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Understanding the Biology of *CRLF2*-Overexpressing Acute Lymphoblastic Leukemia

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Abstract

Recent genomic analyses of childhood and adult B-precursor acute lymphoblastic leukemia (ALL) samples have identified novel genetic alterations in essential lymphoid development and signal transduction pathways, providing insight into the pathogenesis of high risk ALL associated with treatment failure. Particular advances have been made in unraveling the genetics of ALL associated with overexpression of the cytokine receptor-like factor 2 gene (*CRLF2*), which is frequently accompanied by simultaneous activating mutations in genes encoding Ikaros (*IKZF1*), Janus kinase 1 (*JAK1*) and Janus kinase 2 (*JAK2*), and/or the IL-7 receptor alpha chain (*IL7RA*). Children and adults with high risk *CRLF2*-overexpressing ALL have high rates of relapse and dismal overall survival. Various groups have thus attempted to characterize the biochemical consequences of these genetic lesions via preclinical models with the goal of identifying targets for new therapies. These studies provide early data suggesting the promise of signal transduction inhibitors (STIs) of the JAK/STAT and PI3K pathways for *CRLF2*-overexpressing ALL. Additional research efforts continue to elucidate these aberrant signaling networks to provide rationale for bringing STIs into the clinic for these high risk patients. This review highlights the current knowledge of the incidence, prognostic significance, and biology of *CRLF2*-overexpressing ALL and the future directions for development of targeted therapies.

Keywords

acute lymphoblastic leukemia; *CRLF2*; *JAK2*; signal transduction; TSLP; TSLPR

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I. IDENTIFICATION OF NEW GENETIC LESIONS IN ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

B-precursor acute lymphoblastic leukemia is the most common malignancy of childhood; approximately 3000 new cases are diagnosed annually in the United States in children younger than 21 years of age. Significant improvements in relapse-free and overall survival have occurred during the past 50 years, largely secondary to the introduction of central nervous system prophylaxis, multi-agent chemotherapy regimens, and the recognition that certain subsets of patients require higher doses of these agents to achieve cure. In addition, the recognition of the prognostic significance of leukemia cell-specific cytogenetic and chromosomal abnormalities, as well as studies that have shown the value of early response to therapy, have together improved our ability to refine therapy.^{1–3} Approximately 80–85% of children with ALL are cured using these strategies with current multi-agent therapy. However, up to 20% of children will fail our current therapies, and most of these patients will die from their disease. In addition, adults with ALL have generally worse prognoses with an overall survival of 35–50%. One-quarter to one-third of pediatric ALL patients have no identifiable leukemia-associated genetic alterations that are predictive of outcome,⁴ and that statistic is even higher in adult ALL.⁵ Identification of patients who remain at high risk of treatment failure despite contemporary therapies thus remains a priority for basic scientists and clinical investigators with the ultimate hope that these discoveries will lead to new therapies that can improve outcomes. Indeed, a paradigm for such a therapeutic benefit has occurred for children with *BCR/ABL1*-positive ALL; these patients previously experienced a 5-year event-free survival (EFS) of less than 40% when treated with intensive chemotherapy alone, but now experience an EFS greater than 80% with the addition of the tyrosine kinase inhibitor imatinib in combination with chemotherapy.⁶

With the advent of sophisticated genome-wide analytic technologies, it is now possible to profile the biology of primary human leukemias in a sensitive and high-throughput manner. Recently, several groups have utilized modern microarray-based analyses of genetic alterations and gene expression to identify mutations in key lymphoid development and signaling genes in high-risk childhood ALL. In particular, high frequencies of mutations have been described in genes involved in B lymphocyte development (*e.g.*, *EBF1*, *IKZF1*, *PAX5*) and signaling (*e.g.*, *BTLA*, *CD200*, *RAS*) and in cell cycle regulation (*e.g.*, *CDKN2A/B*, *PTEN*, *RB*, *TP53*). Additionally, genomic analyses of paired diagnosis and relapse ALL samples have also shed light upon the early origin and clonal nature of such leukemia-associated mutations.^{7, 8} A comprehensive discussion of these genetic alterations is beyond the scope of this review and has been described elsewhere in greater detail.^{9–13}

