An Insight about Cytokine Receptor Like Factor 2 Expression in Philadelphia Like Acute Lymphoblastic Leukemia

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Abstract

Philadelphia chromosome-like B-cell acute lymphoblastic leukemia (Ph-like ALL) accounts for 15% to 30% of B-cell acute lymphoblastic leukemia in older children, adolescents, and adults and is associated with high rates of conventional treatment failure and relapse. Current clinical trials are assessing the efficacy of the addition of tyrosine kinase inhibitors (TKIs) to chemotherapy for children and adults with Ph-like ALL harboring ABL class translocations or Cytokine Receptor Like Factor 2(CRLF2) rearrangements and other JAK pathway alterations. In this article we highlight CRLF2possible role in Diagnosis of Philadelphia Like Acute Lymphoblastic Leukemia.Ph-like ALL is characterized by a wide range of genetic alterations that dysregulate several cytokine receptor and kinase signaling pathways, including CRLF2 rearrangement in half of the cases and translocation of non-receptor tyrosine kinases (predominantly ABL-class and Janus kinases).Overexpression of cytokine receptor-like factor 2 (CRLF2) resulting from its genomic rearrangement is the most frequent genetic alteration found in Philadelphia chromosome-like (Ph-like) B-cell acute lymphoblastic leukemia (B-ALL), a high-risk leukemia. Detection of CRLF2 expression by multiparameter flow cytometry has been proposed as a screening tool for the identification of Ph-like B-ALL. However, the prognostic relevance of flow cytometric expression of CRLF2 in pediatric B-ALL is not very clear. Additionally, its association with common copy number alterations (CNA) has not been studied in detail.**Conclusion:**The mechanisms of CRLF2 overexpression in leukemia are not fully understood but several studies show that it has a prognostic impact. JAK and mTOR inhibitors have been explored in preclinical models as potential therapies targeting CRLF2-rearranged ALL.

Keywords: Cytokine Receptor Like Factor 2, Philadelphia Like Acute Lymphoblastic Leukemia

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Introduction

The pathogenesis of ALL involves the abnormal proliferation and differentiation of a clonal population of lymphoid cells. Studies in the pediatric population have identified genetic syndromes that predispose to a minority of cases of ALL, such as Down syndrome, Fanconi anemia, Bloom syndrome, ataxia telangiectasia and Nijmegen breakdown syndrome ***(1).*** Other predisposing factors include exposure to ionizing radiation, pesticides, certain solvents or viruses such as Epstein-Barr Virus and Human Immunodeficiency Virus. However, in the majority of cases, it appears as a de novo malignancy in previously healthy individuals. Chromosomal aberrations are the hallmark of ALL, but are not sufficient to generate leukemia. Characteristic translocations include t(12;21) [ETV6-RUNX1], t(1;19) [TCF3-PBX1], t(9;22) [BCR-ABL1] and rearrangement of MLL ***(1).*** More recently, a variant with a similar geneexpression profile to (Philadelphia) Ph-positive ALL but without the BCR-ABL1 rearrangement has been identified. In more than 80% of cases of this so-called Ph-like ALL, the variant possesses deletions in key transcription factors involved in B-cell development including IKAROS family zinc finger 1 (IKZF1), transcription factor 3 (E2A), early B-cell factor 1 (EBF1) and paired box 5 (PAX5) ***(2).***

