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The prognostic significance of cytokine receptor-like factor 2 expression and *JAK2* mutation in pediatric B-cell acute lymphoblastic leukemia: A prospective cohort study

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Acute lymphoblastic leukemia (ALL) is the most common pediatric malignancy. Philadelphia (Ph)-like B-cell acute lymphoblastic leukemia (B-ALL) is defined by a gene expression profile similar to Ph-positive B-ALL and shows a large number of genetic alterations in the cytokine receptor and kinase-signaling pathway genes that contribute to its aggressive phenotype and frequent disease recurrence – the main cause of death in affected children. Here, we aimed to correlate CRLF2 expression and *JAK2* mutations in B-ALL patients with other prognostic factors and the patients' outcomes as well as to evaluate their prognostic significance. The study was approved by the local institutional review board and written consents were obtained from a parent of each child before their enrolment. We included 54 newly diagnosed B-ALL pediatric patients (median age: 9.0 (2.0–18.0)) who were stratified either into a standard-risk (SR) or high-risk (HR) group and treated according to the modified Berlin-Frankfurt-Münster 90 protocol (ALL-BFM 90). Fresh bone marrow samples were used to determine CRLF2 expression as well as to search for the *JAK2* V617F mutation. Normal CRLF2 expression was reported in the SR patients much more often than in the HR group, while its overexpression was more common in the HR patients than in the SR ones (22 vs 6 and 18 vs 8, respectively, $p < 0.001$). CRLF2 was also more often overexpressed in the MRD-positive cases than in the negative ones (17 vs 9, $p < 0.001$), while normal CRLF2 expression was more common in the MRD-negative patients compared to the MRD-positive ones (24 vs 4, $p < 0.001$) which supports the unfavorable prognostic value of CRLF2 in relation to MRD positivity at the end of the induction treatment. *JAK2* mutation was detected only in 2 patients belonging to the CRLF2 overexpression group which made the assessment of the prognostic significance of this mutation impossible. Notably, none of the patients with normal CRLF2 expression ended up relapsing while 4 patients with overexpressed CRLF2 developed a relapse ($p = 0.031$). The study subjects were followed up for up to 24 months, and we did not find CRLF2 overexpression to negatively influence overall survival, however, it did have an adverse effect on relapse-free survival. In summary, CRLF2 overexpression was found to be an unfavorable prognostic factor in childhood ALL as it was expressed more in high-risk patients and in those with poor treatment response. The analysis of CRLF2 expression in B-ALL pediatric patients may help in risk stratification and can potentially offer new treatment options based on novel CRLF2 inhibitors.

Key words: *B-cell acute lymphoblastic leukemia, CRLF2, JAK2, MRD, overexpression*

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Acute lymphoblastic leukemia (ALL) is the most common pediatric malignancy characterized by long-term survival rates approaching 80% in developed countries [1, 2]. Nevertheless, relapse represents the main reason behind death [3]. High-resolution genome-wide profiling and sequencing studies demonstrate that the risk of ALL relapse is associated with specific biological features of the leukemic cells including gene mutations, copy number variations and gene fusions [4].

Philadelphia (Ph)-like B-cell acute lymphoblastic leukemia (B-ALL), also known as B-lymphoblastic leukemia/lymphoma BCRABL1–like, is defined by a gene expression profile similar to Ph chromosome–positive B-ALL (Ph⁾ BALL) but lacks the rearrangement of t(9;22) (q34;q11;2)/ BCR-ABL1. It shows large number of genetic alterations in the cytokine receptor and kinase-signaling pathway genes that contribute to its aggressive phenotype and frequent disease recurrence [5].

Cytokine receptor–like factor 2 (CRLF2), located on chromosome Xp22.3 and Yp11 has been found to be overexpressed in approximately half of the Ph-like B-ALL [6]. The frequency of *JAK2* mutations in ALL has been reported to be about 10% in pediatric high-risk (HR) ALL. Investigation of Janus Kinase 2 (*JAK2*) mutation status showed association of *JAK2* mutations (most notably JAK2-R683G) in about half of cases with CRLF2 overexpression in DS-ALL. This strong association of CRLF2 overexpression and *JAK2* mutation suggested that these proteins might cooperate to transform cells, especially because CRLF2

is a JAK-binding, Box 1 motif–containing cytokine receptor [7].