A. Discovery of *CRLF2*, *JAK1* and *JAK2*, and *IL7RA* Alterations in Acute Lymphoblastic Leukemia (ALL)

In 2008 and 2009, research groups in Israel and Europe and in the United States first identified somatic mutations in the Janus family of kinases (JAK) in pediatric B-precursor ALL cases via various strategies employing candidate gene sequencing, gene expression profiling (GEP), and single nucleotide polymorphism arrays.^{9, 14–18} Missense and insertional mutations were detected in highly conserved regions of the *JAK1* pseudokinase

domain, *JAK2* pseudokinase and kinase domains, and *JAK3* (later determined to be a non-driver mutation), but not in *TYK2*.^{15, 19} Point mutations within the *JAK2* pseudokinase domain were by far the most common, particularly *JAK2* R683G. Notably, these mutations were distinct from the *JAK2* V617F commonly associated with myeloproliferative neoplasms, which is also located in the pseudokinase domain. In particular, *JAK2* mutations occur frequently in children with Down syndrome-associated ALL (DS-ALL) with an incidence of approximately 20%.^{14, 16, 17, 20} Screening of pediatric non-DS-ALL cases demonstrated a 3–10% rate of *JAK* mutations, which was highest in patients classified as “high risk” by either the Oxford Hazard Score (OHS) or the National Cancer Institute (NCI)-Rome risk criteria (age > 10 years or white blood cell count > 50,000/uL at diagnosis). In one study, *JAK* mutations were also noted to be highly associated with *IKZF1* mutations, *CDKN2A/B* deletions, *BCR-ABL1*-like gene expression profiles, and conferred a poor clinical prognosis.¹⁵

Shortly thereafter, additional genome-wide analyses of DNA copy number alterations (CNAs), transcriptional profiling, and sequencing of these and other DS-ALL and non-DS-ALL cases detected alterations in the *cytokine receptor-like factor 1* gene (*CRLF2*), which encodes the thymic stromal lymphopoietin receptor (TSLPR). Russell *et al.* first described deregulation of various cytokine receptors, such as the erythropoietin receptor, mediated via juxtaposition to immunoglobulin heavy chain (*IGH@*) transcriptional enhancers in approximately 3% of B-precursor ALL cases.^{21, 22} Subsequently, the group used serial fluorescent *in situ* hybridization and long distance inverse-polymerase chain reaction to identify recurrent *CRLF2* abnormalities in B-precursor ALL.²³ They discovered that *CRLF2* alterations resulted either from chromosomal breakages and translocation of *CRLF2* (located on the pseudoautosomal region of the sex chromosomes; Xp22.23 or Yp11.32) with *IGH@* (14q32.3) or from interstitial deletion of Xp22.3/Yp11.3 resulting in fusion of *CRLF2* with the *G-protein-coupled purinergic receptor P2Y8* gene (*P2RY8*).²³ Both alterations place *CRLF2* under alternate transcriptional control, resulting in *CRLF2* overexpression.^{23–25} GEP of these leukemias revealed a *BCR-ABL1*-like kinase signature, although the *BCR-ABL1* translocation was not detected in any *CRLF2*-overexpressing ALL samples.^{24, 25} *CRLF2* alterations have been detected in 5–15% of pediatric and adult non-DS-ALL, depending upon the risk status of the population studied,^{23, 25, 26} and, strikingly, in 50–60% of children with DS-ALL.^{20, 24} In general, the *P2RY8-CRLF2* fusion occurs more frequently in younger pediatric patients, while the *IGH@-CRLF2* translocation is more common in adolescents and adults with ALL.^{23–26} More recently, Yoda *et al.* described an activating somatic point mutation in *CRLF2* itself resulting in a phenylalanine-to-cysteine change at amino acid 232 (F232C) in a small number of adult ALL patients,²⁶ which also has been detected infrequently in pediatric ALL patients.^{20, 27} In general, *CRLF2*-overexpressing ALL samples tend to possess either the *P2RY8-CRLF2* fusion or the *IGH@-CRLF2* translocation, though there are cases that appear to lack these alterations. Although usually mutually exclusive, the fusion and translocation or one alteration in conjunction with *CRLF2* F232C have been detected in a minority of patient samples. It is not yet known whether both mutations occur in a single leukemia cell or in two distinct clonal populations²⁵ (and Tasian & Loh, unpublished observations).

