Prognostic significance of cytokine receptor-like factor 2 alterations in acute lymphoblastic leukemia: a meta-analysis

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Meta-analysis

Background: **Cytokine receptor-like factor 2 (CRLF2) has been shown to play a role in the pathogenesis of acute lymphoblastic leukemia (ALL). Studies have examined the relationship between CRLF2 alterations such as over-expression or deregulation and clinical outcome in childhood ALL, but the results are conflicting. This meta-analysis aimed to explore the association between CRLF2 alterations and survival of pediatric patients with ALL.**

Methods: **Electronic databases updated to March 2014 were searched for relevant studies. A meta-analysis was made of twelve studies including 5945 patients to evaluate the prognostic significance of CRLF2 alterations on survival in childhood ALL. Hazards ratios (HRs) with 95% confidence intervals (CIs) were pooled across the studies using a fixed-effects model.**

Results: **CRLF2 over-expression in childhood ALL was associated with poor prognosis in terms of relapse-free survival (RFS; HR=1.70, 95% CI=1.28-2.24, *P*=0.000), event-free survival (EFS; HR=1.78, 95% CI=1.05-3.01, *P*=0.032), and overall survival (OS; HR=2.28, 95% CI=1.42-3.65, *P*=0.001). The combined data also suggested that CRLF2 deregulation in childhood ALL was correlated with poor EFS (HR=1.95, 95% CI=1.46-2.61, *P*=0.000), RFS (HR=2.20, 95% CI=1.53-3.18, *P*=0.000), and OS (HR=1.89, 95% CI=1.24-2.87, *P*=0.003). Subgroup analysis on multivariate HRs showed that CRLF2 deregulation independently predicted a poor prognosis for childhood ALL.**

Conclusions: **The present meta-analysis reveals that both CRLF2 over-expression and deregulation are associated with poor prognosis in pediatric patients with ALL.**

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Key words: **acute lymphoblastic leukemia; cytokine receptor-like factor 2; meta-analysis; prognosis**

Introduction

Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy and one of the major causes of cancer-related mortality in children. Survival rates for childhood ALL have increased dramatically over the last four decades as a result of risk-adjusted treatment, which implements risk factors such as an early treatment response and cytogenetic abnormalities, as well as rationally designed phases in the treatment backbone including remission induction, central nervous system prophylaxis, and intensification and maintenance phases of treatment.[1] However, despite these major improvements, ALL therapy still fails in approximately 20% of children, and surviving patients often experience serious toxicities.[2,3] This underscores the need to identify specific biomarkers that could serve as prognostic factors for ALL and stratify different risk groups for appropriate treatment.

The deregulated expression of cytokine receptor-like factor 2 (CRLF2), also known as thymic stromal lymphopoietin receptor (TSLPR), in B-cell precursor ALL was first described by Russell et al[4] in 2009. Two primary CRLF2 genomic lesions have been identified: a cryptic chromosomal translocation that juxtaposes CRLF2, located in the pseudoautosomal region (PAR1) of chromosome X or Y, to the immunoglobulin heavy chain locus (IGH@); and a PAR1 deletion upstream (centromeric) of CRLF2 that juxtaposes the first noncoding exon of P2RY8 to the entire coding region of CRLF2, resulting in a P2RY8-CRLF2 fusion. Recent

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studies[4-6] have also revealed that over-expression of CRLF2 is driven by its juxtaposition to either the IGH@ enhancer (IGH@-CRLF2) or the P2RY8 promoter (P2RY8-CRLF2). High expression levels of CRLF2 are thought to contribute to leukemogenesis by activation of the JAK-STAT pathway, leading to increased cell proliferation.

To date, several studies[1,6-17] have examined the prognostic impact of elevated CRLF2 expression or CRLF2 deregulation (CRLF2-d) in childhood ALL; however, whether CRLF2 represents a prognostic biomarker remains controversial. Because limited sample sizes in some studies might cause variations in the clinical significance of the results, we performed a systematic review and a meta-analysis to estimate the prognostic importance of CRLF2 alterations in ALL. We assessed the association of better clinical decision-making with the improvement of patient survival in childhood ALL.