In this study we aimed to identifying the frequency of CRLF2 and *JAK2* mutations among pediatric B-ALL patients, determining their association with other risk factors and detecting their prognostic significance in response to treatment protocol.

MATERIALS AND METHODS

Study design and patients' enrollment

A prospective Cohort study was conducted in a Pediatric Oncology Unit, Mansoura University Oncology Center, including all newly diagnosed B-ALL patients (n = 54) who are less than 18 years old over a period of 1 year (from January 2018 till December 2018). The study was approved by the local institutional review board and written consents were obtained from a parent of each child before their enrolment.

Methods

Leukemia diagnosis

Bone marrow (BM) examination was the basis of ALL diagnosis, whenever BM exhibits blast > 25% [8] with additional positive ALL diagnosis confirmations by flowcytometric analysis.

Patients risk stratification

Patients were categorized to standard risk (SR) and HR groups according to National Cancer Institute (NCI) criteria [9]: Patients with an age ranging from more than 1 year or less than 10 years, initial WBC count < 50 × 10^9/L, absence of extramedullary disease during presentation in addition to negative minimal residual disease (MRD) after induction of remission were classified as being SR ALL subjects, otherwise they were considered to be HR ALL patients.

Detection of CRLF2 expression and *JAK2* V617F mutation

One millimetre of EDTA fresh BM samples were obtained from each one child included in our study in order to determine CRLF2 expression as well as *JAK2* V617F mutation detection.

CRLF2 expression by flowcytometry

For CRLF2 analysis by flowcytometry, the stain/lyse/wash technique was used. Briefly, in single tube, we add fresh BM samples (100 µL) with the surface MoAb, 10 µl of the CRLF2-PE MoAb, 10 µl of CD10-FITC MoAb, and 10 µl of CD45 APC, PerCP-CY5.5/CD19 mixed well, and incubated for 20 minutes at room temperature. The cells were then washed twice with phosphate-buffered saline (PBS); 2 ml lysing solution was added, mixed, and left for 15 minutes in the dark, and then the cells were washed twice with PBS. After the last wash, the cells were suspended in 500 µl of PBS, and then analyzed using a flow cytometer (FACS Canto flow cytometer with Cell Quest software; Becton Dickinson, CA, USA). At least 10,000 events/tube were measured. The blast gate was defined based on CD45 dim expression and side-scatter characteristics and calculated as a percentage of total gated events. For analysis of CD CRLF2 expression, measurements included mean fluorescence intensity (MFI) on leukemic blasts (adjusted for background fluorescence using negative internal controls) and relative MFI ratio (divide the MFI values of defined leukemic blasts by lymphocytes events).

Interpretation of the results

Regarding the CRLF2 staining, the negative control was initially defined as the mature lymphocytes of the BM samples analyzed. A cutoff of 10% of positive cells for the CRLF2 as recommended by Dworzak et al. [10]. Patients have ≥ 10% CRLF2 expression were considered positive.

JAK2 V617F mutation detection

DNA extraction and quantification

Genomic DNA extraction was carried out from EDTA blood samples using extraction kit obtained from (Genejet genomic DNA extraction kit (Thermo Fisher Scientific) made in USA). The concentration and quality of extracted DNA was tested by Nano drop (Applied Biosystems, USA). The concentration of extracted DNA was assessed by spectrophotometry at wave length 260:280 and 260: 280. DNA sample whose ratio was <1.8 were excluded. The DNA samples integrity were assessed by agarose gel electrophoresis.

