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Research paper

# *CRLF2* overexpression identifies an unfavourable subgroup of adult B-cell precursor acute lymphoblastic leukemia lacking recurrent genetic abnormalities

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ABSTRACT

*Background:* A deregulated *CRLF2* (d-*CRLF2*) expression was described in B-cell acute lymphoblastic leukemia without recurrent fusion genes (B-NEG ALL). While the role of d-*CRLF2* in children has been extensively described, little is known about its role and impact in adult ALL.
*Methods:* Expression levels of *CRLF2* were evaluated by quantitative real-time PCR in 102 newly-diagnosed adult B-NEG ALL and correlated with the clinico-biological characteristics and outcome. Incidence and clinical impact of the *P2RY8/CRLF2* transcript was also assessed.
*Results:* High *CRLF2* levels, as continuous variable, were significantly associated with hyperleucocytosis ($p = 0.0002$) and thrombocytopenia ($p = 0.005$); when a cut-point at $\Delta Ct \leq 8$ was applied, 35 cases (34.3%), mostly males (80%), proved positive for *CRLF2* expression. High *CRLF2* levels, as continuous or categorical variable, were associated with a worse disease-free ($p = 0.003$ and $p = 0.015$) and overall survival ($p = 0.017$ and 0.0038). Furthermore, when *CRLF2* was analyzed as a categorical variable, a high statistical association was found with *IKZF1* deletion and mutations in the JAK/STAT pathway ($p = 0.001$ and $p < 0.0001$, respectively). Finally, the *P2RY8/CRLF2* transcript, identified in 8/102 patients (7.8%), was associated with a poor outcome.
*Conclusions:* In adult B-NEG ALL, high *CRLF2* expression is associated with distinct clinico-biological features and an unfavourable prognosis in both univariate and multivariate analysis; similarly, *P2RY8/CRLF2* positivity correlates with a poor outcome. The quantification of *CRLF2* is an important prognostic marker in adult B-lineage ALL without known genetic lesions.



## 1. Introduction

Acute lymphoblastic leukemia (ALL) is the most common cancer in children, while it is relatively rare in adolescents and adults. Despite significant improvements in the management of the disease in children, with overall survival rates of 80–85%, the outcome in adult patients is still unsatisfactory, with long-term survival rates that generally do not exceed 40–50% [1,2].

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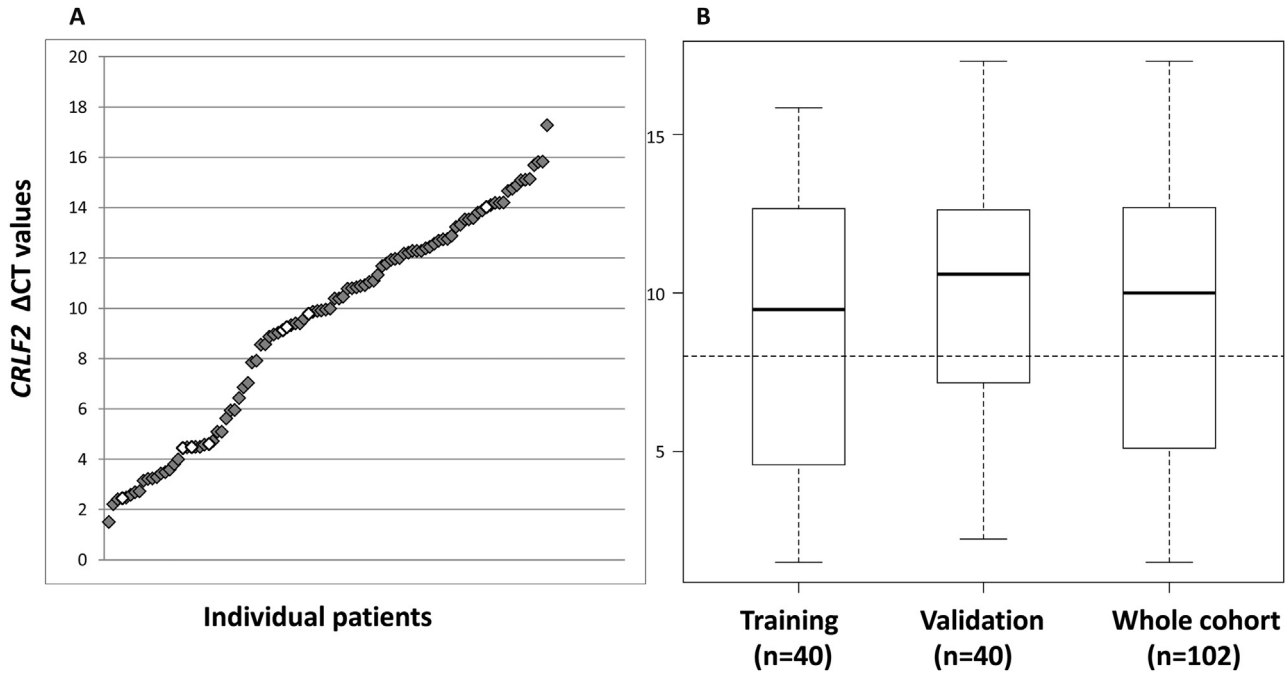


**Fig. 1.** (A) *CRLF2* expression levels among the 102 patients analyzed, represented by ΔCT values. Lower ΔCT values correspond to higher mRNA expression levels. White diamonds represent cases with the *P2RY8/CRLF2* transcript. (B) Box plots of the training and validation cohorts, as well as of the whole population; box plots define the median values, 25–75% of values around the median, and the range of values.