Methods

Search strategy and study selection

The electronic databases PubMed, Embase, Web of Science, the Cochrane Library, ClinicalTrials.gov, China National Knowledge Infrastructure, and Wanfang Data were searched for studies to be included in the present meta-analysis. The last search was updated on March 2014, and no lower date limit was used. Search terms included "acute lymphoblastic leukemia" or "lymphoblastic lymphoma" or "precursor cell lymphoblastic leukemia-lymphoma [Mesh]", "CRLF2" or "TSLPR", "variant" or "deregulation" and "prognosis" or "outcome". The references cited by the identified studies were also screened for any other potential studies.

Studies eligible for inclusion in the meta-analysis had to meet the following criteria: 1) case-control or cohort study; 2) describing the prognostic value of CRLF2 over-expression or deregulation in childhood patients with ALL; 3) CRLF2 dichotomized as "high" and "low" in a CRLF2 over-expression study group, and as "CRLF2-normal" and "CRLF2-d" status in a CRLF2-d group; 4) investigating the association between CRLF2 alterations and patient prognosis relapse-free survival (RFS), and/or event-free survival (EFS), and/or overall survival (OS); 5) providing sufficient information about survival data; and 6) containing a 95% confidence interval (CI) for the hazard ratio (HR), or sufficient data to calculate these numbers. Two authors (Ming Jia and Yong-Min Tang) independently determined the study eligibility, and any disagreements were resolved by consensus. The following studies were excluded: 1) review articles, editorials, correspondences, case reports, meeting abstracts, and animal studies; 2) studies with insufficient data to calculate a HR; and 3) multiple articles based on the same population and published by the same research team. In such cases, only the latest or the largest population study was included.

Data extraction and quality assessment

Data retrieved from the selected studies included first author name, publication year, country, recruitment time, sample size, gender distribution, age range, ethnicity, National Cancer Institute (NCI) risk group, treatment, follow-up period, detection method of CRLF2 alterations, cut-off value, CRLF2 over-expression (%), subtype of CRLF2-d (IGH@-CRLF2 or P2RY8-CRLF2), cases with CRLF2-d, and HR and its 95% CI of relevant outcomes (EFS, RFS or OS) (Tables 1 and 2). Items were treated as "not specified" when data from any of the above categories were not reported in the primary study. We also contacted authors of some meeting abstracts for additional or unreported information. We did not use pre-specified quality-related inclusion or exclusion criteria, and did not weigh each study by a quality score because this has not received general agreement for use in a meta-analysis, especially in observational studies.[18]

Statistical analysis

Included studies were divided into two groups for analysis: those with data on CRLF2 over-expression and those regarding CRLF2-d. The main outcomes were RFS, EFS, and OS, comparing ALL patients with high CRLF2 expression to those with low expression or measuring the impact of CRLF2-d status on prognosis between the two survival distributions of ALL patients. HRs and corresponding 95% CIs were combined across the studies. When these variables were not available in an article, we estimated them from given data using methods reported by Tierney et al.[19] Some of the following data were collected to summarize the HR: HR rate, variance, patients in each experimental arm, observed events, expected events, total events, follow-up time, Kaplan-Meier curves, and *P* value of the log-rank test. Additionally, we used Engauge Digitizer 4.1 software to read survival curve data and calculated survival data using the HR calculation sheet previously published.[19]

Heterogeneity between trials was checked by the Chi-square test according to Peto's method.[20] Additionally, I^2 statistics was calculated for heterogeneity tests. Heterogeneity was defined as $P<0.10$ or $I^2>50\%$. If no heterogeneity was identified, the fixed-effects model

Table 1. Main characteristics and results of the eligible studies of CRLF2 over-expression