ASO-PCR technique for *JAK2* V617F mutation detection

Allele Specific oligonucleotide probe (ASO-PCR) was applied using a common reverse primer *JAK2* R (5'-CTGAATAGTCCTACAG TGTTTTCAGTTTCA-3') and 2 forward primers named JAK2 F Mut (5'-AGCATTTGGT-TTTAAATTATGGAGTATATT-3') specific for the mutant allele containing an intentional mismatch at third nucleotide from the 3' end and *JAK2* F WT (5'-ATCTAT-AGTCATGCTGAAAGTAGGAGAAAG-3') as internal control which amplifies the wild-type allele. PCR was performed, after an initial denaturation of 1 min to 95°C, 35 cycles of denaturation, annealing and extension of 1 min each with the temperatures of 95, 58 and 72°C respectively. The PCR amplified products were run agarose gel 2% along with 100 bp ladder, the gels were stained by ethidum bromide, To confirm findings Singer sequencing (ABI 310 genetic analyzer, Applied Biosystems) was done for PCR products of random positive selected samples [11].

Treatment protocol

B ALL patients were treated using modified Berlin-Frankfurt-Münster 90 (ALL-BFM 90) [12].

Assessment of response to induction chemotherapy on day 28

Patients were considered in complete remission (CR) when BM aspirate showed lymphoblasts < 5% with normal haematopoiesis and MRD using flow cytometry was negative (defined as having < 0,01% leukemic cells in BM) [13].

Follow up of treatment outcome

Patients were followed during chemotherapy treatment for at least 2 years. Patients were relapsed when BM was reinfiltrated with ≥ 25% blasts or presence of blasts in an extra-medullary site [8].

Ethical consideration

The study was conducted in accordance with the declaration of Helsinki [14]. Written consents were obtained from either parent of each child before being enrolled in the study. The study was approved by local the institutional review board on 7th of March, 2019.

Statistical analysis and data interpretation

Data were fed to the computer and analyzed using IBM SPSS software package version 26 (IBM, Inc, Chicago, USA). Patients' data were compared using Chi-squared (χ^2), Fisher's exact, Mann Whitney U or Student t-tests appropriately according to the type of data. Overall survival (OS) was estimated from the time of diagnosis till the time of death or the last follow up. Event free survival (EFS) was determined from the time of diagnosis till the occurrence of the first event (i.e., relapse). Both OS and EFS were calculated by Kaplan–Meier analysis and differences were compared using the log-rank test. The diagnostic performance of a test, or the accuracy of a test to discriminate diseased cases from non-diseased cases is evaluated using Receiver Operating Characteristic (ROC) curve analysis. Sensitivity and Specificity were detected from the curve and Positive predictive value, negative predictive value and accuracy were calculated through cross tabulation.

RESULTS

The study included 54 newly diagnosed B ALL pediatric patients with the median age in years was 9.0 (2.0–18.0), 35 patients were males and 19 were females. Median TLC × 10^9/L at diagnosis was 6 (0.5–220). The mean percentage of blasts in the BM was 88.20 ± 8.95. The median CRLF2 was 16.9 (1.2–98.9) and *JAK2* mutation was found in only 2 patients (*table 1*).

Table 2 illustrates CRLF2 expression according to patients' risk stratification, *JAK2* mutation and treatment response. Normal CRLF2 expression was significantly more among SR patients than HR group, while over expression was more reported in HR patients than SR ones (22 V_s 6 and 18 V_s 8, $p < 0.001$). Negative MRD patients showing normal CRLF2 expression were more than MRD positive ones, while CRLF2 overexpression was more among MRD positive group when they were compared to MRD negative cases with statistical significance (24 V_s 4 and 17 V_s 9, $p < 0.001$ respectively). Furthermore, all patients with normal CRLF2 expression 28 (100%) did not exhibit relapse but 4 (15.4%) patients with overexpression relapsed with significant *p* value (0.03). *JAK2* mutation was detected only in 2 patients belonging to CRLF2 overexpression group without statistical significance ($p = 0.135$).