Similarly, kinase-activating mutations are seen in 90% of the Ph-like ALL. The most common of these include rearrangements involving ABL1, JAK2, PDGFRB, CRLF2 and EPOR, activating mutations of IL7R and FLT3 and deletion of SH2B3, which encodes the JAK2-negative regulator LNK. This has significant therapeutic implications as it suggests that Ph-like ALL, which tends to carry a worse prognosis, may respond to kinase inhibitors ***(3).***In fact, It was showed that cell lines and human leukemic cells expressing ABL1, ABL2, CSF1R and PDGFRB were sensitive in vitro and in vivo human xenograft models to second-generation TKIs (for example, dasatinib.); those with EPOR and JAK2 rearrangements were sensitive to JAK kinase inhibitors (for example, ruxolitinib); and those with ETV6-NTRK3 fusion were sensitive to ALK inhibitors crizotinib***(1).*** Furthermore, Holmfeldtand his colleagues described that the genetic basis of another subset with poor outcomes was hypodiploid ALL. In near-haploid (24–31 chromosomes) ALL, alterations in tyrosine kinase or Ras signaling was seen in 71% of cases and in IKAROS family zinc finger 3 (IKZF3) in 13% of cases. In contrast, low-hypodiploid (32–39 chromosomes) ALL, alterations in p53 (91%), IKZF2 (53%) and RB1 (41%) were more common. Both near haploid and low-hypodiploid exhibited activation of Ras- and PI3K-signaling pathways, suggesting that these pathways may be a target for therapy in aggressive hypodiploid ALL ***(4).***

Most of the clinical manifestations of ALL reflect the accumulation of malignant, poorly differentiated lymphoid cells within the bone marrow, peripheral blood, and, extramedullary

sites. Presentation can be nonspecific, with a combination of constitutional symptoms and signs of bone marrow failure (anemia, thrombocytopenia, leukopenia). Common symptoms include 'B symptoms' (fever, weight loss, night sweats), easy bleeding or bruising, fatigue, dyspnea and infection *(1)*. Involvement of extramedullary sites commonly occurs and can cause lymphadenopathy, splenomegaly or hepatomegaly in 20% of patients. CNS involvement at time of diagnosis occurs in 5–8% of patients and present most commonly as cranial nerve deficits or meningismus. T-cell ALL also may present with a mediastinal mass *(5)*