First author	Year	Country	Total number	Male (%)	Age (y)	Ethnicity	NCI risk group	Treatment
van der Veer[1]	2013	Netherlands/Germany	507	53.5	1-18	Caucasian	Unselected	DCOG-ALL-8/9/10 regimen COALL-97/03 regimen
Chen[7]	2012	USA	1061	51.6	1-18	Caucasian	HR/SR	COG-Trial-P9905/9906 regimen
Cario[9]	2010	Germany	555	52.4	1-18	Caucasian	Unselected	ALL-BFM-2000 regimen ALL-XH-99 protocol
Mi[8]	2012	China	752	62.5	1-18	Asian	Unselected	Chinese ALL-88 protocol ALL-2005 protocol
Palmi[10]	2012	Italy/Germany	464	51.5	1-18	Caucasian	Unselected	AIEOP-BFM-2000 protocol
Yamashita[11]	2013	Japan	194	49.0	1-18	Asian	HR/SR	JCCLSG regimen
Hertzberg[6]	2010	Israel/Italy/Germany	53	52.8	1-18	Caucasian	Unselected	ALL-BFM-90/95/99/2000 regimen COALL-06-97/07-03 protocol AIEOP-BFM-2000/AIEOP-R2006 regimen

Continued from Table 1

First author	Follow-up (mon)	Detection method	Cutoff	Number of CRLF2-h	Outcome	Multivariate/ univariate	HR (95% CI)
van der Veer[1]	NS	QRT-PCR	90th percentile	51 (10.0%)	EFS	Univariate	1.80 (1.00-3.50)
Chen[7]	96	QRT-PCR	Arbitrary value	186 (17.5%)	RFS	Multivariate	1.76 (1.28-2.41)
Cario[9]	NS	QRT-PCR	Arbitrary value	49 (9.0%)	RFS	Multivariate	1.76 (0.87-3.54)
Mi[8]	NS	QRT-PCR	5 SDs above the median	59 (16.6%)	OS	Surv curve	2.35 (1.44-3.81)
Palmi[10]	60	QRT-PCR	20 times above the median	22 (4.7%)	RFS	Multivariate	1.05 (0.37-2.97)
					OS	Surv curve	1.36 (0.19-9.90)
Yamashita[11]	72 (median)	QRT-PCR	10 times above the median	15 (10.0%)	EFS	Multivariate	2.24 (0.72-6.95)
Hertzberg[6]	NS	QRT-PCR	NS	33 (62.0%)	EFS	Surv curve	0.85 (0.13-5.57)

ALL: acute lymphoblastic leukemia; CRLF2: cytokine receptor-like factor 2; NS: not specified; HR/SR: high-risk/standard risk; CRLF2-h: CRLF2-high expression; QRT-PCR: quantitative reverse transcription-polymerase chain reaction; SDs: standard deviations; RFS: relapse-free survival; EFS: event-free survival; OS: overall survival; Surv Curve: survival curve; HR: hazard ratio; CI: confidence interval; DCOG: Dutch childhood oncology group; COALL: co-operative study group for childhood acute lymphoblastic leukemia; BFM: Berlin Frankfurt Münster; XH: XinHua; AIEOP: Italian association of pediatric hematology and oncology; JCCLSG: Japanese children's cancer & leukemia study group; COG: children's oncology group.

Table 2. Main characteristics and results of the eligible studies of CRLF2 deregulation

First author	Year	Country	Total number	Male (%)	Age (y)	Ethnicity	NCI risk group	Treatment
Ensor[12]	2011	England	865	54.3	1-18	Caucasian	HR/SR	MRC-ALL-97 regimen
Morak[13]	2012	Austria	268	53.4	1-18	Caucasian	Unselected	ALL-BFM-2000 regimen
Cario[9]	2010	Germany	70	NS	1-18	Caucasian	Unselected	ALL-BFM-2000 regimen
Palmi[14]	2013	Italy	410	52.2	1-17	Caucasian	Unselected	AIEOP-BFM-2000 regimen
Dörge[15]	2013	Germany	694	57.6	1-18	Caucasian	Unselected	ALL-BFM-2000 regimen
Buitenkamp[16]	2012	Netherlands/England	122	63.9	1-18	Caucasian	Unselected	DCOG-ALL-8/9/10 regimen UK ALL-97/99/03 regimen