In the last decades, the use of array-based approaches and next-generation sequencing techniques allowed to identify an increasing number of genetic alterations. These include deletions of B-cell differentiation, cell cycle control genes and mutations of genes involved in key signalling pathways [3]. Among the novel lesions identified in both pediatric and adult cohorts, a deregulated expression of the cytokine receptor-like factor 2 (d-*CRLF2*) was described in B-lineage ALL lacking recurrent genetic aberrations (herein defined as B-NEG ALL), while it is virtually absent in other molecularly defined subgroups [4,5]. The *CRLF2* gene is located in the pseudoautosomal region (PAR1) of sex chromosomes at Xp22.3/Yp11.3 and encodes the cytokine receptor-like factor 2 (alias thymic stromal lymphopoietin receptor) which heterodimerises with the IL7 receptor alpha (IL7Rα) to form a receptor for thymic stromal lymphopoietin [6]. An elevated *CRLF2* expression has been ascribed to two main distinct genomic lesions: a cryptic chromosomal translocation, that juxtaposes *CRLF2* to the immunoglobulin heavy chain locus (IGH) [7] or an interstitial deletion of part of the PAR1 region centromeric to *CRLF2*, resulting in *CRLF2* positioning under the control of the *P2RY8* promoter [8]. Other mechanisms leading to an elevated *CRLF2* expression are the presence of additional copies of the *CRLF2* locus, presumably through supernumerary X chromosomes, or a rare point mutation at codon 232 (F232C), which substitutes a phenylalanine with a cysteine [9,10].

It was shown that an increased *CRLF2* expression is associated with activating *JAK1/JAK2* mutations and *IKZF1* lesions, and it seems to correlate with a BCR/ABL1-like genomic profile and a poor outcome [11,12]. Furthermore, d-*CRLF2* is observed in about 50% of children with Down syndrome and B-lineage ALL, but in this subset of patients it does not appear to confer a worse prognosis [13–15].

While the frequency and prognostic relevance of the abnormal *CRLF2* expression was extensively investigated in pediatric cases, little is known about its role in adult ALL [4,5]. In this study, *CRLF2* expression levels were analyzed in 102 newly-diagnosed adult B-NEG ALL cases to evaluate if *CRLF2* expression levels differ among young adults, adults and elderly, and to identify a correlation between increased *CRLF2* expression, regardless the underlying molecular lesion, the clinico-biological characteristics and outcome of adult B-NEG ALL.

## 2. Methods

### 2.1. Patients' characteristics

Peripheral blood or bone marrow samples with ≥70% blasts from 102 adults (median age: 45 years, range: 19–81) with newly-diagnosed B-NEG ALL, according to the WHO classification [16], were analysed. Only samples that proved negative for the recurrent fusion genes *BCR/ABL1*, *ETV6/RUNX1*, *E2A/PXB1*, *MLL/AFF1*, *MLL1/ENL*, *SIL/TAL1*, *SET/NUP214*, *NUP98/RAP1GDS1* [17] were included. Sixty-eight patients were males and 34 females; median white blood cell (WBC) and platelet (PLT) counts were $16.3 \times 10^9$/L and $44 \times 10^9$/L, respectively, and mean hemoglobin (Hb) level was 8.9 g/dl (Table A.1). Flow-cytometry showed a pro-B stage in 7 cases, a common B-ALL in 55 and a pre-B in 10. Four age-cohorts were considered: 18–34 years ($n = 35$); 35–44 years ($n = 20$); 45–55 years ($n = 18$); >55 years ($n = 29$).

Patients were enrolled into different GIMEMA protocols (Table A.2). The study was approved by the local IRB and conducted in accordance with the Declaration of Helsinki. All patients gave their written informed consent to blood collection and biologic analyses.

### 2.2. *Quantitative* CRLF2 *expression and detection of the* P2RY8/CRLF2 *rearrangement*

One microgram of total RNA was retrotranscribed using the Advantage RT-for-PCR Kit (Clontech, Mountain View, CA). To evaluate the expression levels of *CRLF2*, a real-time quantitative polymerase chain reaction (Q-PCR) analysis was performed with an ABI PRISM 7300 sequence detection system and the SYBR green dye (Applied Biosystems, Foster City, CA) (Additional Materials and Methods in Supplementary data). *CRLF2* quantitative values were expressed by means of ΔCt.