The predictive validity of CRLF2 for mortality and relapse was evaluated using ROC curve (*figure 1, 2*) respectively. Area under curve for CRLF2 is fair with the best detected cut off point for mortality and relapse is 40% and 50.15% yielding accuracy of 64.8% and 78% respectively.

Table 1
Demographic and laboratory characteristics of the studied group

Parameter	Value
Data	*n* = 54
Age, median (min–max), years	9.0 (2.0–18.0)
Sex: male female	 35 (64.8%) 19 (35.2%)
TLC at diagnosis, median (min–max) mm^3	6000 (500–220 000)
Blast cells (mean ± SD), % in BMA	88.20 ± 8.95
CRLF2, median (min–max)	16.9 (1.2–98.9)
JAK2 mutation: present absent	 2 (3.7%) 52 (96.3%)

Notes. TLC – total leucocyte count; BMA – BM aspirate; CRLF2 – cytokine receptor-like factor2.

Table 2
CRLF2 expression according to patients' risk stratification, *JAK* mutation and treatment response

Parameter	Normal CRLF2 expression (*n* = 28)	Overexpressed CRLF2 (*n* = 26)	Test of significance and *p* value
Risk category: SR HR	 22 (78.6%) 6 (21.4%)	 8 (30.8%) 18 (69.2%)	$\chi^2 = 12.476$ $p < 0.001$*
MRD: positive negative	 4 (19%) 24 (72.7%)	 17 (81%) 9 (27.3%)	FET = 14.812 $p < 0.001$*
Treatment Response: relapse no relapse	 0 (0%) 28 (100%)	 4 (15.4%) 22 (84.6%)	FET = 4.652 $p = 0.031$*
JAK2 mutation: non-mutant mutant	 28 (100%) 0 (0%)	 24 (92.3%) 2 (7.7%)	FET = 2.237 $p = 0.135$

*Notes. FET – Fischer's exact test, χ^2 – chi-square test; * – significant p value*

Figure 1
ROC curve of CRLF2 to predict mortality (area under curve is fair with the best detected cut off point for mortality is 40 yielding accuracy of 64.8%)

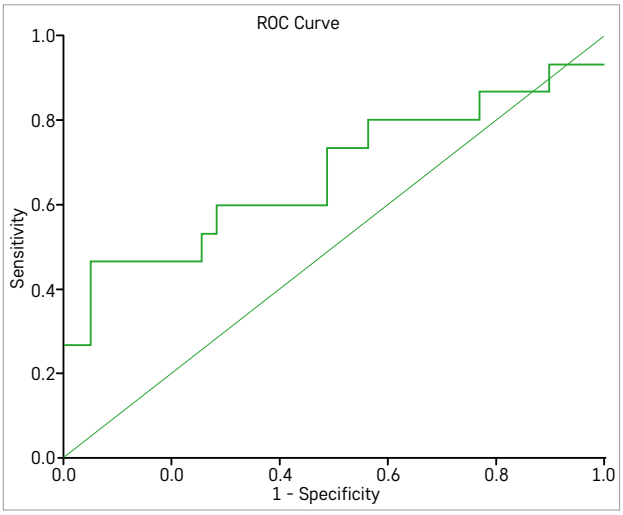


Figure 2
ROC curve of CRLF2 to predict relapse (area under curve is fair with the best detected cut off point for relapse is 50 yielding accuracy of 78%)

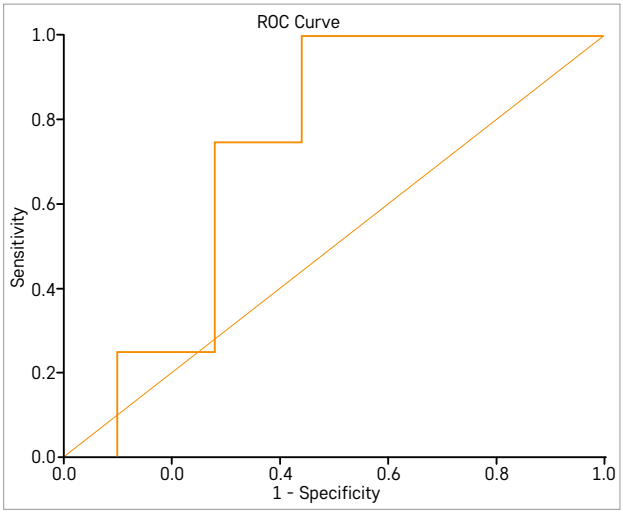


Figure 3
Kaplan–Meier curve for OS among patients with normal and overexpressed CRLF2