Continued from Table 2

First author	Follow-up (mon)	Detection method	Subtype	Number of CRLF2-d	Outcome	Multivariate/ univariate	HR (95% CI)
Ensor[12]	EFS: 95 (median)				EFS	Multivariate	1.45 (0.88-2.39)
	RFS: 93 (median)	Fish	CRLF2-d*	52 (6%)	RFS	Multivariate	1.67 (0.96-2.91)
	OS: 95 (median)				OS	Multivariate	1.90 (1.08-3.36)
Morak[13]	EFS: 62 (median)	QRT-PCR	P2RY8-CRLF2	67 (25%)	EFS	Surv curve	2.39 (1.26-4.52)
	OS: 62 (median)				OS	Surv curve	1.90 (0.63-5.70)
Cario[9]	NS	QRT-PCR	P2RY8-CRLF2	21 (30%)	RFS	Multivariate	3.11 (1.40-6.92)
Palmi[14]	NS	RT-PCR	P2RY8-CRLF2	19 (5.1%)	EFS	Multivariate	3.25 (1.54-6.85)
					RFS	Multivariate	3.73 (1.75-7.93)
Dörge[15]	NS	NS	P2RY8-CRLF2	27 (3.89%)	EFS	Multivariate	2.04 (0.94-4.41)
Buitenkamp[16]	68 (median)	Fish	CRLF2-d	64 (54.7%)	EFS	Multivariate	1.69 (0.83-3.44)
					RFS	Multivariate	1.14 (0.39-3.36)
					OS	Multivariate	1.86 (0.87-3.94)

ALL: acute lymphoblastic leukemia; CRLF2-d: CRLF2-deregulation; HR/SR: high-risk/standard risk; NS: not specified; Fish: fluorescence *in situ* hybridization; QRT-PCR: quantitative reverse transcription-polymerase chain reaction; RFS: relapse-free survival; EFS: event-free survival; OS: overall survival; Surv curve: survival curve; HR: hazard ratio; CI: confidence interval; NCI: national cancer institute; MRC: medical research council; BFM: Berlin Frankfurt Münster; AIEOP: Italian association of pediatric hematology and oncology; DCOG: Dutch childhood oncology group. *: this subgroup comprises patients with IGH@-CRLF2 and P2RY8-CRLF2.

was applied. Otherwise, a random-effects model was used. By convention, an observed HR >1 implied worse survival for the analysis group with corresponding factors. For CRLF2-d, subgroup analysis was performed to investigate the prognostic impact of P2RY8-CRLF2 fusion on ALL patients. Two parallel analyses were performed, one focusing on univariate HRs and the other on multivariate HRs, to determine whether CRLF2 is an independent prognostic factor of different survival endpoints.

Finally, publication bias was assessed by Begg's funnel plot[21] and Egger's test.[22] *P* values <0.05 were regarded as statistically significant. Statistical analyses were performed by STATA 12.0 software (StataCorp, College Station, TX).

Results

Study selection and characteristics

The process of identifying eligible studies is shown in Fig. 1. Following stepwise selection, twelve studies[1,6-16] published in the period of 2010-2013 were eligible for meta-analysis. The total number of patients included was 5945, ranging from 53 to 1061 patients per study (median, 486). Seven studies reported the prognostic value of CRLF2 over-expression for survival in childhood ALL (Table 1). These studies were conducted in seven countries: the Netherlands, the United States, Germany, China, Italy, Japan, and Israel. Of these seven studies, two were performed in Asian populations, and the remaining five focused on non-Asian patients. The treatment options in the seven studies differed from each other.

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was used to assess CRLF2 expression in these seven studies. However, the "high" CRLF2 expression group was defined by using different cut-off values between the studies. The proportion of patients exhibiting CRLF2 over-expression ranged from 4.7% to 62.0% in individual studies.