CRLF2 alterations are further highly associated with *JAK* mutations in both DS-ALL and non-DS-ALL. Approximately half of patients whose leukemias have *CRLF2* alterations also harbor *JAK* mutations, particularly at the *JAK2* R683 residue. Conversely, virtually all *JAK*-mutated ALL samples reported in the literature overexpress *CRLF2* via the *P2RY8-CRLF2* fusion or *IGH@-CRLF2* translocation, suggesting the cooperative nature of these genetic events in leukemogenesis.^{23, 24}

Most recently, gain-of-function somatic mutations in the interleukin-7 receptor alpha chain (IL-7R α ; *IL7RA*) have also been described by Shochat *et al.* in a small number of patients with *CRLF2*-overexpressing ALL and in T cell ALL. These mutations occur as S185C point mutations, located in the extracellular domain of the IL-7R α , or as in-frame insertion and deletion mutations within the transmembrane domain. Three of the eight *CRLF2*-overexpressing B-precursor ALL samples with *IL7RA* mutations also had *JAK2* mutations, which further emphasizes the complex association of these genetic events.

Importantly, it should be noted that ALL-specific alterations in *CRLF2*, *JAK1* and *JAK2*, and *IL7RA* have not been detected in leukemia cells with known prognostic cytogenetic abnormalities, such as high hyperdiploidy, *ETV6-RUNX1*, *TCF-ECF1*, *MLL* rearrangements, or *BCR-ABL1*,^{4, 23, 28} with the exception of intrachromosomal amplification of chromosome 21 in some non-DS-ALL patients.^{23, 29, 30} *CRLF2* and associated alterations thus seem to comprise a new cytogenetic subtype of B-lineage ALL (Table 1).

B. NCI High Risk Patients with *CRLF2*-Overexpressing ALL Have Poor Outcomes

Following the initial discovery of *IGH@-CRLF2* and *P2RY8-CRLF2* alterations,²³ other groups also identified *CRLF2* alterations via high-resolution genomic profiling of cohorts of pediatric ALL patients who had experienced extremely poor clinical outcomes.²⁴

Subsequent GEP and CNA microarray analysis by Harvey *et al.* of 207 high risk patients treated uniformly with an augmented Berlin-Frankfurt-Münster (BFM) regimen on the legacy Children's Oncology Group study P9906 revealed a 14% incidence of *CRLF2* alterations (29 patients), only two of whom had Down Syndrome. *CRLF2* alterations were highly associated with *JAK* and *IKZF1* mutations (69% and 80%, respectively) and with Hispanic/Latino ethnicity. Patients with *CRLF2*-overexpressing ALL responded slowly to chemotherapy and had high rates of minimal residual disease (MRD) at the end of induction. Furthermore, nearly 70% of these patients relapsed. Relapse-free survival (RFS) was 35.3% for *CRLF2* overexpressors vs. 71.3% for non-overexpressors treated on this trial at four years. Interestingly, there was no significant effect of the presence of simultaneous *JAK* or *IKZF1* mutations upon MRD or RFS, although the number of patients with *CRLF2*-overexpressing *JAK* wild-type *IKZF1* wild-type ALL in this study was small.²⁵

