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ORIGINAL ARTICLE

Status of *IKZF1* Deletions in Diagnose and Relapsed Pediatric B-ALL Patients

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Abstract

IKZF1 deletions (*ΔIKZF1*) are common in precursor B-cell acute lymphoblastic leukemia (B-ALL) and are assumed to have a prognostic impact. We aimed to determine the prognostic implications of *ΔIKZF1* and *CRLF2* overexpression in pediatric B-ALL. Furthermore, we sought to compare the multiplex polymerase chain reaction (PCR) assay with standard multiplex ligand-dependent probe amplification (MLPA) methods to ascertain *IKZF1* status in a clinical context. Seventy-nine diagnoses and 43 relapse B-ALL samples were evaluated for deletions of *IKZF1* Δ2-7, Δ4-7, and Δ4-8 by conventional PCR and then sequenced by targeted sequencing. Subsequently, MLPA analysis was performed for *ΔIKZF1* detection, and *CRLF2* expression was evaluated in 42 diagnose time B-ALL patients by QRT-PCR. *ΔIKZF1* was detected in 10 out of 79 diagnose samples (12.66%) and eight of the 43 first relapsed materials (18.60%). Our results revealed no association between survival outcomes with *ΔIKZF1* or *CRLF2* overexpression status in pediatric B-ALL patients. However, we found Δ*IKZF1* was more frequent among relapsed samples, and the deletions showed consistency between diagnose-first/second relapse pairs of samples. These results suggest that *ΔIKZF1* may contribute to the development of treatment failure in B-ALL. Furthermore, we demonstrated methodological adjustments in conventional PCR and MLPA for selected alterations in *ΔIKZF1*.

Keywords IKZF1 · CRLF2 · B-ALL · Prognosis

Abbreviations

B-ALL	B-cell acute lymphoblastic leukemia
PAX5	*Paired Box 5*
IKZF1	*IKAROS*

Extended author information available on the last page of the article

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EBF1	*Early B-Cell Factor 1*
CDKN2A/B	*Cyclin-Dependent Kinase Inhibitor 2A/*B
PAR1	*Pseudoautosomal Region 1 of the sex chromosomes*
CRLF2	*Cytokine Receptor-Like Factor 2*
BFM	The Berlin–Frankfurt–Munster regimen
EGIL	European Group for the Immunological Characterization of Leukemias
MLPA	Multiple ligation-dependent probe amplification
CNVs	Copy number variations
EFS	Event-free survival
OS	Overall survival

Introduction

Precursor B-cell acute lymphoblastic leukemia (B-ALL) is the most common childhood cancer. Although five-year survival rates exceed 90% in childhood ALL, long-term side effects of chemotherapy and poor treatment response and survival rate after relapse are among the major problems of ALL (Inaba and Mullighan 2020; Nguyen et al. 2008). Gene expression differences, genomic variations, fusion transcripts, and copy-number alterations provide a basis to define the B-ALL subtype with clinical relevance. Genetic alterations in lymphoid transcription factor genes such as *PAX5*, *IKZF1*, and *EBF1* were reported in most of B-ALL patients (Mullighan et al. 2007).

The *IKZF1* gene encodes a zinc finger DNA-binding protein which promotes differentiation, inhibits proliferation, and modulates the expression of early B-cell-specific genes. Gene deletions (*ΔIKZF1)* and less commonly missense variations in *IKZF1* can cause loss of function and cellular mis-localization of the protein (Conserva et al. 2023; Olsson and Johansson 2015). Patients with *IKZF1* alterations show differential expression profiles for DNA repair genes, self-renewal, and JAK-STAT signaling pathways (Mullighan et al. 2009).

ΔIKZF1 heterogeneously distributed within the ALL subgroups and has been reported in 75% of *BCR::ABL1*-positive ALL, 12–15% of childhood, and 30–50% of adult B-ALL (Mullighan et al. 2007). Several studies sought to analyze the clinical importance of *IKZF1* aberration and *ΔIKZF1* have been correlated with poor prognosis in childhood B-ALL (Clappier et al. 2015; Kuiper et al. 2010; Moorman et al. 2014; Mullighan et al. 2009; Sutton et al. 2018; Yang et al. 2011; Ayón-Pérez et al. 2019). Stanulla et al. defined a prognostic profile termed *IKZF1*^{plus} group which was characterized with *ΔIKZF1* that co-occurred with at least one additional deletion in *CDKN2A/B*, *PAX5*, and/or *PAR1* in the absence of *ERG* deletion. The *IKZF-1*^{plus} group was suggested as a minimal residual disease (MRD) dependent very poor prognostic profile in B-ALL (Churchman et al. 2018; Zhang et al. 2016).

CRLF2 rearrangements or variations were detected 7% of B-ALL (Russell et al. 2009). *P2RY8::CRLF2* fusions which is caused by illegitimate V(D)J-mediated recombination and *IGH::CRLF2* translocations result in over expression of *CRLF2*. *P2RY8::CRLF2* fusions often carry additional alterations in JAK/STAT pathway

genes. So, it would not be surprising that abnormal kinase signaling accompanied by aberrant expression of *IKZF1* has been associated with increased relapse risk and treatment failure.

Several biomarkers correlated with poor therapy response in ALL. However, for detecting all these markers, combined molecular tools and relatively complex bioinformatic analysis are needed to be performed. Unfortunately, applying all these tests in routine diagnosis is not feasible, especially for developing and non-developed countries. Through this perspective, we aimed to assess the frequency and the prognostic impact of *ΔIKZF1* and *CRLF2* expression in childhood B-ALL.