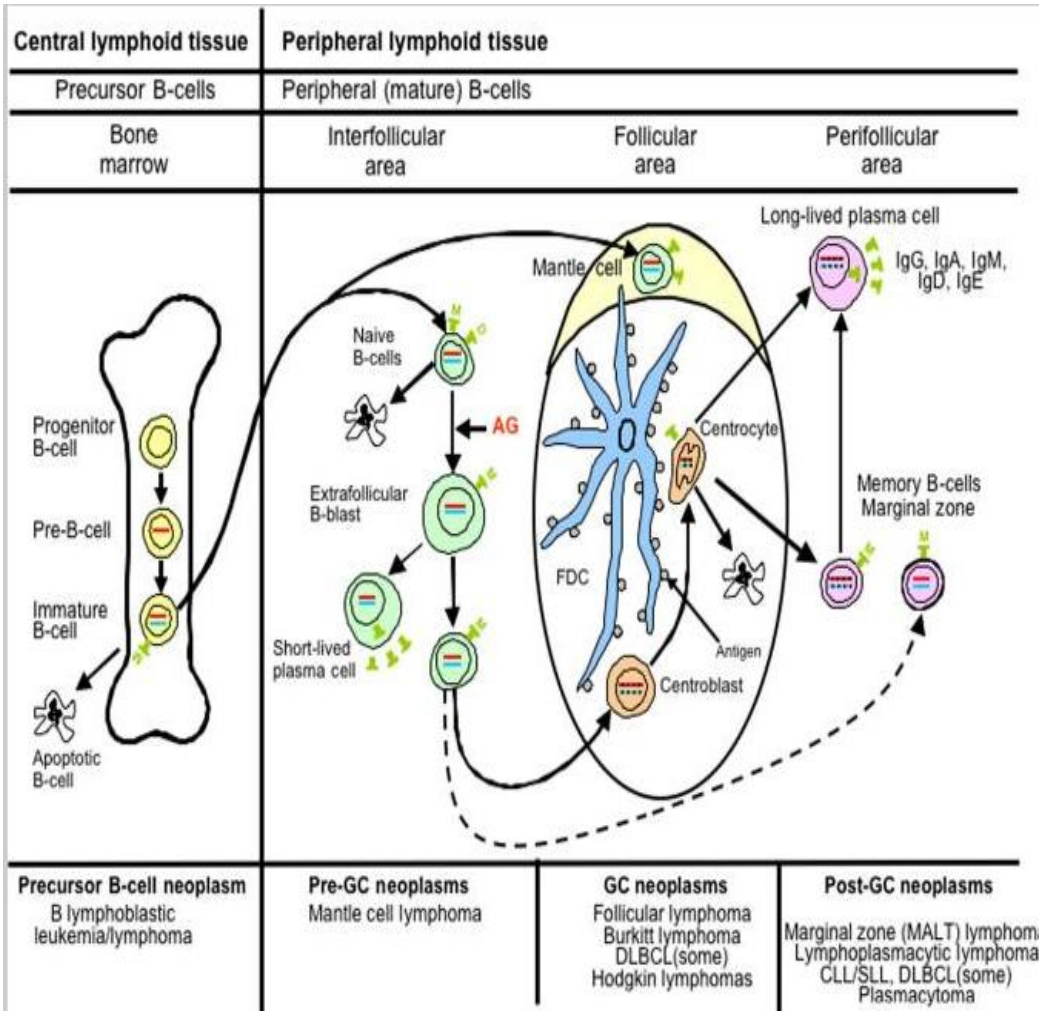

Figure (1): B-cell development

Diagnosis is established by the presence of 20% or more lymphoblasts in the bone marrow or peripheral blood. Evaluation for morphology, flow cytometry, Immunophenotyping and cytogenetic testing is valuable both for confirming the diagnosis and risk stratification. Lumbar puncture with CSF analysis is standard of care at the time of diagnosis to evaluate for CNS involvement *(6)*. If the CNS is involved, brain MRI should be performed. Other evaluation includes complete blood count with differential and smear to evaluate the other hematopoietic

cell lines, coagulation profiles and serum chemistries. Baseline uric acid, calcium, phosphate and lactate dehydrogenase should be recorded to monitor for tumor lysis syndrome ***(7).***

ALL is a heterogeneous disease characterized by multiple structural variations, mutations and chromosomal rearrangements that affect epigenetic regulation, cell growth and proliferation and eventually perturb normal lymphoid maturation. The widespread use of genome sequencing and profiling has shaped our understanding of the genetic basis of ALL and allowed researchers to identify recurring genetic abnormalities and subsequently to define new subtypes of ALL such as Ph-like ALL. Based on the altered pathways, patients with Ph-like ALL are subdivided into 3 main groups (kinase alterations, cytokine receptor alterations and other less frequent pathways activation like RAS pathway) ***(8).***

Kinase pathway alteration: the majority of Ph-like ALL cases (90%) have activating kinase alterations, particularly deletions of IKAROS Family Zinc Finger 1 (IKZF1) which are found in up to 80% of cases. Normally IKZF1 is involved in B cell differentiation, and alterations of IKZF1 function portend a poor prognosis in Pre B- ALL. The 5' part of the fusion transcript leads to constitutive tyrosine kinase activation with no need for receptor stimulation or ligand binding, and the 3' part of the fusion transcript determines the cascade of downstream signal transduction, and potentially which inhibitors could inhibit the activated cascade and therefore inhibit leukemic cell growth. ABL gene rearrangements and fusions with different partner genes eventually leading to ABL kinase activation and leukemo-genesis are also commonly identified in Ph-like ALL cases (9.8%-12.6%). Other less common altered kinases include: platelet-derived growth factor receptor (PDGFR A and B), colony stimulating factor 1 receptor (CSF1R), fms-related tyrosine kinase 3 (FLT3), diacylglycerol kinase eta (DGKH), neurotrophic receptor tyrosine kinase 3 (NTRK3), protein tyrosine kinase 2 beta (PTK2B) and B-cell linker (BLNK) ***(9).***

