Clinical characterization and prognosis of T cell acute lymphoblastic leukemia with high CRLF2 gene expression in children

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Abstract

It has been reported that overexpression of the CRLF2 gene is associated with poor outcomes in pediatric B cell acute lymphoblastic leukemia (B-ALL), but the incidence rates, clinical characteristics and outcomes of CRLF2 gene overexpression in pediatric T cell ALL (T-ALL) have not been systematically analyzed. In this study, CRLF2 mRNA expression levels and clinical and laboratory parameters in 63 pediatric T-ALL patients were detected at the Children’s Hospital of Chongqing Medical University and Children’s Hospital of Xianyang between February 2015 and June 2018. The patients were treated according to the modified St. Jude TXV ALL protocol, and early treatment responses (bone marrow smear and MRD level) and prognoses in the enrolled patients were assessed. CRLF2 overexpression was detected in 21/63 (33.33%) patients. Statistical differences were not found for clinical or laboratory parameters (including sex, age, initial WBC count, incidence mediastinal involvement, abnormal karyotype and fusion genes) between patients with high CRLF2 expression and patients with low expression of CRLF2 (P>0.05). One patient died of tumor lysis syndrome and renal failure, and the treatment response was monitored on day 19 (TP1) of remission in 62 patients. One patient quit treatment because of family decisions, and 61 patients underwent treatment response evaluation on day 46 (TP2) of remission. Significant differences were not found between patients with high CRLF2 expression and patients with low CRLF2 expression in terms of the treatment responses at TP1 or TP2 (P>0.05). Following October 2018, 12 patients among the 61 evaluable patients relapsed (relapse rate: 19.67%), 3 patients died from chemotherapy, and the treatment-related mortality (TRM) rate was 4.92%. Secondary tumors occurred in 1 patient. The 3-year prospective EFS rate was 54.1±11.2% and 77.7±6.6% for the 61 evaluable patients and 58 patients without TRM. Patients with low CRLF2 expression had longer EFS durations than patients with high CRLF2 expression (61 evaluable patients: 35.91±2.38 months vs 23.43±2.57 months; 58 patients without TRM: 37.86±2.08 months vs 24.55±2.43 months, P<0.05). CRLF2 expression levels were also monitored in 13 patients at TP1 and TP2, and the MRD level did not.
vary with the CRLF2 expression level. Our data suggest that clinical features, laboratory findings and treatment responses in the pediatric T-ALL population do not vary based on the overexpression of CRLF2 but that CRLF2 overexpression can contribute to a high risk of relapse in pediatric T-ALL. Thus, CRLF2 expression levels should not be used as biomarkers for monitoring MRD.

Introduction

T cell acute lymphoblastic leukemia (T-ALL) accounts for approximately 15–20% of ALL in children [1]. Despite improvements in intensive chemotherapy and allogenic hematopoietic stem cell transplantation (allo-HSCT), 15–20% and 20–40% of pediatric T-ALL patients die of relapse or treatment failure in developed and developing countries, respectively [2,3]. The Cytokine receptor-like factor 2 (CRLF2) gene encodes a member of the type I cytokine receptor family, and the encoded protein is a receptor for thymic stromal lymphopoietin (TSLP) [4]. Together with the interleukin 7 receptor (IL7R), the CRLF2 protein and TSLP activate the STAT3, STAT5, and JAK2 pathways, which control processes such as proliferation of cells and development of the hematopoietic system [5,6]. Rearrangement of this gene with immunoglobulin heavy chain gene (IGH) on chromosome 14 or with P2Y purinoceptor 8 gene (P2RY8) on the same X or Y chromosome is associated with B-progenitor ALL (B-ALL) [7] and Down syndrome-related ALL [8].

Overexpression of CRLF2 in B-ALL has been reported and shown to be correlated with poor outcomes in pediatric and adult B-ALL [7,9]. However, the correlation between CRLF2 expression level and the clinical features and prognoses of T-ALL patients has not been thoroughly investigated [10]. This study prospectively assessed the influence of CRLF2 overexpression on the clinical features and prognoses of T-ALL in children and adolescents.