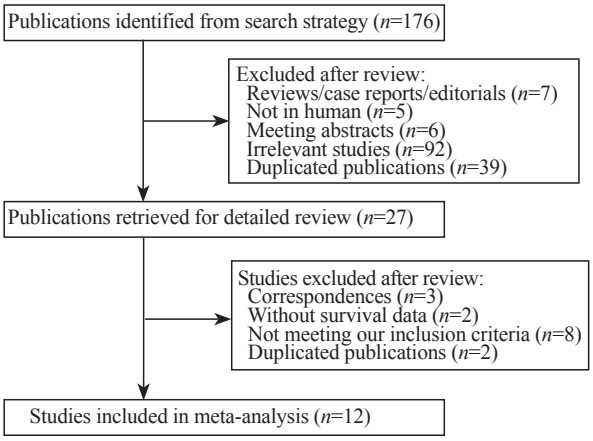

Fig. 1. Flow diagram of the meta-analysis.

The main characteristics of the six eligible publications that evaluated the prognostic value of CRLF2 deregulation for survival in childhood ALL are summarized in Table 2. These studies were conducted in five countries: England, Austria, Germany, Italy, and the Netherlands, and involved Caucasian patients. Fluorescence *in situ* hybridization analyses were used in two studies to assess CRLF2-d, while qRT-PCR/RT-PCR was used in the other three studies. Different treatment options were used in all six studies.

Two studies focused on CRLF2-d, based on IGH@-CRLF2 and P2RY8-CRLF2, while the other four studies focused on the prognostic significance of subtype P2RY8-CRLF2. The positive ratio of CRLF2-d in individual studies ranged from 3.89% to 54.7%. Five studies measured HRs and 95% CIs derived directly from multivariate analyses, and only one study reported HRs and 95% CIs derived from survival curves with different outcomes (EFS/OS).

CRLF2 over-expression and ALL prognosis

A total of seven studies including 3586 cases were evaluated for the impact of CRLF2 over-expression on the prognosis of childhood ALL. A fixed-effects model was used to combine HR values. The results of the meta-analysis are shown in Fig. 2A. Three studies were evaluated for the impact of CRLF2 over-expression on the RFS of ALL patients. The pooled HR value was 1.70 (95% CI=1.28-2.24, *P*=0.000), without evidence of heterogeneity (*P*=0.645, I^2=0.0%), suggesting that CRLF2 over-expression is associated with decreased RFS in ALL patients. The combined data on EFS showed that patients with CRLF2 over-expression had a shorter EFS (HR=1.78, 95% CI=1.05-3.01, *P*=0.032) than those without CRLF2 over-expression, with no evidence of data heterogeneity (*P*=0.686, I^2=0.0%). The pooled HR of CRLF2 over-expression on ALL patient survival for OS was 2.28 (95% CI=1.42-3.65, *P*=0.001) without evidence of heterogeneity (*P*=0.598, I^2=0.0%).

We then conducted two parallel analyses on univariate HRs and multivariate HRs. The combined HR for three eligible studies evaluating CRLF2 over-expression on RFS by multivariate analysis was 1.70 (95% CI=1.28-2.24), with no significant heterogeneity (*P*=0.645, I^2=0.0%). The fixed-effects model was adopted (Fig. 2B). These results suggested that CRLF2 over-expression is an independent prognostic factor of poor RFS for childhood ALL. However, a pooled analysis according to data type could not be performed for EFS and OS because of the lack of original data.

