-Walker LM, Martineau M, Vance GH, Cherry AM, Higgins RR, Fielding AK, *et al*: Karyotype is an independent prognostic factor in adult acute lymphoblastic leukemia (ALL): Analysis of cytogenetic data from patients treated on the Medical Research Council (MRC) UKALLXII/Eastern cooperative oncology group (ECOG) 2993 trial. Blood 109: 3189-3197, 2007.
26. Landau H and Lamanna N: Clinical manifestations and treatment of newly diagnosed acute lymphoblastic leukemia in adults. Curr Hematol Malig Rep 1: 171-179, 2006.
27. Druker BJ, Sawyers CL, Kantarjian H, Resta DJ, Reese SF, Ford JM, Capdeville R and Talpaz M: Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. N Engl J Med 344: 1038-1042, 2001.
28. Schrappe M, Hunger SP, Pui CH, Saha V, Gaynon PS, Baruchel A, Conter V, Otten J, Ohara A, Versluys AB, *et al*: Outcomes after induction failure in childhood acute lymphoblastic leukemia. N Engl J Med 366: 1371-1381, 2012.
29. Collins EC and Rabbitts TH: The promiscuous MLL gene links chromosomal translocations to cellular differentiation and tumour tropism. Trends Mol Med 8: 436-442, 2002.
30. Cayuela JM, Baruchel A, Orange C, Madani A, Auclerc MF, Daniel MT, Schaison G and Sigaux F: TEL-AML1 fusion RNA as a new target to detect minimal residual disease in pediatric B-cell precursor acute lymphoblastic leukemia. Blood 88: 302-308, 1996.

31. Sutcliffe MJ, Shuster JJ, Sather HN, Camitta BM, Pullen J, Schultz KR, Borowitz MJ, Gaynon PS, Carroll AJ and Heerema NA: High concordance from independent studies by the Children's Cancer Group (CCG) and Pediatric Oncology Group (POG) associating favorable prognosis with combined trisomies 4, 10, and 17 in children with NCI Standard-Risk B-precursor Acute Lymphoblastic Leukemia: A Children's Oncology Group (COG) initiative. Leukemia 19: 734-740, 2005.
32. Yeoh EJ, Ross ME, Shurtleff SA, Williams WK, Patel D, Mahfouz R, Behm FG, Raimondi SC, Relling MV, Patel A, *et al*: Classification, subtype discovery, and prediction of outcome in pediatric acute lymphoblastic leukemia by gene expression profiling. Cancer Cell 1: 133-143, 2002.
33. Conter V, Bartram CR, Valsecchi MG, Schrauder A, Panzer-Grümayer R, Möricke A, Aricò M, Zimmermann M, Mann G, De Rossi G, *et al*: Molecular response to treatment redefines all prognostic factors in children and adolescents with B-cell precursor acute lymphoblastic leukemia: Results in 3184 patients of the AIEOP-BFM ALL 2000 study. Blood 115: 3206-3214, 2010.
34. Zhao MY, Yu Y, Xie M, Yang MH, Zhu S, Yang LC, Kang R, Tang DL, Zhao LL and Cao LZ: Digital gene expression profiling analysis of childhood acute lymphoblastic leukemia. Mol Med Rep 13: 4321-4328, 2016.
35. van Dongen JJ, van der Velden VH, Brüggemann M and Orfao A: Minimal residual disease diagnostics in acute lymphoblastic leukemia: Need for sensitive, fast, and standardized technologies. Blood 125: 3996-4009, 2015.