Cytokine receptors pathway alterations: CRLF2 alteration is a frequent abnormality in adult Ph-like ALL (50%-60%) and tends to occur in older patients presenting with higher white blood cell count as compared to non CRLF2 rearranged Ph-like ALL. Additionally CRLF2 appears to cluster in Hispanic patients as compared to other ethnicities (78% of patients with CRLF2 overexpression were Hispanic), with the majority of rearrangements involving IGH-CRLF2 (57.6%-76%), followed by P2RY8-CRLF2 (17%-21%) ***(3).*** **CRLF2** encodes cytokine receptor-like factor 2 monomers, which in combination with IL7R-alpha subunit, form a heterodimeric receptor for thymic stromal lympho-poetin (TSLP)***(10).***

Cytokine receptor-like factor 2 (CRLF2), also known as TSLPR, encodes for a receptor protein that participates in activating STAT, possibly through JAK pathways. These pathways are important in immune system regulation. In cancer, CRLF2 rearrangements and one recurring mutation leading to CRLF2 over expression have been identified in a subset of patients with

high-risk acute lymphoblastic leukemia who have an exceptionally dismal prognosis.The cytokine receptor-like factor2 (CRLF2) gene mapped to pseudoautosomal region, Xp22.3 and Yp11.3 that encodes a subunit of thymic stromal lymphopoietin (TSLPR) receptor which is highly expressed by early B-and T- cell progenitors, mast cells and dendritic cells. **(11).**

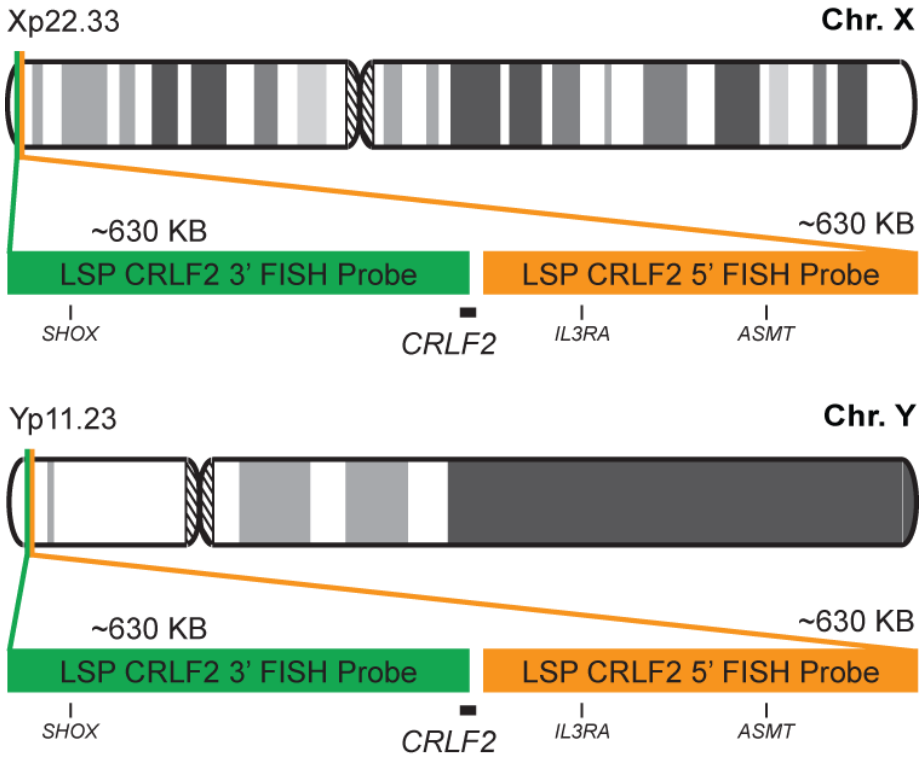

Figure (2): Genetic map of CRLF2 gene.