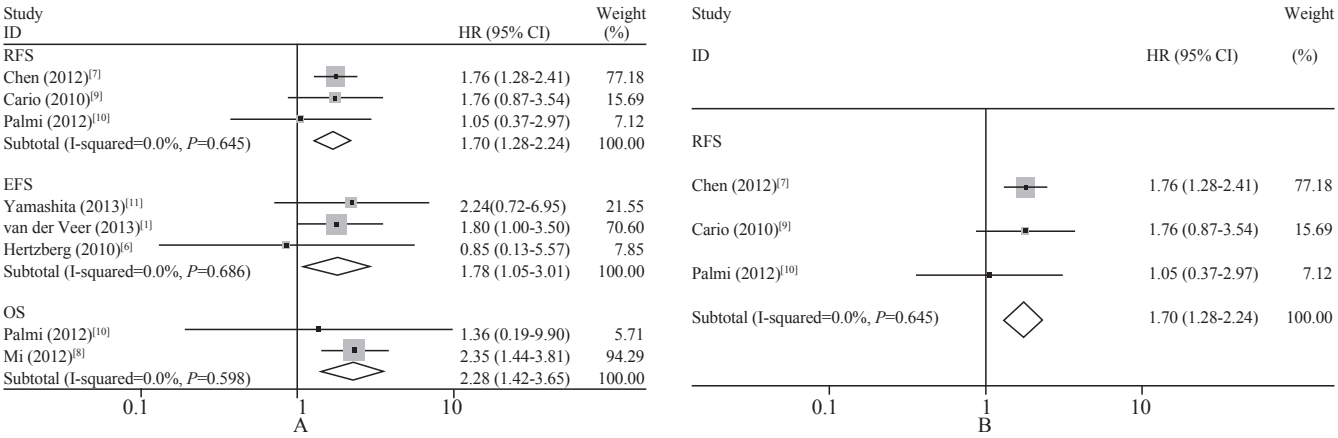

Fig. 2. A: Meta-analysis (forest plot) of the seven evaluable studies assessing CRLF2 over-expression in ALL stratified by outcome (RFS, EFS, and OS); **B:** Subgroup analysis assessing CRLF2 over-expression with RFS prognosis involving multivariate HRs. Studies are shown with first author name and HR with 95% CIs. Summary HRs and 95% CIs are also shown (overall). CRLF2: cytokine receptor-like factor 2; ALL: acute lymphoblastic leukemia; CIs: confidence intervals; HR: hazards ratio; EFS: event-free survival; RFS: relapse-free survival; OS: overall survival.

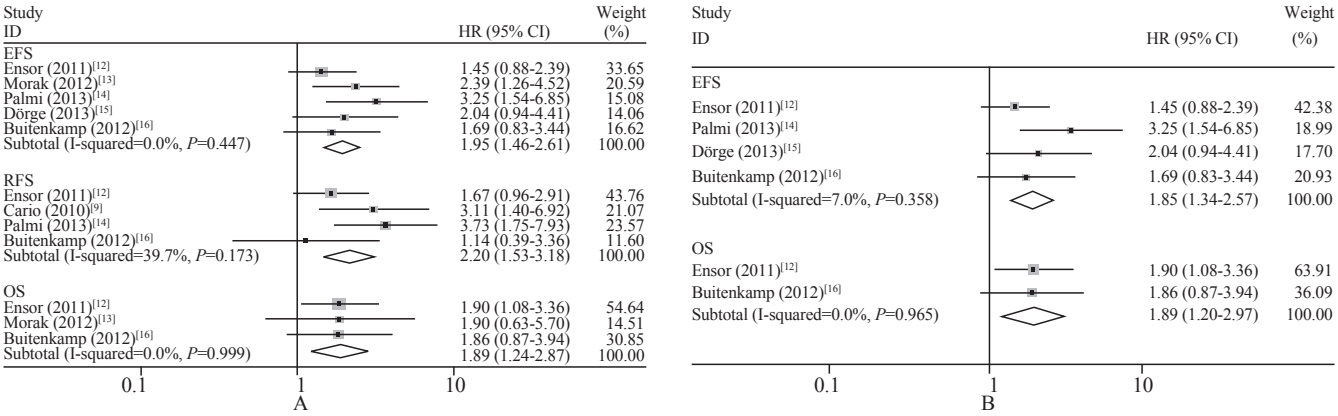

Fig. 3. A: Meta-analysis (forest plot) of the six evaluable studies assessing CRLF2 deregulation in ALL stratified by outcome (EFS, RFS, and OS); **B:** Subgroup analysis assessing CRLF2 deregulation with EFS and OS prognosis involving multivariate HRs. Studies are shown with first author name and HR with 95% CIs. Summary HRs and 95% CIs are also shown (overall). CRLF2: cytokine receptor-like factor 2; ALL: acute lymphoblastic leukemia; CIs: confidence intervals; HR: hazards ratio; EFS: event-free survival; RFS: relapse-free survival; OS: overall survival.