Materials and methods

Patients

A total of 63 newly diagnosed T-ALL (aged ≤15 years) patients were enrolled in this study. The patients were treated following a modified St. Jude TXV protocol [11] (S1 Table) at the Children’s Hospital of Chongqing Medical University (CHCMU) and Children’s Hospital of Xianyang between February 2015 and June 2018. Patients who were diagnosed with BCR/ABL1-positive T-ALL, early T precursor ALL (ETP-ALL), or secondary leukemia or patients who had received chemotherapy before hospitalization were excluded. Ethical approval for the treatment was granted by the Ethics Commission of CHCMU, and informed consent was obtained from the patients or their guardians. Clinical features, laboratory findings and prognostic data for the enrolled patients were collected and analyzed from clinical records.

The initial diagnosis of T-ALL was based on FAB morphological classification detected by cytomorphological observation in BM smears and immuno-phenotype detection by flow cytometry (FCM) according to protocol [11]; chromosomal karyotyping and fluorescence in situ hybridization (FISH) of chromosomal translocation including ETV6/RUNX1, MLL rearrangements (MLLr), BCR/ABL and TCF3/PBX1 were analyzed as reported in the literature [12]. Twenty common fusion genes, including ETV6/RUNX1, TCF3/PBX1, TCF3/HLF, BCR/ABL1, MLL/AF10, MLL/AF4, MLL/AF9, MLL/ENL, MLL/AF1p, MLL/AF1q, MLL/AF6, MLL/AFX, dupMLL, SIL/TAL1, ETV6/ABL1, SET/NUP214, EBF1/PDGFRB, TLS/ERG, SET/CAN...
and HOX11, were detected by multiplex nested reverse transcription polymerase chain reaction (multiplex RT-PCR), and positive results were confirmed by split RT-PCR as reported in the literature [13,14].

Treatment protocol and therapeutic evaluation

The protocol was divided into four phases: remission induction, consolidation, continuation and maintenance (S1 Table); patients received triple intrathecal injection (TiT) and high-dose methotrexate (HD-MTX) for prophylaxis of central nervous system (CNS) involvement following the protocol.

Early treatment responses based on cytomorphological detection of bone marrow (BM) were conventionally conducted at two time points (TP): TP1 (day 19 of remission induction) and TP2 (day 46 of remission induction). BM smear status was recorded as in [11]: M1 (<5% leukemic cells) or complete remission (CR), M2 (5–25% leukemic cells), and M3 (≥25% leukemic cells). Minimal residual disease (MRD) level was detected by FCM and described according to the literature report [11]. MRD level of ≥1×10^{-4} was considered positive.

Central nervous system leukemia (CNSL) or testicular leukemia (TL) were defined and recorded as in the literature [15]. Event was defined as each of the following situations [11]: refractory disease (BM status M2 or M3 at TP2), relapse (BM relapse and/or extramedullary relapse), death (any reason) or diagnosis of secondary malignancy.

Detection of CRLF2 expression levels

Total RNA from BM samples was converted to cDNA, and the expression of CRLF2 was detected by real-time quantitative polymerase chain reaction (qRT-PCR) assays and performed as reported in the literature [10]. Expression of the gene Eukaryotic translation elongation factor 2 (EEF2) was chosen as an internal control. The ΔCt value was calculated and the expression ratio was defined by the percentage of ΔCt value in the ΔCt table of CRLF2 of a standard cohort from the detection laboratory. High expression of CRLF2 was defined as an expression ratio ≥90% and low expression of CRLF2 was defined as an expression ratio <90% [9].

Statistical analysis

Following October 2018, data on the clinical features, laboratory findings, treatment responses, treatment-related mortality (TRM) and event-free survival (EFS) rates of these patients were collected and analyzed. EFS was calculated from the date of diagnosis to the last follow-up, loss of follow-up or loss of the first event. SPSS 19.0 (IBM Corp., Armonk, NY) software was employed for statistical analysis. Survival curves were plotted according to the Kaplan-Meier method and compared using the log-rank test. Proportional differences between patient groups were analyzed by Pearson’s chi-square (χ²) tests or Fisher’s exact tests. A P value <0.05 was regarded as significantly different.