Materials and Methods

Patients and Treatment

The patient's cohort comprised 87 diagnose and 43 first and 2 subsequent second relapse samples of childhood B-ALL (16 paired diagnostic-relapse samples) who were treated according to BFM (the Berlin–Frankfurt–Munster regimen) or relapse BFM protocols between 2000 and 2012. Bone marrow materials were available for 85 diagnose and 45 relapse samples, two diagnose patient's peripheral blood samples with > 90% blast were included in the study. Immunophenotypic characterization of samples was done by flow cytometry based on the European Group for the Immunological Characterization of Leukemias (EGIL) criteria (Bene et al. 1995).

In AIEOP-BFM ALL 2000, patients were stratified into three risk groups (Vora et al. 2014). Patients with prednisone poor response, ≥ 5% leukemic blasts in the bone marrow on day 33, or positivity for t(4;11) or t(9;22) were stratified into the high-risk (HR) group. Patients who have no HR criteria and initial WBC count ≥ 20 000/μL and/or age at diagnosis ≥ 6 years were classified into intermediate-risk (MR) group. Patients who have no HR criteria and initial WBC count < 20 000/μL, and age at diagnosis between 1–6 years and no T-ALL were stratified into standard-risk (SR) group. The diagnostic cohort included HR ($n = 12$), MR ($n = 30$), SR ($n = 20$) groups, and unclassified patients ($n = 25$, risk group classifications were not available). The median blast percentage at the time of diagnosis was 95% (range, 30–100%) and median follow-up time was 52 months (range, 1- 258 months).

More than 25% blast in peripheral blood or ≥ 5% blast in bone marrow aspirate with CNS and/ or testicular involvement at any point after remission was defined as relapse. The first relapse cohort was compromised 32 early relapsed (< 36 months) and 11 late relapsed (≥ 36 months). The patient's clinical data were summarized in Supplement Table 1.

Multiple Ligation-Dependent Probe Amplification (MLPA) Analysis

The total DNA was isolated from diagnose ($n = 79$) and relapsed ($n = 43$) patients' bone marrow or peripheral blood samples using QIAamp DNA Blood Midi Kit (Qiagen). Multiple ligation-dependent probe amplification (MLPA) analysis was

performed using the MLPA SALSA kit P335 (Bedics et al. 2023). The assay covers the whole *IKZF1* exons. For MLPA experiment, 100 ng of DNA was incubated with the SALSA probe mix at 60 °C for 17 h. Following this hybridization step, ligation was conducted using Ligase-65 enzyme at 54 °C for 15 min. The ligase reaction was then terminated by heating the samples to 98 °C for 5 min to inactivate the enzyme. For the amplification, the samples were subjected to 35 cycles of PCR using SALSA primers and polymerase under the following thermal cycling conditions: initial denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s, with a final extension at 72 °C for 20 s. The fragments were analyzed using Coffalyser v9.4 software. Copy number alterations (CNVs) of *EBF1*, *CDKN2A/B*, *PAX5*, *ETV6*, *BTG1*, *RB1*, and the *PAR1* region in a group of our cohort were published in the previous paper combining with *JAK2*, *CRLF2*, *IL7R,* and *PAX5* variations (Erbilgin et al. 2019).

Genomic PCR for Δ2–7, Δ4–7, and Δ4–8 and Deep Sequencing

IKZF1 deep sequencing plates were designed by IRON-II study consortium and covered Δ2–7, Δ4–7, and Δ4–8 variants (Kohlmann et al. 2012). Twenty ng DNA were amplified in a final volume of 25 µL containing 1 × buffer (FastStart High Fidelity Reaction Buffer, Roche, Penzberg, Germany), 1.25-µl DMSO, 4.5-mM $MgCl_2$, 0.2-mM dNTP, 0.4 µM of each primer, and 0.2U FastStart High Fidelity Enzyme (Roche, Penzberg, Germany). The cycling protocol was a denaturation step at 95 °C for 10 min followed by 30 s at 94 °C, 30 s at 57 °C, 2 min at 72 °C for 35 cycles, and a final extension phase of 10 min at 72 °C. The primers located for deletion break point for exon 2: Forward 5′-AAGTGACCCATCCTTTGAA-3′, for exon 7 Reverse: %2-TGAATAGACCAAGATACATCCT-3′, for exon 4: Forward 5′-GAAGTCTGG AGTCTGTGAAG-3′, and exon 8 Reverse: 5′-GAGTGTGGAGCTAGGAGAT-3′. PCR products were loaded to 2% agarose gel and amplified samples were sequenced using GS Junior 454 (Roche Applied Science, Penzberg, Germany). To check the DNA quality, *PAX5* gene was used as a reference gene. Amplicon sequences were analyzed using the SEQ powered by Genomize software (https://seq.genomize.com). The minimum read depth cut-off was set as 500 reads (for both strands).

CRLF2 Expression Analysis

Forty-two diagnostic and six relapse time patient materials were available for RNA isolation. RNA isolation was performed by RNeasy Mini Kit (Qiagen) and RNA quality was evaluated with spectrophotometry (NanoDrop 2000c Technologies, USA). A total of 1 µg of total RNA from diagnostic leukemic cells were converted to cDNA using MMLV-RT and random hexamers. *CRLF2* and *Cyclophilin A* (internal control) transcript levels were measured by qRT-PCR using the Universal Probe Library using LC480 Roche Real-Time PCR System (Roche) with the following protocol: initial step at 95 °C for 10 min, then 45 cycles at 95 °C for 10 s and at 60 °C for 20 s and at 72 °C for 1 s, and final cooling at 40 °C for 10 s. Each cDNA sample (20 ng RNA equivalent) was run duplicate, and amplification reaction performed in

a final volume of 15 µl, containing 1xLC480 Hybr Prob Master Mix (Roche), 200-nM UPL probe, and 400-nM primer from each one. Relative gene expression was quantified by delta Ct method (Schmittgen and Livak 2008).