To validate these results and to evaluate *CRLF2* overexpression in standard risk patients, further analyses were performed of 896 ALL samples from children enrolled on the COG 9905 study, comprised of both NCI high risk (HR) and standard risk (SR) patients. One hundred seventeen patients demonstrated high levels of *CRLF2* expression, which was confirmed by specific mutational analysis. Of the *CRLF2* overexpressors, only those patients classified as NCI HR had a significantly poorer RFS (<40%), which was in sharp contrast to those initially classified as NCI SR (RFS >80%).²⁷

Additional analyses of the prognostic significance of *CRLF2* expression have also been performed by Cario *et al.* in 555 leukemia samples from children treated on the ALL-BFM 2000.³¹ Specimens were classified as *CRLF2*-high (42 patients) vs. *CRLF2*-low (513 patients) based upon prior published gene expression data^{23, 24} and further stratified by HR and non-high risk (NHR) clinical groups (classified by *in vivo* response to prednisone). Predictably, DS-ALL patients were enriched in the *CRLF2*-high group. In contrast to the COG studies, these analyses demonstrated no prognostic effect of *CRLF2* overexpression in the HR cohort, whereas *CRLF2*-high NHR patients had worse event-free survival (EFS) than *CRLF2*-low NHR patients (73% and 87%, respectively).³¹

The Medical Research Council (MRC) has also published data regarding the prognostic significance of *CRLF2* overexpression in OHS SR children.²⁸ Fifty-two of 865 studied patients treated on the MRC ALL97 trial had evidence of deregulated *CRLF2* in their leukemia cells (6%), although this rate was markedly higher in DS-ALL patients (54%; 14 of 26 DS patients).²⁸ Ensor and colleagues noted that *CRLF2*-overexpressing ALL patients were more likely to have hepatosplenomegaly at the time of leukemia diagnosis, although there was no association with age, gender, or initial white blood cell count. *CRLF2* alterations were also associated with *IKZF1*, *CDKN2A/2B*, *PAX5*, and *JAK2* mutations, but to a lesser degree than previously observed in the COG cohorts.^{24, 25} While univariate analysis demonstrated inferior EFS (but not inferior RFS) of patients with *CRLF2*-overexpressing ALL, this phenomenon did not retain significance in a multivariate analysis. This study also confirmed the association of *IGH@-CRLF2* translocation with older age observed by others^{23–26} and noted a non-statistically significant trend of worse EFS for patients with *IGH@-CRLF2* vs. those with *P2RY8-CRLF2*. Ultimately, however, no significant difference in RFS or overall survival (OS) was detected between *CRLF2* overexpressors and non-expressors in this SR cohort, as has now been observed in the COG P9905 analysis.²⁷ The authors concluded that *CRLF2* overexpression in OHS SR ALL patients thus conferred an intermediate, not high risk, prognosis.²⁸

It is important to note the differences among the P9905, P9906, ALL-BFM 2000, and MRC ALL-97 protocols, which may account for the observed variability in outcomes. In particular, striking differences exist between the characteristics of patients treated on COG 9906 and on MRC ALL-97. The P9906 patients were significantly older (median age = 12.8 years), had a higher incidence of *IGH@-CRLF2* translocations and *IKZF1* mutations, and were more likely to be Latino/Hispanic. MRC ALL-97 enrolled patients who were younger, had a five-fold higher rate of *P2RY8-CRLF2* fusions than *IGH@-CRLF2*, and had a higher percentage of DS-ALL patients. In addition, the optimal threshold to define *CRLF2* overexpression by QT-PCR has not yet been standardized across international consortia, but these efforts are currently ongoing. Despite these differences, comprehensive data from these analyses do suggest that *CRLF2* overexpression likely portends a poor prognosis for HR, but not SR, ALL patients treated with current therapies. The reason for the discrepant clinical outcomes of HR vs. SR *CRLF2*-overexpressing non-DS-ALL patients remains unknown. Additionally, Hertzberg and colleagues noted a trend towards worse OS in DS-ALL *CRLF2* overexpressors,^{16, 20} while Gaikwad *et al.* observed no difference in EFS between *CRLF2*-overexpressing and non-overexpressing DS-ALL patients.¹⁶