Results

Clinical features of the whole cohort

Data on clinical characteristics, including age at diagnosis, sex, white blood cell (WBC) count in peripheral blood, chromosomal karyotype, existence of fusion genes and mediastinal involvement, were collected and are described in Table 1. Of the 63 patients enrolled in this study, 50 were male and 13 were female. The age at diagnosis of the enrolled patients ranged from 19 months to 178 months (median age: 97.22±5.71 months; average age: 97.22±5.71
months), and 23 (36.51%) patients were more than 10 years old. The initial WBC count was 1.05–543.79 × 10^9/L (median value: 62.51 × 10^9/L; average value: 131.41 ± 19.42 × 10^9/L); 24 (38.10%) patients had initial WBC counts < 100 × 10^9/L, and 15 (23.81%) patients had initial WBC counts < 10 × 10^9/L. All 63 patients underwent karyotype detection, and normal karyotype was found in 48 patients, whereas abnormal karyotype was found in 12 patients. Samples from 3 patients could not be analyzed due to the lack of mitotic cells. The DNA index was all ≥ 1.0 for the 60 analyzed patients. Recurrent cytogenetics and molecular genetic features were determined by FISH and multiplex RT-PCR, respectively: 44 (69.84%) patients were negative; SIL/TAL fusion gene was positive in 16 (25.40%) patients; BCR/ABL1, SET/CAN and MLL/ENL fusion genes were detected in 1 patient. TL and CNSL were also confirmed in 1 patient. Chest CT scans were performed, and mediastinal involvement was found in 37 (58.73%) cases.

In this study, 42 (66.67%) patients presented with low CRLF2 expression, and 21 (33.33%) patients presented with high CRLF2 expression. Clinical characteristics were compared between patients grouped by high and low CRLF2 expression (Table 1). Statistical differences were not found based on sex, age at diagnosis, initial WBC count, mediastinal involvement, karyotype, chromosomal translocations or SIL/TAL fusion gene between the two groups.

### Treatment responses and prognosis

The 63 enrolled patients received treatment according to a modified St. Jude TXV protocol. Except for 1 patient who died of tumor lysis syndrome and secondary renal failure during the 1st week of remission induction, BM smears and MRD levels were recorded for 62 patients at TP1. Cytomorphological detections of BM smears showed 53 (85.48%), 6 (9.68%) and 3 (4.84%) patients with BM status M1, M2 and M3, respectively. MRD level was also detected by FCM at TP1; 29 (46.77%) patients had MRD level < 1×10^-4 (negative MRD), and 33 (53.23%) patients had MRD level ≥ 1×10^-4 (positive MRD). Among the 33 patients who presented with positive MRD, 7 patients had MRD levels < 1%, 10 patients had MRD levels 1%-10%, and 16 patients had MRD levels ≥ 10%. One patient quit treatment because of family decisions, and BM smears and MRD levels were evaluated in 61 patients at TP2. All 61 patients achieved CR; MRD level was also detected; 51 (83.61%) patients were MRD-negative, 10 (16.39%) patients were MRD-positive, and 4 of the 10 patients with positive MRD had MRD levels < 1%, and 6 had MRD levels 1%-10% (Table 2).

Following October 2018, among the 61 evaluable patients, 3 patients died of sepsis due to myelosuppression caused by chemotherapy, and the TRM rate was 4.92%; 12 patients relapsed (CNS relapse: 5 patients; BM relapse: 4 patients; combined CNS and BM relapse: 2 patients; and lymph node relapse: 1 patient), and the relapse rate was 19.67%. Langerhans cell histiocytosis occurred in 1 patient, and 3 patients were lost to follow-up after treatment. The three-

### Table 1. Comparison between cases with high and low CRLF2 expression based on clinical features.

| Feature                       | High expression of CRLF2 | Low expression of CRLF2 | t or Chi-square value | P value |
|-------------------------------|--------------------------|-------------------------|-----------------------|---------|
| Age (months)                  | 103.43±11.72             | 94.12±6.30              | t = 0.7661 df = 61    | 0.4465  |
| Sex (male:female)             | 14:6                     | 36.7                    | χ² = 1.5691           | 0.2103  |
| Mediastinal mass (n, %)       | 11 (55.00%)              | 28 (65.12%)             | χ² = 0.59241          | 0.4415  |
| WBC count (×10^9/L)           | 175.5 ± 41.94            | 109.4 ± 19.77           | t = 1.626 df = 61     | 0.1091  |
| Abnormal karyotype (n, %)     | 4 (20.00%)               | 10 (25.00%)             | χ² = 0.16801          | 0.6819  |
| Positive fusion gene (n, %)   | 6 (30.00%)               | 12 (27.91%)             | χ² = 3.0641           | 0.0800  |
| SIL/TAL fusion gene (n, %)    | 6 (30.00%)               | 10 (23.26%)             | χ² = 0.32771          | 0.5670  |