Statistical Analysis

Event-free survival (EFS) was calculated from the date of remission to any event (relapse, death, or second malignant condition). Overall survival (OS) was calculated from the date of diagnosis to the date of death or last follow-up.

Categorical variables were compared by the Chi-Square test, Fisher's exact test, and Fisher Freeman Halton test. Kaplan–Meier test was used for survival time and survival curves were compared by the Log-Rank test. Analyses were performed using SPSS (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp) software.

Results

IKZF1 Deletions in Childhood B-ALL

Overall, *ΔIKZF1* was detected in 10 out of 79 (12.66%) diagnose samples and eight of the 43 (18.60%) first relapsed materials (Fig. 1). Two patients harbored more than one *ΔIKZF1*. Among the deletions, Δ4–7 was the most frequent both at diagnose (50% of deletions, $n=5$) and first relapse time (75% of deletions, $n=6$).

In total, 15 samples showed *ΔIKZF1* by genomic PCR and sequenced by deep sequencing. The predicted average coverage per amplicon was 2165 reads (min 688, max 4291) and a threshold was set as > 1% of variant detection in both strands. We did not find any additional point variations in samples that had Δ2–7, Δ4–7, or

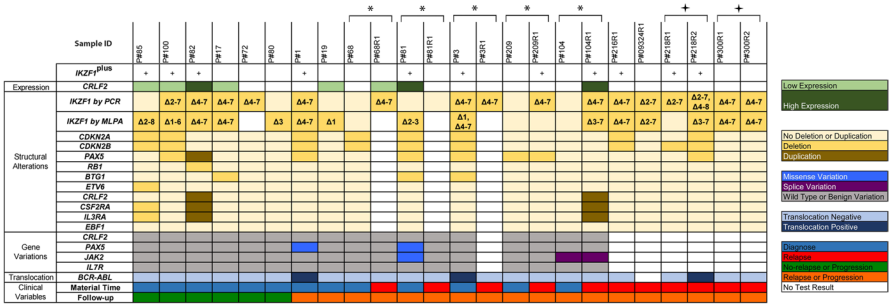


Fig. 1 Overview of *IKZF1* deletions and other genetic alterations identified in B-ALL patients. Single-nucleotide variations, CNVs for 16 most altered genes, *BCR::ABL* translocation and *CRLF2* expression are presented. The patients who were classified in *IKZF*^{plus} group was indicated in first line with "+." The patient cohort is annotated according to the material time and follow-up. Each column corresponds to one sample. R1 means first relapse, R2 means second relapse material. *Matched diagnose relapse; four-point star indicates first–second relapse samples of the patient

Δ4–8. The breakage site was varied between the samples, but each sample had a monoclonal pattern for the deletion point.

Deletions that were detected by genomic PCR were confirmed by MLPA analyses. However, we observed discordance between MLPA and PCR results in three relapse samples. A relapse sample P#104R was reported as Δ4–7 by PCR, and Δ3–7 by MLPA (Fig. 1 and Supplement Fig. 1). We detected Δ2–7 only by PCR in P#218R1. P#218R1 is a first relapse sample and matched second relapse sample (P#218R2) were found to carry Δ2–7 and Δ4–8 by PCR and Δ3–7 by MLPA (Fig. 1 and Supplement Fig. 2). In addition to genomic PCR results, rare deletions Δ1, Δ1–6, Δ2–8, Δ2–3, and Δ3 were also determined in six independent cases by MLPA.

We did not know the *ERG* deletion status of the patients, but we could stratify the samples as *IKZF1*^{plus} according to co-occurrences of *CDKN2A*, *CDKN2B, PAX5*, or *PAR1* variations together with *ΔIKZF1*. Accordingly, six patients were classified as *IKZF1*^{plus} at diagnosis and four patients at relapse time (Fig. 1).

Five patients of 16 paired diagnose-relapse samples harbored *ΔIKZF1*. Three of the patients (P#68, P#209, P#104) were wild type for *ΔIKZF1* at diagnosis but gained Δ4–7 at relapse. P#68 and P#104 developed early relapse and P#209 had late relapse. P#3 developed early relapse and carried Δ4–7 both in diagnosis and relapse time. Two patients matched first and second relapses were available for the analysis, first relapse sample P#218R1 had Δ2–7 and matched second relapse P#218R2 harbored additional Δ4–8 deletion (Fig. 1). Another matched first and second relapse samples, P#300R1 and P#300R2 both accumulated Δ4–7 deletion.

CRLF2 Expression in B-ALL

The diagnose and relapse samples of B-ALL patients were evaluated for *CRLF2* overexpression. We set the *CRLF2* high expression threshold as described in *Chen* et al. paper (Cario et al. 2010; Chen et al. 2012; Yano et al. 2014). To define the *CRLF2*^{high} expression group, the lowest ΔCt values for *CRLF2* expression (ΔCt = 9.59) in a patient with *CRLF2* genomic lesions were determined as a threshold. The patients with ΔCt ≤ 9.59 values were classified as *CRLF2*^{high} (Fig. 2). Accordingly, *CRLF2* overexpression was detected in 30.95% ($n = 13$) of the diagnose samples.

Clinical Characteristics According to *IKZF1* Status and CRLF2 Expression

Comparing leukemic and clinical characteristics of diagnose and relapsed patients with and without *ΔIKZF1*, no significant differences ($p > 0.05$) were observed concerning clinical variables (Table 1).