No evidence for significant publication bias was identified for RFS (Begg's test, P=0.296; Egger's test, P=0.437), EFS (Begg's test, P=1.000; Egger's test, P=0.627), or OS (Begg's test, P=1.000; Egger's test P value could not be calculated) in the whole group analysis.

CRLF2 deregulation and ALL prognosis

In six studies of 2429 cases, the survival data (EFS, RFS, and OS) were assessed for the impact of CRLF2-d on the prognosis of childhood ALL. Fig. 3A shows the forest plot of the association between CRLF2-d and prognosis in childhood ALL. A fixed-effects model was used to combine the HR values. The pooled HR for EFS showed that CRLF2-d carriers had a worse EFS than non-carriers (HR=1.95, 95% CI=1.46-2.61, P=0.000). No heterogeneity was observed (P=0.447, I^2=0.0%). Combined RFS data showed that patients with CRLF2-d had a shorter RFS than those without CRLF2-d (HR=2.20, 95% CI=1.53-3.18, P=0.000), and that no significant heterogeneity was observed in the data (P=0.173, I^2=39.7%). We identified three studies assessing the OS of CRLF2-d carriers and the pooled HR of the CRLF2-d effect on ALL patient survival for OS was 1.89 (95% CI=1.24-2.87, P=0.003). There was no evidence of heterogeneity (P=0.999, I^2=0.0%).

Fig. 3B shows the results of the subgroup analysis according to data type. The fixed-effects model was adopted to combine the HR values. In studies reporting HRs based on multivariate analysis, CRLF2-d was significantly associated with poor EFS in ALL patients (HR=1.85, 95% CI=1.34-2.57, P=0.000). The same result was observed for OS (HR=1.89, 95% CI=1.20-2.97, P=0.006). No significant heterogeneity was observed in the studies on EFS (P=0.358, I^2=7.0%) and OS (P=0.965, I^2=0.0%). These results suggest that CRLF2-d is an independent prognostic factor of poor prognosis for childhood ALL.

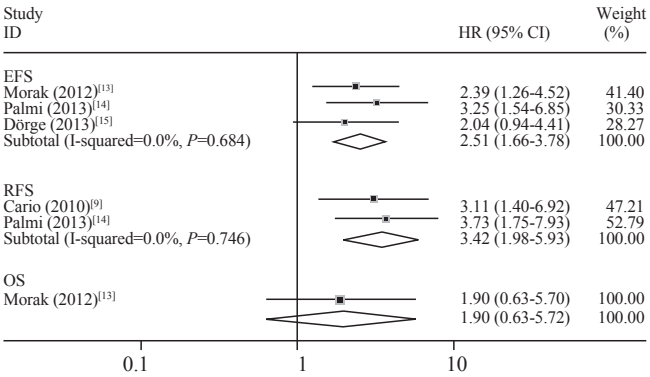

Fig. 4. Meta-analysis (forest plot) of eligible studies evaluating the association between P2RY8-CRLF2 and survival in ALL. Studies are shown with first author name and HR with 95% CIs. Summary HRs and 95% CIs are also shown (overall). CRLF2: cytokine receptor-like factor 2; ALL: acute lymphoblastic leukemia; CIs: confidence intervals; HR: hazards ratio; EFS: event-free survival; RFS: relapse-free survival; OS: overall survival.

To assess whether P2RY8-CRLF2 is associated with the prognosis of childhood ALL, we pooled the data into a subgroup analysis (Fig. 4). This stratified analysis indicated that P2RY8-CRLF2 was associated with a poor ALL prognosis in terms of both EFS and RFS, with a pooled HR of 2.51 (95% CI=1.66-3.78, P=0.000) and 3.42 (95% CI=1.98-5.93, P=0.000), respectively, using a fixed-effects model with no significant heterogeneity (I^2=0.0%, P=0.684 and I^2=0.0%, P=0.746, respectively).