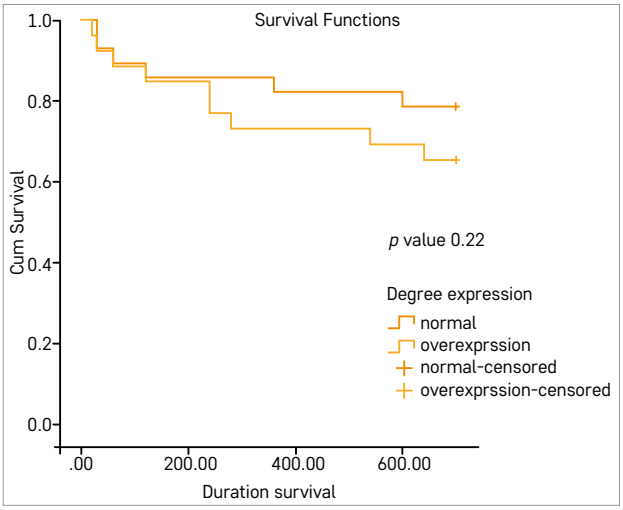
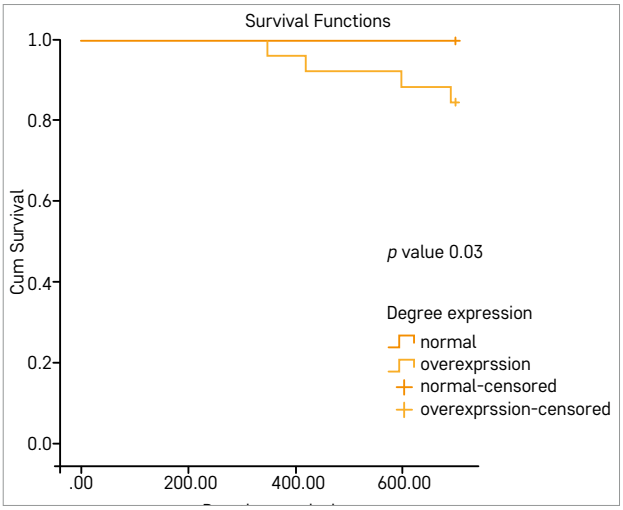


Figure 4
Kaplan–Meier curve for RFS among patients with normal and overexpressed CRLF2



OS was defined as the time from the diagnosis to death from any cause or the last follow-up (OS) using Kaplan–Meier analysis did not differ significantly among patients with overexpressed CRLF2 and those with normal expression (p = 0.22) (*figure 3*), however patients with CRLF2 overexpression had significantly worse relapse free survival (RFS) than patients with normal expression (p = 0.03) (*figure 4*).

Discussion

One of the major achievements in cancer therapy has been the increased cure rates for ALL thanks to better understanding and assessment of conventional prognostic factors as well as identification of molecular markers that are associated with a better response to therapy. Suitable risk stratification has permitted a more personalized treatment, selecting patients for receiving standard or intensified therapy, alone or in combination with ALL specific target therapies, and together with an enhanced supportive care have contributed to the increase in the event-free survival (EFS) rates [15].

In the current study CRLF2 overexpression was more among patients with HR B-ALL (69%) than in the patients with SR B-ALL (31%). *CRLF2* overexpression has been described at various rates in the literature. Our results are consistent with Yamashita et al. [17] who found higher *CRLF2* gene expression in HR-ALL than in SR-ALL patients. However, previous results from research by the United States Children's Oncology Group and a study of Japanese pediatric B-ALL series showed less CRLF2 overexpression (17–18%) of unselected B-ALL patients [18, 19]. The difference may be attributed to lack of risk categorization in the latter studies.