Diagnose patients' OS mean was 200.31 ± 11.88 months and EFS survival mean was 134.73 ± 8.07 months. Relapsed patients' OS mean was 126.98 ± 25.65 months. The survival analysis, *ΔIKZF1* were not found associated with inferior OS rate ($p = 0.169$) or inferior EFS rate ($p = 0.331$) for diagnose samples (Supplement Fig. 3). Furthermore, we evaluated *IKZF1*^{plus} group for the survival rate and there was a non-significant trend toward OS and EFS for patients with *IKZF1*^{plus} (OS;

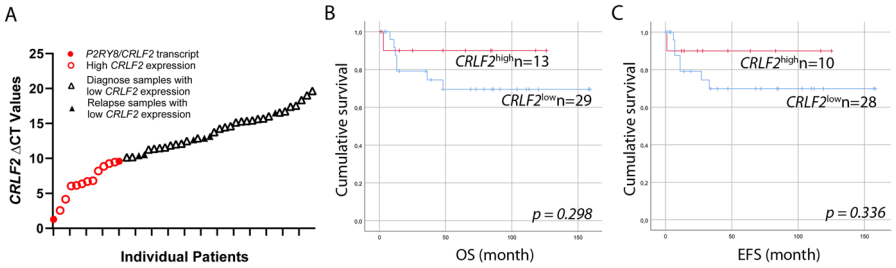


Fig. 2 *CRLF2* expression and survival analysis. **A** *CRLF2* expressions were evaluated in 42 diagnose and six relapse time samples. The patients with $\Delta Ct \leq 9.59$ values were classified as high *CRLF2* expressed group and represented in red ($n = 13$). **B** Overall survival according to *CRLF2* expression. **C** Event-free survival according to *CRLF2* expression

$p = 0.294$, EFS; $p = 0.452$). Similarly, in relapse cohort, we did not observe survival rate differences for *ΔIKZF1* alone (OS; $p = 0.655$, EFS; $p = 0.331$).

There were no significant associations between *CRLF2*^{high} and other clinical variables (Supplement Table 2). Diagnose *CRLF2*^{high} B-ALL patients did not show any differences for EFS and OS compared to the *CRLF2*^{low} cases (EFS; $p = 0.336$, OS; $p = 0.298$). Similarly, Kaplan–Meier survival analysis did not reveal any significant differences in EFS and OS in relapsed B-ALL patients with *CRLF2* high and low expression (EFS; $p = 0.795$, OS; $p = 0.792$) (Fig. 2).

Discussion

We investigated the incidence and prognostic impact of *ΔIKZF1* and *CRLF2* overexpression in diagnose and relapsed pediatric B-ALL patients. 13% of diagnose patients and 19% of first relapse B-ALL samples were carried *ΔIKZF1*. These results are consistent with previous reports that detected the overall frequency range of *ΔIKZF1* as 4–15% at diagnose time in childhood ALL (Clappier et al. 2015; Dörge et al. 2013; Hamadeh et al. 2019; Kicinski et al. 2023; Ma et al. 2015). *IKZF1* deletions results in loss of IKAROS activity and increased hematopoietic stem-cell like phenotype. Both common and rare *IKZF1* alterations were detected in our cohort. Total deletion of *IKZF1* and Δ4–7 were reported in about 60% of childhood B-ALL patients and Δ2–3, Δ2–7, and Δ4–8 were detected less frequently (Clappier et al. 2015). Similarly, Δ4–7 was the most common alteration both in our diagnose and relapse groups which results in expression of the IK6 isoform (Mullighan et al. 2008, 2009; Olsson and Johansson 2015). This isoform is nonfunctional and leads cytoplasmic localization of IKAROS and shows dominant negative effects in transcription. Besides that, *IKZF1* deletions Δ1–6, Δ2–3, Δ2–7, Δ2–8, and Δ4–8 cause reduced levels of IKAROS and haploinsufficiency (Sun et al. 1999a, b, c; Sun et al. 1999a, b, c; Sun et al. 1999a, b, c). Rare variants that cover exon 1 deletion (in our cohort, Δ1 and Δ1–6) were associated with the loss of *IKZF1* promoter. Besides that, rare *IKZF1* deletion variants may impact the patient outcome and detection of these alterations can contribute to risk assessment of the patients.

Table 1 Diagnose and relapse patient's characteristics and outcomes according to *IKZF1* status

Feature	Diagnose patients			Relapse patients		
	IKZF1 not deleted	Δ*IKZF1*	*p* value	*IKZF1* not deleted	Δ*IKZF1*	*p* value
	n (%)	*n* (%)		*n* (%)	*n* (%)	
Number of samples	69 (87.34)	10 (12.66)		35 (81.40)	8 (18.60)	
Sex			0.738[a]			0.665[a]
Male	43 (62.31)	7 (70.00)		26 (74.29)	5 (62.50)	
Female	26 (37.69)	3 (30.00)		9 (25.71)	3 (37.50)	
A*ge at diagnosis or relapse (years)*			0.173[a]			0.445[a]
1–9	60 (86.96)	7 (70.00)		19 (55.88)	3 (37.50)	
$<1, \geq 10$	9 (13.04)	3 (30.00)		15 (44.12)	5 (62.50)	
Presenting WBC (count/μl)			0.120[a]			0.116[a]
$<50{,}000$	53 (76.81)	5 (50.00)		25 (86.21)	4 (57.14)	
$\geq 50{,}000$	16 (23.19)	5 (50.00)		4 (13.79)	3 (42.86)	
Hemoglobin			0.456[b]			1.000[b]
≤ 10	22 (31.88)	4 (40.00)		3 (8.56)	0 (0.00)	
>10	8 (11.59)	2 (20.00)		1 (2.86)	0 (0.00)	
NA	39 (56.52)	4 (40.00)		31 (88.57)	8 (100.00)	
Platelet			0.353[b]			1.000[b]
$\leq 50{,}000$	13 (18.84)	4 (40.00)		1 (2.86)	0 (0.00)	
$>50{,}000$	19 (27.54)	2 (20.00)		3 (8.57)	0 (0.00)	
NA	37 (53.62)	4 (40.00)		31 (88.57)	8 (100.00)	
Risk group			0.636[b]			1.000[a]
MRG	26 (50.98)	2 (33.33)		3 (18.75)	0 (0.00)	
HRG	10 (19.61)	2 (33.33)		13 (81.25)	3 (100.00)	
SRG	15 (29.41)	2 (33.33)				