*: Three patients failed karyotype analysis.
The three-year EFS rate for the 58 patients who did not die from chemotherapy (without TRM) was 77.7 ± 6.6%, and the mean EFS duration for the 58 patients without TRM was 33.34 ± 2.20 months (95% CI: 29.02–37.66 months, Fig 1). The 63 patients were classified by CRLF2 expression levels including 42 patients with low CRLF2 expression and 21 patients with high CRLF2 expression. The CRLF2 overexpression rate was 33.33%. Early response indices for chemotherapy (BM smears or MRD levels) were evaluated at TP1 and TP2, and significant differences were not found between patients with high CRLF2 expression and patients with low CRLF2 expression. The outcomes of the patients were also analyzed and are demonstrated in Table 2 and Fig 1. Patients with low CRLF2 expression had longer EFS durations (35.91 ± 2.38 months vs 23.43 ± 2.57 months) than patients with high CRLF2 expression (P < 0.05, Table 2 and Fig 2a). One patient and 2 patients died from treatment in the low CRLF2 expression and high CRLF2 expression group, respectively. For the 58 patients without TRM, the EFS duration in the low CRLF2 expression group (39 patients) was also superior to that in the high CRLF2 expression group (19 patients) (37.86 ± 2.08 months vs 24.55 ± 2.43 months, P < 0.05, Table 2 and Fig 2b).

At TP1 and TP2, CRLF2 expression levels were monitored in 13 patients, including 4 patients with low CRLF2 expression 9 patients with high CRLF2 expression at diagnosis. The MRD level did not vary with the CRLF2 expression level (Table 3). CRLF2 expression level was also detected in BM samples from 5 of the 13 relapsed patients, and no clear relationships were observed between CRLF2 expression levels and prognoses at TP1 or TP2 or the relapse status (Table 3).

Discussion

T-ALL is a neoplasm of immature T-cell lymphoblasts and is characterized by several genetic alterations and poor prognoses [16]. T-ALL accounts for approximately 15–20% and 20–25% of newly diagnosed ALL cases in children and adults, respectively [1, 17]. T-ALL is an aggressive hematologic malignancy, and outcomes for T-ALL have historically remained poor [1]. With intensive chemotherapy and allo-HSCT, the EFS rates for T-ALL have increased to 85% in developed countries [16,17], whereas the outcomes of T-ALL in developing counties have remained poor. The 3-year EFS rate for this study was 77.7 ± 6.6%, which is similar to that in the literature [3]. The lower EFS rate in our study than that in developed countries may be partially due to the enrolled patients receiving a lower intensity of chemotherapy than the intensive protocols used for T-ALL patients in developed countries [16].

Somatic mutations in the CRLF2 gene mediated via juxtaposition to the immunoglobulin heavy chain gene (IGH) transcriptional enhancers are present in 7% of B-ALL cases [7] and 50% of DS-ALL patients [8]. TSLP, the encoded protein, activates the STAT3, STAT5, and JAK2 pathways, which participate in processes of lymphocyte proliferation and development.

Table 2. Comparison between cases with high and low CRLF2 expression based on treatment responses or outcomes.

| Feature                        | High expression of CRLF2 | Low expression of CRLF2 | t or Chi-square value | P value |
|--------------------------------|--------------------------|-------------------------|-----------------------|--------|
| BM smear (M1:M2+M3) at TP1     | 15:5                     | 38:3                    | χ² = 3.689            | 0.0548 |
| MRD level at TP1 (positive rate, %) | 11:9                     | 20:21                   | χ² = 0.2081           | 0.6483 |
| MRD level at TP2 (positive rate, %) | 5:15                     | 5:36                    | χ² = 1.608            | 0.2048 |
| EFS (months)                   | 23.43±2.57               | 35.91±2.38              | χ² = 4.646            | 0.0311 |
| EFS without TRM (months)       | 24.55±2.43               | 37.86±2.08              | χ² = 5.496            | 0.0191 |

https://doi.org/10.1371/journal.pone.0224652.t002

year prospective EFS (EFS) rate for the 61 evaluable patients was 54.1±11.2%, and the mean EFS duration for the 61 patients was 31.80± 2.25 months (95% CI: 27.40–36.20 months, Fig 1);
It has been demonstrated that the overexpression of CRLF2 results in poor outcomes in B-ALL [9]. The incidence rates, clinical features and prognoses of the T-ALL in patients with CRLF2 overexpression have not been fully discussed [10].