C. Adults with *CRLF2*-Overexpressing ALL Have Worse Rates of Survival

While various groups have begun to characterize the outcomes of pediatric ALL patients with *CRLF2* alterations, less is currently known about the prognoses of adults with *CRLF2*-overexpressing ALL. In one study of adult B-precursor ALL patients whose leukemias lacked prognostic cytogenetic abnormalities, 15 of 90 (16.7%) samples demonstrated *CRLF2* overexpression.²⁶ *CRLF2* overexpressors and non-overexpressors did not differ significantly in age, gender, or presenting white blood cell count, but disease-free and overall survival were markedly worse in the *CRLF2* overexpressors (17.8 months vs. 37.8 months and 25.5 months vs. >100 months, respectively).²⁶ Although adult patients with ALL have notably poorer outcomes than children, generally ascribed to the higher incidence of *BCR/ABL1*-positive disease and to their inability to tolerate intensive multi-agent chemotherapy, *CRLF2* overexpression also appears to confer a worse prognosis in this population. Larger analyses of RFS and OS in adults with *CRLF2*-overexpressing ALL are indicated and will likely provide important rationale for the identification and development of targeted therapies with potentially fewer toxicities, especially for patients who are unable to tolerate traditional therapy. Various cooperative research groups have thus sought to understand and characterize the biochemical consequences of these genetic lesions with the goal of identifying new therapeutic targets.

II. ELUCIDATING THE BIOCHEMICAL SEQUELAE OF *CRLF2* ALTERATIONS IN ALL

A. The Role of the Thymic Stromal Lymphopoietin Receptor (TSLPR) in ALL

CRLF2 encodes the TSLPR subunit, which heterodimerizes with the IL-7R α subunit to form the functional TSLPR. Upon binding of its ligand, thymic stromal lymphopoietin (TSLP), the TSLPR is known to induce phosphorylation of the signal transducer and activator of transcription factor 5 (STAT5) in normal dendritic and T cells in response to allergic or inflammatory stimuli, which is likely mediated by antecedent phosphorylation of *JAK2* (pJAK2).^{32–37} TSLP has also been shown to promote early development of B cells *in vitro*.³⁴

Little is currently known about the role of the TSLPR and TSLP-mediated signal transduction in leukemia, however. Initial studies by Brown *et al.* of transgenic murine non-*CRLF2*-overexpressing B-precursor ALL cell lines demonstrated TSLP- and IL-7-induced phosphorylation of STAT5 (pSTAT5), as well as dose-dependent cellular proliferation in culture.³⁸ TSLP and IL-7 also induced phosphorylation of S6 (pS6) and 4EBP1 (p4EBP1), which could be inhibited by rapamycin, a mammalian target of rapamycin (mTOR) inhibitor. TSLP further rescued rapamycin-treated primary human ALL cells from apoptosis in short-term culture.³⁹

B. *CRLF2* and *JAK* Mutations Confer Gain-of-Function and Result in Aberrant Signal Transduction

Several groups have created preclinical models of human *CRLF2* and *JAK* mutations using retrovirally-transduced Ba/F3 cells, an IL-3-dependent murine lymphoma cell line, to evaluate the biochemical effects of these genetic lesions. In an initial study by Mullighan *et*