Table 1 (continued)

Feature	Diagnose patients			Relapse patients		
	IKZF1 not deleted	Δ*IKZF1*	*p* value	*IKZF1* not deleted	Δ*IKZF1*	*p* value
	n (%)	*n* (%)		*n* (%)	*n* (%)	
CNS positivity			1.000[a]			1.000[a]
No	59 (98.33)	7 (100.00)		13 (81.25)	2 (100.00)	
Yes	1 (1.67)	0 (0.00)		3 (18.75)	0 (0.00)	
BCR::ABL1			0.080[a]			1.000[a]
Negative	65 (97.02)	8 (80.00)		30 (96.77)	7 (100.00)	
Positive	2 (2.98)	2 (20.00)		1 (3.23)	0 (0.00)	
ETV6::RUNX1			0.587[a]			1.000[a]
Negative	57 (90.48)	10 (100.00)		26 (96.30)	7 (100.00)	
Positive	6 (9.52)	0 (0.00)		1 (3.70)	0 (0.00)	
MLL::AF4			1.000[a]			1.000[a]
Negative	64 (95.52)	10 (100.00)		27 (96.43)	7 (100.00)	
Positive	3 (4.48)	0 (00.00)		1 (3.57)	0 (0.00)	
CRLF2 expression			1.000[a]			0.250[a]
High expression	8 (28.57)	2 (33.33)		0 (0.00)	1 (50.00)	
Low expression	20 (71.43)	4 (66.67)		6 (100.00)	1 (50.00)	
33-day response			1.000[a]			0.516[a]
Good	60 (96.77)	10 (100.00)		10 (83.33)	2 (66.67)	
Poor	2 (3.23)	0 (0.00)		2 (16.67)	1 (33.33)	
Final event			0.441[a]			0.434[a]
Alive	55 (79.71)	7 (70.00)		16 (45.71)	2 (25.00)	
Ex	14 (20.29)	3 (30.00)		19 (54.29)	6 (75.00)	
EGIL subtype			*0.040*[c]			

Table 1 (continued)

Feature	Diagnose patients			Relapse patients		
	IKZF1 not deleted	Δ*IKZF1*	*p* value	*IKZF1* not deleted	Δ*IKZF1*	*p* value
	n (%)	*n* (%)		*n* (%)	*n* (%)	
BI	12 (20.34)	4 (40.00)				
BII	44 (74.58)	4 (40.00)				
BIII	3 (5.08)	2 (20.00)				

The significance *p* value is given in italics

[a]Fisher's exact test, [b]Fisher Freeman–Halton test

CRLF2 rearrangements, indels, and CNVs were detected in ALL and overexpression of *CRLF2* expression was reported approximately 7–42% of B-ALL patients (Cario et al. 2010; Chen et al. 2012; Yano et al. 2014). In concordance with the previous reports, we detected 31% (13/42) of patients with *CRLF2* overexpression. High *CRLF2* expression is frequently accompanied by activated variations of *IKZF1, JAK1, JAK2,* and *IL7RA* (Forero-Castro et al. 2017; Harvey et al. 2010; Tasian et al. 2017). In our cohort, two patients carried *ΔIKZF1* and one patient had concomitant *JAK2* variation and *ΔIKZF1*. But we did not find any differential presentation of *IKZF1* alterations in *CRLF2* high expressed subtype. The prognostic value of the high *CRLF2* expression was found controversial in different ALL study cohorts (Cario et al. 2010; Chen et al. 2012; Ensor et al. 2011). We did not find any statistically significant association between *CRLF2*^{high} expressed patients for survival rates. A pediatric study reported a significant correlation between *CRLF2*^{high} expression and shorter EFS only within high-risk children (Chen et al. 2012). In our cohort, high-risk group of patients' number was limited, and further prospective studies are required to understand the prognostic value of the *CRLF2* overexpression.

Various studies suggested *IKZF1* alterations as an inferior prognostic marker in pediatric ALL (Clappier et al. 2015; Kuiper et al. 2010; Moorman et al. 2014; Mullighan et al. 2009; Sutton et al. 2018; Yang et al. 2011). However, we did not find any correlation between *ΔIKZF1, IKZF1*^{plus} or *CRLF2* overexpressed groups and survival outcomes. *IKZF1*^{plus} group was suggested as unfavorable factor in pediatric B-ALL and frequently related with *BCR::ABL*, *BCR::ABL*-like, and *KMT2A* rearranged ALL (Stanulla et al. 2018). The poor prognosis associated with *IKZF1*-altered B-ALL may be influenced by coexisting other high-risk factors like low hypodiploidy or other genetic changes. For example, patients who carried *ERG* deletions co-occurred with *IKZF1* alterations did not show an inferior therapy response (Clappier et al. 2014; Zaliova et al. 2014). Especially *ΔIKZF1* combined with MRD levels are associated with increased risk of relapse and poor outcome in the literature (Stanulla et al. 2018). However, limited patient number and lack of MRD based follow-up data, hypodiploidy status and *ERG* deletion information were the limitations of the study.