Different CRLF2 overexpression incidences observed in different groups are likely attributed in part to different methods used to define the cut-off point for CRLF2 overexpression. Some set the cut-off at the lowest level of CRLF2 expression in a patient with a known CRLF2 rearrangement (P2RY8-CRLF2 or IGH-CRLF2) [18, 20] while others defined the threshold as over certain folds (e.g. 10- or 20-fold) of overall median expression value [17, 21–23] In this study, we adopt Dworzak et al. [10] definition of CRLF2 over expression cut- off point as 5 folds or more over the median expression.

In the present study, we could not find prognostic significance of *JAK2* mutation because of the rarity of its occurrence among the studied group (in only 2 patients). Asai et al. [23] also failed to identify *JAK2* mutations in 202 unselected B-ALL cases in a Japanese cohort. This may be attributed to the ethnic differences as *JAK2* mutations are more prevalent in Hispanic patients with B-ALL than in Caucasian patients [21]. Although *JAK2* mutation is only detected in 2 patients but both belonged to CRLF2 overexpression group. This was emphasized by the results of Konoplev et al. [24] showing that *JAK2* was frequently mutated in CRLF2$^+$ B-ALL and did not test its mutation in CRLF2 – B-ALL as it has been shown in previously published studies that *JAK2* mutation is infrequent in CRLF2– cases [25, 26].

MRD's clinical significance is now generally acknowledged, and it is often recognized as the most important prognostic factors in current ALL treatment. MRD monitoring based on a specific marker can help us to predict leukemia relapse and determine the best-individualized treatment. In the present work, CRLF2 level was significantly higher among MRD positive group. Furthermore, most of the patients with positive MRD belong to CRLF2 over expression. This supports the unfavorable prognostic value of *CRLF2* in relation to MRD positivity at the end of the induction.

Similarly, Chen et al. [27] studied the association of high CRLF2 expression with end induction MRD and observed that ALL cases with high CRLF2 gene expression are associated with higher MRD level at the end of induction and poor RFS. However, Palmi et al. [12], who classified his patients' groups into a HR group, intermediate-risk group, and SR group, found no statistically significant difference between CRLF2 overexpression and MRD across the different risk subgroup ($p = 0.09$).

In the present work, patients were followed for the treatment response up to 24 months, and we did not find an influence of CRLF2 overexpression on OS. These results go hand in hand with the findings of Dworzak et al. [10] who also did not report negative impact of CRLF2 overexpression on OS ($p = 0.35$). This may be attributed to the heterogenicity and plenty of factors that affect the mortality in ALL patients other than treatment response (e.g., serious infections, drug toxicities and others).

Unlike OS, CRLF2 overexpression had an adverse effect on RFS. This highlights the poor prognostic significance of CRLF2 evidenced by significantly higher relapse rate among patients with overexpression of CRLF2. This is consistent with Yamashita et al. [17] who reported that 4-year EFS for the patients with high CRLF2 expression was significantly worse than those with low expression ($p = 0.003$) and difference was recognized only in HR-ALL patients. Also, Dou et al. [28] found that patients with CRLF2 overexpression had shorter EFS ($p = 0.004$).

In summary, CRLF2 overexpression was found to be unfavorable prognostic factor for childhood ALL as it was expressed more in HR patients and in those with poor treatment response i.e., higher relapse rate. However, we could not find prognostic significance of *JAK2* mutation as it was reported only in 2 patients belonging to CRLF2 over expression group. This emphasizes the role of CRLF2 overexpression in the prediction of treatment response and encourages its involvement in risk stratification-based therapy. Preceding MRD detection at the end of induction chemotherapy, CRLF2 expression can be endorsed for tailoring the initial treatment plan for patients according to its level of expression.