Cytokine Function in Normal B-cells

For the complex process of B-cell differentiation to occur, developing B-cells depend on extrinsic factors that are present in the BM microenvironment including non-hematopoietic cells such as stromal cells and the factors that they secrete. **(12)**

In the bone marrow, B-cells associate with bone marrow stromal cells (BMSCs), these BMSCs support the development of hematopoietic (blood) cells including B- cells by secreting cytokines (growth factors) that are required for the B-cells to develop.

Cytokines are described as peptides or glycoproteins that stimulate cell growth, differentiation and survival. CXCL12, Flt3, and interleukin 7 (IL-7) are among cytokines that are produced by BMSCs and are required for the development of B-cells **(13)**

IL-7 induces the expansion of human Pro-B cells, which is crucial for providing large cell numbers to progress through the stages of B-cell differentiation. **(14).**

The cytokine Thymic Stromal Lymphopoietin (TSLP), which is produced by epithelial cells of the lung and skin as well as BMSCs has also been shown to contribute to B-development. **(15)**

In addition to activating dendritic cells, regulating inflammation and contributing to asthma and allergic responses; TSLP also induces proliferation in human pro-B cells and promotes B-cell survival by protecting cells from apoptosis. **(16)**

TSLP and IL-7 Signaling in Normal B-cells

TSLP performs its function in B-cells by binding to its receptor, which comprises of two subunits: IL-7Rα and CRLF2 . Upon binding to its receptor, TSLP traditionally activates the JAK-STAT pathway**. (17)**

CRLF2 binds its receptor subunits, which dimerize leading to the recruitment and activation of Janus kinases (JAK1 and JAK2) via cross-phosphorylation followed by phosphorylation of tyrosine residues found in the cytoplasmic domain of the receptors. **(18).**

Signal transducers and activators of transcription-STAT (primarily STAT5) proteins-bind to the tyrosine-phosphorylated receptors via their SH2 domains and become phosphorylated by the JAKs. STATs then dissociate from the receptors, form dimers, translocate to the nucleus and bind to the promoter of target genes in order to regulate gene transcription. Studies have shown that STAT5 regulates B-cell survival and differentiation; a process that is mediated by the Bcl2 family members .**(19).**