The pathogenic effect of *ΔIKZF1* may vary depending on the lost site; *IKZF1* Δ2–7, Δ2–8, Δ4–7 alterations were associated with adverse clinical outcomes (Bedics et al. 2023; Boer et al. 2016). In our cohort, a diagnose sample with Δ2–8 alteration had a good response to chemotherapy, and no recurrency or other events were observed during the follow-up period. On the other hand, two of five patients with *IKZF1* Δ4–7 alteration developed disease progression or relapse. Three relapse patients who did not show any *IKZF1* alteration at diagnosis, they gained Δ4–7 alteration at relapse and the patients preserved Δ4–7 alteration in the second relapse. Moreover, *IKZF1* Δ2–7 alterations were only detected in relapsed samples and showed persistency in a patient's second relapse as well. Accordingly, *ΔIKZF1* seemed to occur more often in relapse than diagnosis and preserved at the time of relapse strongly indicating that *ΔIKZF1* appears as a secondary hit during leukemogenesis. Our data support the possible contribution of *IKZF1* Δ4–7, Δ2–7 alterations to therapy failure in B-ALL.

MLPA is commonly used for detection of *IKZF1* deletions due to cost-effectiveness and accurate results. In parallel to MLPA analysis, we amplified Δ2–7, Δ4–7, and Δ4–8 alterations by PCR. The PCR screening revealed additional Δ*IKZF1* that were not detected by MLPA analysis for the corresponding amplicons. Mismatches in MLPA primer binding site or lower tumor load can be the reason of MLPA failure. A discordancy between MLPA and PCR results was detected in a relapse sample who reported Δ4–7 by PCR and Δ3–7 by MLPA. This sample might harbor Δ3 in addition to Δ4–7 alteration. MLPA has the advantage of detecting other region/gene deletions but, the PCR method is cheap and has higher sensitivity than MLPA. Mitchell et al. demonstrated that PCR is a powerful technique for detection of *IKZF1* deletion (Mitchell et al. 2021). Therefore, PCR can be used to detect selected region alterations of *IKZF1* especially in low-middle-income countries where it might be hard to access MLPA analysis.

In conclusion, our results revealed that *ΔIKZF1* is more common in relapsed samples which supports the therapy resistance in B-ALL. On the other hand, no association identified between survival outcomes of childhood B-ALL patients and *ΔIKZF1*, *IKZF1*^{plus}, or *CRLF2* overexpression groups. Further large-scale cohort studies are required to clarify the prognostic value of *ΔIKZF1* and other accompanied alterations in pediatric ALL. Besides the prognostic value of these markers, new targeted drugs, drug repurposing studies and/or combinational therapy approaches are important for the development of more efficient ALL therapy. Therefore, robust screening of common genetic alterations such as *ΔIKZF1* is valuable in ALL, and PCR based method is an efficient and rapid way to detect *IKZF1* deletions in a cost-efficient way.

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Author Contributions Yücel Erbilgin, Sinem Firtina, Ugur Ozbek, Ozden Hatirnaz Ng, and Müge Sayitoglu designed the study; Yücel Erbilgin, Sinem Firtina, and Khusan Khodzhaev performed sequencing and MLPA analysis, Elif Kırat performed CRLF2 expression analysis; Yücel Erbilgin and Ozlem Toluk did statistical analysis; Yücel Erbilgin and Müge Sayitoglu wrote the manuscript; Khusan Khodzhaev prepared the figures; and all authors participated in collecting clinical and/or molecular data, helped analyze and interpret data, and reviewed and approved the final version of the manuscript.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no competing financial interests.

Ethical Approval The study was approved by the Ethical Committee of Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey (Number: 2020/124). Written informed consents were obtained from patients and/or their parent(s)/legal guardian(s).

References

Ayón-Pérez MF, Pimentel-Gutiérrez HJ, Durán-Avelar MJ, Vibanco-Pérez N, Pérez-Peraza VM, Pérez-González Ó et al (2019) IKZF1 gene deletion in pediatric patients diagnosed with acute lymphoblastic leukemia in Mexico. Cytogenet Genome Res 158(1):10–16. https://doi.org/10.1159/000499641

Bedics G, Egyed B, Kotmayer L, Benard-Slagter A, de Groot K, Bekő A et al (2023) PersonALL: a genetic scoring guide for personalized risk assessment in pediatric B-cell precursor acute lymphoblastic leukemia. Br J Cancer 129(3):455–465. https://doi.org/10.1038/s41416-023-02309-8

Bene MC, Castoldi G, Knapp W, Ludwig WD, Matutes E, Orfao A, van't Veer MB (1995) Proposals for the immunological classification of acute leukemias. European Group for the Immunological Characterization of Leukemias (EGIL). Leukemia 9(10):1783–1786

Boer JM, van der Veer A, Rizopoulos D, Fiocco M, Sonneveld E, de Groot-Kruseman HA et al (2016) Prognostic value of rare IKZF1 deletion in childhood B-cell precursor acute lymphoblastic leukemia: an international collaborative study. Leukemia 30(1):32–38. https://doi.org/10.1038/leu.2015.199

Cario G, Zimmermann M, Romey R, Gesk S, Vater I, Harbott J et al (2010) Presence of the P2RY8-CRLF2 rearrangement is associated with a poor prognosis in non-high-risk precursor B-cell acute lymphoblastic leukemia in children treated according to the ALL-BFM 2000 protocol. Blood 115(26):5393–5397. https://doi.org/10.1182/blood-2009-11-256131