CONCLUSION

CRLF2 overexpression is associated with poor outcome in pediatric B-ALL, so that it can be used as a prognostic factor for predicting patient response to treatment. Involvement of CRLF2 expression in diagnostic workup of B-ALL may help in risk stratification and offer potential therapy based on novel CRLF2 inhibitors.

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CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

References

1. Maloney K.W., Devidas M., Wang C., Mattano L.A., Friedmann A.M., Buckley P., et al. Outcome in children with standard-risk B cell acute lymphoblastic leukemia: results of Children's Oncology Group Trial AALL0331. J Clin Oncol 2020; 38 (6): 602–12.
2. Pieters R., de Groot-Kruseman H., Van der Velden V., Fiocco M., van den Berg H., Bont E.D., et al. Successful therapy reduction and intensification for childhood acute lymphoblastic leukemia based on minimal residual disease monitoring: Study ALL10 from the Dutch Childhood Oncology Group. J Clin Oncol 2016; 34 (22): 2591–601.
3. Gaynon P.S. Childhood acute lymphoblastic leukaemia and relapse. Br J Haematol 2005; 131 (5): 579–87.
4. Germano G., Del Giudice L., Palatron S., Giarin E., Cazzaniga G., Biondi A., et al. Clonality profile in relapsed precursor-B-ALL children by GeneScan and sequencing analyses. Consequences on minimal residual disease monitoring. Leukemia 2003; 17 (8): 1573–82.
5. Harvey R.C., Tasian S.K. Clinical diagnostics and treatment strategies for Philadelphia chromosome–like acute lymphoblastic leukemia. Blood Adv 2020; 4 (1): 218–28
6. Iacobucci I., Roberts K.G. Genetic Alterations and Therapeutic Targeting of Philadelphia-Like Acute Lymphoblastic Leukemia. Genes 2021; 12 (5): 687.
7. Jerchel I. Signaling Pathways as Therapeutic Targets in Pediatric B-cell Precursor Acute Lymphoblastic Leukemia; 2018.
8. Lanzkowsky P. Leukemias. In: Lanzkowsky P. eds. Manual of PediatricHematology and Oncology. 3rd ed. San Diego: Academic Press; 2005. Pp. 411–8.
9. Smith M., Arthur D., Camitta B., Carroll A.J., Crist W., Gaynon P., et al. Uniform approach to risk classification and treatment assignment for children with acute lymphoblastic leukemia. J Clin Oncol 1996; 14: 18–24.
10. Dworzak M., Buldini B., Gaipa G., Ratei R., Hrusak O., Luria D., et al. AIEOP-BFM consensus guidelines 2016 for flow cytometric immunophenotyping of pediatric acute lymphoblastic leukemia: iBFM-flow standards for immunophenotyping of pediatric ALL. Cytometry B Clin Cytom 2018; 94 (1): 82–93.
11. Syeed N. *JAK2* and Beyond: *JAK2*V617 Mutational Study of Myeloproliferative Disorders and Haematological Malignancies. Asian Pac J Cancer Prev 2019; 20 (12): 3611–5.
12. Schrappe M., Reiter A., Sauter S., Ludwig W.D., Wörmann B., Harbott J., et al. Concept and interim result of the ALL-BFM 90 therapy study in treatment of acute lymphoblastic leukemia in children and adolescents/ the significance of initial therapy response in blood and bone marrow. Klin Padiatr 1994; 206: 208–21.
13. Campana D. Molecular determinants of treatment response in acute lymphoblastic leukemia. Hematology Am Soc Hematol Educ Program 2008; 366–73.
14. World Medical Association. World Medical Association Declaration of Helsinki. Ethical principles for medical research involving human subjects. Bull World Health Organ 2001; 79 (4): 373–4.