Begg's funnel plot and Egger's test of the whole group analysis indicated that there was no risk of publication bias in this meta-analysis (EFS: Begg's test P=0.462, Egger's test P=0.195; RFS: Begg's test P=1.000, Egger's test P=0.958; OS: Begg's test P=1.000, Egger's test P=0.878).

Discussion

ALL is a markedly heterogeneous disease with a complex spectrum of genetic abnormalities, including recurring chromosomal translocations, and genomic deletions and amplifications.[23-26] The characterization of molecular and cytogenetic abnormalities has not only provided insights into the mechanisms of leukemogenesis, but also led to the establishment of new treatment strategies targeting these abnormalities.

To date, several studies[1,6-17] have examined the prognostic impact on CRLF2 alterations in ALL, however, the use of CRLF2 as a prognostic biomarker remains controversial. To our knowledge, this meta-analysis is the first study to systematically evaluate the prognostic role of CRLF2 alterations in ALL. Our results show that both CRLF2 over-expression and deregulation are associated with poor prognosis in childhood ALL, while subgroup analyses according to multivariate HRs reveal that both CRLF2 over-expression and CRLF2-d are independent prognostic factors of poor prognosis for childhood ALL. Therefore, the detection of CRLF2 alterations may be useful to stratify patients at high risk, guide their clinical surveillance, and to select the most appropriate chemotherapy.

Children with Down syndrome (DS) have a higher rate of associated ALL. DS-ALL has been shown to be a highly heterogeneous disease that is not definable as a unique entity.[27] Recently, CRLF2 abnormalities have been described in approximately 60% of DS-ALL patients. In our study, the conclusion didn't change substantially despite we included two studies[6,16] focused primarily on the prognosis of DS-ALL based on CRLF2 aberrations.

The present meta-analysis has a number of limitations. First, the original studies might be different in patient characteristics such as age, sex distribution, ethnicity, NCI risk group, and treatment received. These may contribute to between-study heterogeneity. A comparative analysis of Italian association of pediatric hematology and oncology-Berlin Frankfurt Münster study group and the UK national cancer research institute-children's cancer and leukemia group[28] revealed that P2RY8-CRLF2+ patients had an intermediate protocol-independent outcome. However, it remains to be determined whether different treatment protocols affect the outcome of patients with CRLF2 alterations. Second, the method of detecting CRLF2 alterations was not standardized between the studies, and the application of different cut-off values for determining high CRLF2 levels is a potential source of bias. Thus, we propose that well-defined standardized methods should be used in the future to reproducibly evaluate biological markers. Third, the method of HR and 95% CI extrapolation is another potential source of bias. In our study, if multivariate survival analysis data were unavailable, we extrapolated them from the survival curves, which could have caused heterogeneity. Fourth, as there was a limited number of prospectively designed prognostic studies concerning biomarkers and our meta-analysis was only concerned with retrospective studies, the results should be confirmed by well-designed prospective studies. Additionally, our findings should be interpreted with caution because of the small number of studies included in each group analysis. Further high-quality and large-sample studies are therefore needed to confirm our results. Finally, publication bias is inevitable, even though not evident from statistical testing, because negative or controversial results might not be reported. To minimize publication bias, we attempted to retrieve all relevant data that are unavailable from the published reports, but

it is likely that some data are still missing.

In conclusion, CRLF2 over-expression and deregulation could both be used as biomarkers to predict the prognosis of patients with childhood ALL. Based on the current findings, an assessment of CRLF2 alterations would provide improved prognostic information and could be used as a novel therapeutic target for patients with ALL. To strengthen our findings, well-designed prospective studies with a standardized assessment of prognostic markers should be conducted to explore the relationship between CRLF2 and survival of pediatric patients with ALL.

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