Chen IM, Harvey RC, Mullighan CG, Gastier-Foster J, Wharton W, Kang H et al (2012) Outcome modeling with CRLF2, IKZF1, JAK, and minimal residual disease in pediatric acute lymphoblastic leukemia: a Children's Oncology Group study. Blood 119(15):3512–3522. https://doi.org/10.1182/blood-2011-11-394221

Churchman ML, Qian M, Te Kronnie G, Zhang R, Yang W, Zhang H et al (2018) Germline genetic IKZF1 variation and predisposition to childhood acute lymphoblastic leukemia. Cancer Cell 33(5):937-948.e938. https://doi.org/10.1016/j.ccell.2018.03.021

Clappier E, Auclerc MF, Rapion J, Bakkus M, Caye A, Khemiri A et al (2014) An intragenic ERG deletion is a marker of an oncogenic subtype of B-cell precursor acute lymphoblastic leukemia with a favorable outcome despite frequent IKZF1 deletions. Leukemia 28(1):70–77. https://doi.org/10.1038/leu.2013.277

Clappier E, Grardel N, Bakkus M, Rapion J, De Moerloose B, Kastner P et al (2015) IKZF1 deletion is an independent prognostic marker in childhood B-cell precursor acute lymphoblastic leukemia, and distinguishes patients benefiting from pulses during maintenance therapy: results of the EORTC Children's Leukemia Group study 58951. Leukemia 29(11):2154–2161. https://doi.org/10.1038/leu.2015.134

Conserva MR, Redavid I, Anelli L, Zagaria A, Tarantini F, Cumbo C et al (2023) IKAROS in acute leukemia: a positive influencer or a mean hater? Int J Mol Sci. https://doi.org/10.3390/ijms24043282

Dörge P, Meissner B, Zimmermann M, Möricke A, Schrauder A, Bouquin JP et al (2013) IKZF1 deletion is an independent predictor of outcome in pediatric acute lymphoblastic leukemia treated according to the ALL-BFM 2000 protocol. Haematologica 98(3):428–432. https://doi.org/10.3324/haematol.2011.056135

Ensor HM, Schwab C, Russell LJ, Richards SM, Morrison H, Masic D et al (2011) Demographic, clinical, and outcome features of children with acute lymphoblastic leukemia and CRLF2 deregulation: results from the MRC ALL97 clinical trial. Blood 117(7):2129–2136. https://doi.org/10.1182/blood-2010-07-297135

Erbilgin Y, Firtina S, Mercan S, Hatirnaz Ng O, Karaman S, Tasar O et al (2019) Prognostic gene alterations and clonal changes in childhood B-ALL. Leuk Res 83:106159. https://doi.org/10.1016/j.leukres.2019.05.009

Forero-Castro M, Robledo C, Benito R, Bodega-Mayor I, Rapado I, Hernández-Sánchez M et al (2017) Mutations in TP53 and JAK2 are independent prognostic biomarkers in B-cell precursor acute lymphoblastic leukaemia. Br J Cancer 117(2):256–265. https://doi.org/10.1038/bjc.2017.152

Hamadeh L, Enshaei A, Schwab C, Alonso CN, Attarbaschi A, Barbany G et al (2019) Validation of the United Kingdom copy-number alteration classifier in 3239 children with B-cell precursor ALL. Blood Adv 3(2):148–157. https://doi.org/10.1182/bloodadvances.2018025718

Harvey RC, Mullighan CG, Chen IM, Wharton W, Mikhail FM, Carroll AJ et al (2010) Rearrangement of CRLF2 is associated with mutation of JAK kinases, alteration of IKZF1, Hispanic/Latino ethnicity, and a poor outcome in pediatric B-progenitor acute lymphoblastic leukemia. Blood 115(26):5312–5321. https://doi.org/10.1182/blood-2009-09-245944

Inaba H, Mullighan CG (2020) Pediatric acute lymphoblastic leukemia. Haematologica 105(11):2524–2539. https://doi.org/10.3324/haematol.2020.247031

Kicinski M, Arfeuille C, Grardel N, Bakkus M, Caye-Eude A, Plat G et al (2023) The prognostic value of IKZF1. Pediatr Blood Cancer 70(6):e30313. https://doi.org/10.1002/pbc.30313

Kohlmann A, Martinelli G, Hofmann W-K, Kronnie G, Chiaretti S, Preudhomme C et al (2012) The Interlaboratory Robustness of Next-Generation Sequencing (IRON) Study Phase II: deep-sequencing analyses of hematological malignancies performed by an international network involving 26 laboratories American Society of Hematology (ASH) 54th Annual Meeting, Atlanta, GA, USA. https://ashpublications.org/blood/article/120/21/1399/89411/The-Interlaboratory-Robustness-of-Next-Generation

Kuiper RP, Waanders E, van der Velden VH, van Reijmersdal SV, Venkatachalam R, Scheijen B et al (2010) IKZF1 deletions predict relapse in uniformly treated pediatric precursor B-ALL. Leukemia 24(7):1258–1264. https://doi.org/10.1038/leu.2010.87

Ma X, Edmonson M, Yergeau D, Muzny DM, Hampton OA, Rusch M et al (2015) Rise and fall of subclones from diagnosis to relapse in pediatric B-acute lymphoblastic leukaemia. Nat Commun 6:6604. https://doi.org/10.1038/ncomms7604