15. Pui C.H., Pei D., Coustan-Smith E., Jeha S., Cheng C., Bowman W.P., et al. Clinical utility of sequential minimal residual disease measurements in the context of risk-based therapy in childhood acute lymphoblastic leukaemia: a prospective study. Lancet Oncol 2015; 16 (4): 465–74.
16. Chiaretti S., Brugnoletti F., Messina M., Paoloni F., Fedullo A.L., Piciocchi A., et al. CRLF2 overexpression identifies an unfavourable subgroup of adult B-cell precursor acute lymphoblastic leukemia lacking recurrent genetic abnormalities. Leuk Res 2016; 41: 36–42
17. Yamashita Y., Shimada A., Yamada T., Yamaji K., Hori T., Tsurusawa M., et al. *IKZF1* and *CRLF2* gene alterations correlate with poor prognosis in Japanese BCR-ABL1-negative high-risk B-cell precursor acute lymphoblastic leukemia. Pediatr Blood Cancer 2013; 60 (10): 1587–92.
18. Chen I.M., Harvey R.C., Mullighan C.G., Gastier-Foster J., Wharton W., Kang H., et al. Outcome modeling with CRLF2, IKZF1, JAK, and minimal residual disease in pediatric acute lymphoblastic leukemia: A Children's Oncology Group study. Blood 2012; 119: 3512–22.
19. Yano M., Imamura T., Asai D., Moriya-Saito A., Suenobu S., Hasegawa D., et al. An overall characterization of pediatric acute lymphoblastic leukemia with CRLF2 overexpression. Genes Chromosomes Cancer 2014; 53: 815–82.
20. Cario G., Stanulla M., Fine B.M., Teuffel O., Neuhoff N.V., Schrauder A., et al. Distinct gene expression profiles determine molecular treatment response in childhood acute lymphoblastic leukemia. Blood 2005; 105 (2): 821–6.
21. Harvey R.C., Mullighan C.G., Wang X., Dobbin K.K., Davidson G.S., Bedrick E.J., et al. Identification of novel cluster groups in pediatric high-risk B-precursor acute lymphoblastic leukemia with gene expression profiling: correlation with genome-wide DNA copy number alterations, clinical characteristics, and outcome. Blood 2010; 116 (23): 4874–84.
22. Palmi C., Vendramini E., Silvestri D., Longinotti G., Frison D., Cario G., et al. Poor prognosis for P2RY8-CRLF2 fusion but not for CRLF2 over-expression in children with intermediate risk B-cell precursor acute lymphoblastic leukemia. Leukemia 2012; 26 (10): 2245–53.
23. Asai D., Imamura T., Suenobu S., Saito A., Hasegawa D., Deguchi T., et al. IKZF1 deletion is associated with a poor outcome in pediatric B-cell precursor acute lymphoblastic leukemia in Japan. Cancer Med 2013; 2 (3): 412–9.
24. Konoplev S., Lu X., Konopleva M., Jain N., Ouyang J., Goswami M., Roberts K.G., et al. CRLF2-Positive B-Cell Acute Lymphoblastic Leukemia in Adult Patients: A Single-Institution Experience. Am J Clin Pathol 2017; 147 (4): 357–63.
25. Roberts K.G., Morin R.D., Zhang J., Hirst M., Zhao Y., Su X., et al. Genetic alterations activating kinase and cytokine receptor signaling in high-risk acute lymphoblastic leukemia. Cancer Cell 2012; 22: 153–66.
26. Roberts K.G., Li Y., Payne-Turner D., Harvey R.C., Li Yang Y., Pei D., et al. Targetable kinase activating lesions in Ph-like acute lymphoblastic leukemia. N Engl J Med 2014; 371: 1005–15.
27. Chen X., Wood B.L. Monitoring minimal residual disease in acute leukemia: Technical challenges and interpretive complexities. Blood Rev 2017; 31 (2): 63–75.
28. Dou H., Chen X., Huang Y., Su Y., Lu L., Yu J., et al. Prognostic significance of P2RY8-CRLF2 and CRLF2 overexpression may vary across risk subgroups of childhood B-cell acute lymphoblastic leukemia. Genes Chromosomes Cancer 2017; 56 (2): 135–46.