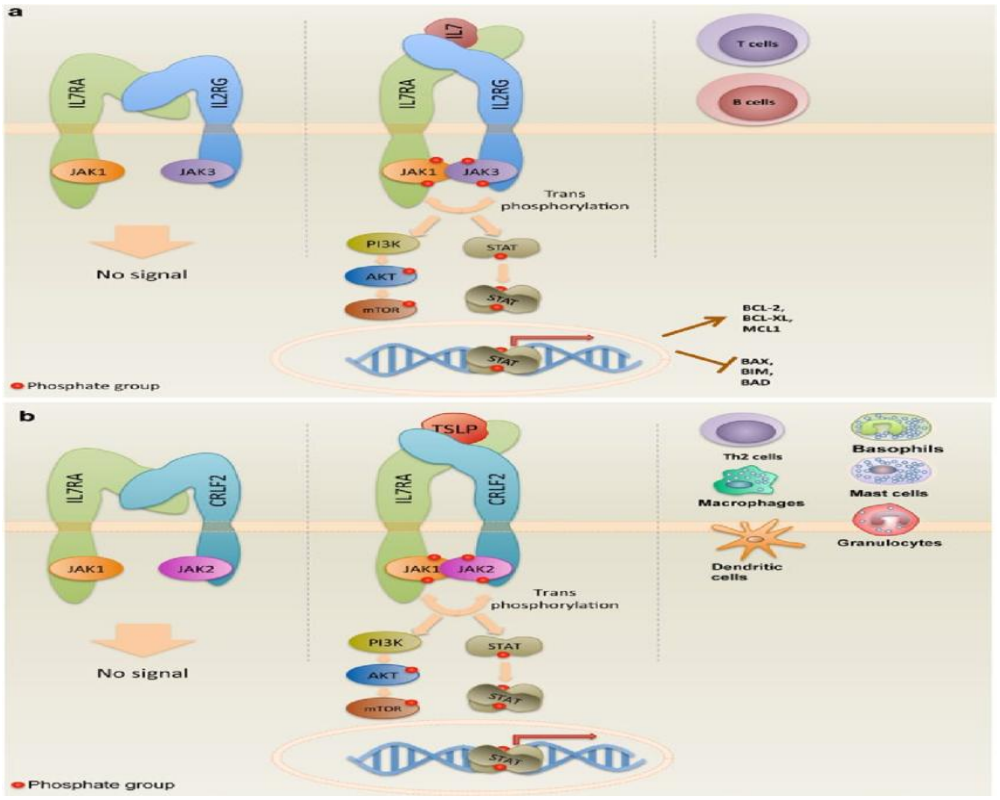

Figure (3): TSLP and IL-7 Signaling in B-cells.

JAK and mTOR inhibitors have been explored in preclinical models as potential therapies targeting CRLF2-rearranged ALL. **(20).**

Since the initial description of deregulated CRLF2 expression in B-cell precursor acute lymphoblastic leukemia (ALL) in 2009, several studies have described various correlations between elevated *CRLF2*mRNA expression, genomic lesions affecting *CRLF2*, clinical features, and treatment outcome.**(21)**

Two genomic lesions have been identified that result in elevated expression of wild-type *CRLF2*: 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@*), or, an interstitial deletion of some of the PAR1 region centromeric to *CRLF2*, resulting in *CRLF2* expression being driven by the *P2RY8*promoter.**(22)**

Another possible mechanism for elevated *CRLF2* expression, although not yet well studied, may be related to the presence of additional copies of the *CRLF2* locus, presumably through chromosomal gain. A less common alteration of *CRLF2* is a point mutation at codon 232 (F232C), which substitutes a phenylalanine with a cysteine. **(23)**

CRLF2 overexpression however, requires cooperating lesions for leukemic transformation, and approximately 50% of CRLF2-rearranged (CRLF2-r) cases harbor activating mutations in JAK2. **(24)**

Patients with CRLF2 overexpression may harbor JAK2 mutations, presenting a subset that can benefit from specific therapeutic strategies targeting these abnormalities, which may improve the prognosis of B-ALL. **(25)**

The JAK2 mutations in BCP-ALL are predominantly missense mutations that cluster in exon 16 within the pseudo kinase domain. These lesions are distinct from the JAK2 V617F mutations associated with myeloproliferative neoplasms, and the expression of CRLF2 with the JAK2 mutations observed in ALL induces factor-independent transformation of cell lines in vitro.**(26)**

Interestingly, the CRLF2 rearranged cases that lack JAK2 mutations commonly harbor CRLF2 or IL7R mutations that promote constitutive receptor dimerization and downstream JAK/STAT signaling **(27).**

CRLF2 high expression is not only caused by the P2RY8/CRLF2fusion but, in a similar number of cases, by additional aberrations, which are only partly known. A translocation involving the immunoglobulin heavy chain (IGH) locus on chromosome leads to CRLF2 high expression **(28)** and, recently, a novel rare chimericCSF2RA/CRLF2 fusion transcript with a deletion of the CRLF2 pro-moter region was identified, suggesting that an enhancer of colony-stimulating factor 2 receptor, alpha, low-affinity (granulocyte-macrophage) (CSF2RA) may control the expression of CRLF2**(8).**

The mechanisms of CRLF2 overexpression in leukemia are not fully understood but several studies show that it has a prognostic impact. JAK and mTOR inhibitors have been explored in preclinical models as potential therapies targeting CRLF2-rearranged ALL**(29).**

No Conflict of interest.

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