Mitchell RJ, Kirkwood AA, Barretta E, Clifton-Hadley L, Lawrie E, Lee S et al (2021) IKZF1 alterations are not associated with outcome in 498 adults with B-precursor ALL enrolled in the UKALL14 trial. Blood Adv 5(17):3322–3332. https://doi.org/10.1182/bloodadvances.2021004430

Moorman AV, Enshaei A, Schwab C, Wade R, Chilton L, Elliott A et al (2014) A novel integrated cytogenetic and genomic classification refines risk stratification in pediatric acute lymphoblastic leukemia. Blood 124(9):1434–1444. https://doi.org/10.1182/blood-2014-03-562918

Mullighan CG, Goorha S, Radtke I, Miller CB, Coustan-Smith E, Dalton JD et al (2007) Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. Nature 446(7137):758–764. https://doi.org/10.1038/nature05690

Mullighan CG, Miller CB, Radtke I, Phillips LA, Dalton J, Ma J et al (2008) BCR-ABL1 lymphoblastic leukaemia is characterized by the deletion of Ikaros. Nature 453(7191):110–114. https://doi.org/10.1038/nature06866

Mullighan CG, Su X, Zhang J, Radtke I, Phillips LA, Miller CB et al (2009) Deletion of IKZF1 and prognosis in acute lymphoblastic leukemia. N Engl J Med 360(5):470–480. https://doi.org/10.1056/NEJMoa0808253

Nguyen K, Devidas M, Cheng SC, La M, Raetz EA, Carroll WL et al (2008) Factors influencing survival after relapse from acute lymphoblastic leukemia: a Children's Oncology Group study. Leukemia 22(12):2142–2150. https://doi.org/10.1038/leu.2008.251

Olsson L, Johansson B (2015) Ikaros and leukaemia. Br J Haematol 169(4):479–491. https://doi.org/10.1111/bjh.13342

Russell LJ, Capasso M, Vater I, Akasaka T, Bernard OA, Calasanz MJ et al (2009) Deregulated expression of cytokine receptor gene, CRLF2, is involved in lymphoid transformation in B-cell precursor acute lymphoblastic leukemia. Blood 114(13):2688–2698. https://doi.org/10.1182/blood-2009-03-208397

Schmittgen TD, Livak KJ (2008) Analyzing real-time PCR data by the comparative C(T) method. Nat Protoc 3(6):1101–1108. https://doi.org/10.1038/nprot.2008.73

Stanulla M, Dagdan E, Zaliova M, Möricke A, Palmi C, Cazzaniga G et al (2018) IKZF1. J Clin Oncol 36(12):1240–1249. https://doi.org/10.1200/JCO.2017.74.3617

Sun L, Crotty ML, Sensel M, Sather H, Navara C, Nachman J et al (1999a) Expression of dominant-negative Ikaros isoforms in T-cell acute lymphoblastic leukemia. Clin Cancer Res 5(8):2112–2120

Sun L, Goodman PA, Wood CM, Crotty ML, Sensel M, Sather H et al (1999b) Expression of aberrantly spliced oncogenic ikaros isoforms in childhood acute lymphoblastic leukemia. J Clin Oncol 17(12):3753–3766. https://doi.org/10.1200/JCO.1999.17.12.3753

Sun L, Heerema N, Crotty L, Wu X, Navara C, Vassilev A et al (1999c) Expression of dominant-negative and mutant isoforms of the antileukemic transcription factor Ikaros in infant acute lymphoblastic leukemia. Proc Natl Acad Sci U S A 96(2):680–685. https://doi.org/10.1073/pnas.96.2.680

Sutton R, Venn NC, Law T, Boer JM, Trahair TN, Ng A et al (2018) A risk score including microdeletions improves relapse prediction for standard and medium risk precursor B-cell acute lymphoblastic leukaemia in children. Br J Haematol 180(4):550–562. https://doi.org/10.1111/bjh.15056

Tasian SK, Loh ML, Hunger SP (2017) Philadelphia chromosome-like acute lymphoblastic leukemia. Blood 130(19):2064–2072. https://doi.org/10.1182/blood-2017-06-743252

Vora A, Goulden N, Mitchell C, Hancock J, Hough R, Rowntree C et al (2014) Augmented post-remission therapy for a minimal residual disease-defined high-risk subgroup of children and young people with clinical standard-risk and intermediate-risk acute lymphoblastic leukaemia (UKALL 2003): a randomised controlled trial. Lancet Oncol 15(8):809–818. https://doi.org/10.1016/S1470-2045(14)70243-8

Yang YL, Hung CC, Chen JS, Lin KH, Jou ST, Hsiao CC et al (2011) IKZF1 deletions predict a poor prognosis in children with B-cell progenitor acute lymphoblastic leukemia: a multicenter analysis in Taiwan. Cancer Sci 102(10):1874–1881. https://doi.org/10.1111/j.1349-7006.2011.02031.x

Yano M, Imamura T, Asai D, Moriya-Saito A, Suenobu S, Hasegawa D et al (2014) An overall characterization of pediatric acute lymphoblastic leukemia with CRLF2 overexpression. Genes Chromosomes Cancer 53(10):815–823. https://doi.org/10.1002/gcc.22190

Zaliova M, Zimmermannova O, Dörge P, Eckert C, Möricke A, Zimmermann M et al (2014) ERG deletion is associated with CD2 and attenuates the negative impact of IKZF1 deletion in childhood acute lymphoblastic leukemia. Leukemia 28(1):182–185. https://doi.org/10.1038/leu.2013.282

Zhang J, McCastlain K, Yoshihara H, Xu B, Chang Y, Churchman ML et al (2016) Deregulation of DUX4 and ERG in acute lymphoblastic leukemia. Nat Genet 48(12):1481–1489. https://doi.org/10.1038/ng.3691

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