In this study, 33.33% of pediatric T-ALL patients presented with CRLF2 overexpression, which was higher than that in B-ALL (7%) [7]. Clinical characteristics in patients with high
CRLF2 expression, including sex, age and WBC count at diagnosis, incidence of mediastinal involvement, chromosomal abnormalities and fusion genes were similar between patients with low and high CRLF2 expression, suggesting that differences in traditional risk factors did not vary according to CRLF2 expression in T-ALL patients.

It has been well reported that a good therapeutic response is related to favorable outcomes in T-ALL, and a poor BM smear status (M2 or M3) or high MRD level (≥1%) at TP1 or TP2 indicates poor prognosis [11]. The pediatric T-ALL cohort in this study was classified by CRLF2 expression levels, and the therapeutic response was evaluated. Significant differences in BM smears and MRD levels at TP1 and TP2 were not observed between patients with high and low CRLF2 expression, suggesting that CRLF2 overexpression was unrelated to the treatment response in pediatric T-ALL patients. The survival data for this cohort (all evaluable patients and patients without TRM) were also analyzed, and patients with low CRLF2 expression presented with longer EFS durations than patients with high CRLF2 expression. These data suggest that CRLF2 overexpression plays an important role in the outcomes of T-ALL.

CRLF2 expression levels were monitored at TP1, TP2 or relapse in 13 patients who received chemotherapy, and no relationship was found among MRD levels, prognoses and CRLF2.

### Table 3. CRLF2 expression level after chemotherapy.

| Pt. | CRLF2 level | CRLF2 expression (%) | MRD | CRLF2 level | CRLF2 expression (%) | MRD | CRLF2 level | CRLF2 expression |
|-----|-------------|----------------------|-----|-------------|----------------------|-----|-------------|------------------|
| 6   | low         | 67.55                | positive | high         | 94.32               | positive | low         | 89.76            |
| 8   | high        | 92.78                | positive | high         | 97.31               | positive | high         | 91.09            |
| 15  | high        | 91.28                | positive | low          | 44.33               | negative | low         | 80.58            |
| 16  | high        | 92.17                | positive | low          | 79.20               | negative | low         | 66.26            |
| 17  | high        | 92.77                | positive | low          | 82.99               | negative | low         | 42.29            |
| 20  | high        | 95.33                | positive | low          | 44.32               | negative | low         | 62.11            |
| 23  | low         | 22.57                | positive | low          | 80.68               | negative | high         | 94.32            |
| 26  | low         | 69.28                | positive | low          | 86.10               | negative | low         | 50.94            |
| 32  | high        | 92.17                | negative | low          | 65.86               | negative | low         | 67.56            |
| 33  | high        | 92.58                | negative | low          | 62.50               | negative | low         | 76.85            |
| 34  | high        | 95.18                | negative | low          | 40.69               | negative | low         | 87.42            |
| 37  | high        | 91.57                | negative | low          | 57.64               | negative | low         | 73.16            |
| 42  | low         | 71.69                | negative | low          | 72.82               | negative | low         | 64.67            |

Pt.: patient; a MRD value ≥0.0001 was positive, MRD value <0.0001 was negative.

https://doi.org/10.1371/journal.pone.0224652.t003
expression levels at TP1, TP2 or relapse. This result revealed that more samples and further studies are needed before concluding CRLF2 expression level as a risk factor for therapeutic response.

In general, in this study, we evaluated the clinical features, treatment responses and prognoses of pediatric T-ALL patients. Our data suggest that clinical features, laboratory findings and treatment responses in the pediatric T-ALL population do not differ according to the expression level of CRLF2 but that CRLF2 high expression at diagnosis can contribute to a high risk of relapse in pediatric T-ALL patients. CRLF2 expression detection at diagnosis might be a good supplement to evaluate the risk stratifications of T-ALL but this index should not be used as biomarkers for monitoring MRD.

Supporting information

S1 Fig. The event free survival curves of the whole cohort. The event free survival curves of the whole cohort (dotted line) and patients without treatment related mortality (red line). (TIF)

S1 File. Pediatric T-ALL with CRLF2 gene expression. (XLSX)

S1 Table. Modified St. Jude TXV protocol. (DOCX)

S2 Table. The clinical and CRLF2 expression information of cohort. (DOCX)

Acknowledgments

We wish to thank all of the physicians and laboratory technicians who participated in T-ALL treatment and diagnosis.

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