al.,¹⁵ Ba/F3 cells transduced with various patient-derived *JAK1* and *JAK2* mutations and the murine erythropoietin (EPO) receptor demonstrated moderate cytokine independent growth, as well as preferential growth inhibition when co-cultured with the chemical JAK inhibitor I. These cell lines also had constitutive levels of pJAK2 and pSTAT5, which were further induced by EPO stimulation.¹⁵ Subsequent work by this group using Ba/F3 cells transduced with the murine IL-7R (Ba/F3-IL-7R), the *P2RY8-CRLF2* fusion, and *JAK2* pseudokinase and kinase domain mutations demonstrated cytokine independence only of the compound *CRLF2/JAK2* mutants, but not of cells transduced with either mutation alone or with wild-type constructs.²⁴ IL-3 independent growth was partially attenuated by short hairpin-RNA knock-down of *CRLF2*. *CRLF2/JAK2* mutant Ba/F3 cells also demonstrated constitutive activation of JAK/STAT signaling and preferential growth inhibition by the pharmacologic JAK inhibitor I.²⁴ Similar observations of constitutive JAK2/STAT5 activation and inhibition with JAK inhibitor I were also made by Hertzberg and colleagues using Ba/F3 cells transduced with wild-type *CRLF2* and *JAK2* R683S, as well as IL-3 independent growth even in the absence of transduced IL-7R.²⁰

Another study of *CRLF2* overexpression in adult ALL patients used Ba/F3-IL-7R cells transduced with wild-type *CRLF2* or *CRLF2* F232C to explore the specific effects of this newly-discovered point mutation.²⁶ Using reducing and non-reducing conditions, Yoda *et al.* demonstrated that mutant, but not wild-type, *CRLF2* likely constitutively homodimerizes via intermolecular disulfide bonds of the cysteine residues. TSLP also stimulated modest proliferation of Ba/F3-IL-7R *CRLF2* F232C cells *in vitro*. Immunoblotting of lysates from Ba/F3 *CRLF2* F232 cells demonstrated some constitutive phosphorylation of ERK 1/2 (pERK) and pSTAT5. Additionally, Ba/F3 cells transduced with wild-type *CRLF2* and *JAK2* R683G or R683S had markedly increased basal levels of pJAK2, pSTAT5, and pERK in comparison to *CRLF2* wild-type/*JAK2* wild-type cells,²⁶ which was concordant with prior observations.^{15, 24} Growth of mutant cell lines was also preferentially inhibited by the JAK inhibitor I.²⁶

Most recently, studies of primary human *CRLF2*-overexpressing ALL samples with and without simultaneous *JAK* mutations demonstrate activation of JAK/STAT and phosphatidylinositol 3-kinase (PI3K) pathway signal transduction with TSLP stimulation *in vitro*. JAK2 inhibition with XL019 (Exelixis) abrogated TSLP-induced pSTAT5 and pS6. Minor activation of TSLP-induced MAPK signaling has also been observed in human *CRLF2*-overexpressing ALL cell lines, but less so in primary samples. Studies are ongoing to define more precisely the aberrant signal transduction networks in human *CRLF2*-overexpressing ALL and to test relevant signal transduction inhibitors *in vitro* (Tasian & Loh, unpublished data) and *in vivo* in preclinical murine xenograft models.⁴⁰

C. IL-7Rα Mutations are Also Transforming and Activate JAK/STAT and PI3K Signal Transduction

Given the heterodimeric structure of the TSLPR, research groups have recently begun to evaluate *CRLF2*-overexpressing ALL samples for the presence of *IL7RA* mutations. The group that first identified these somatic missense mutations in *CRLF2*-overexpressing ALL (and in T cell ALL) also tested whether or not these lesions conferred cytokine independent

growth using Ba/F3 models.⁴¹ Cells transduced with the transmembrane domain in-frame insertional *IL7RA* mutation InsPPCL demonstrated IL-3 independent growth, while simultaneous *CRLF2* wild-type and *IL7RA* S185C constructs were necessary to confer cytokine independence, suggesting biologic differences between the subtypes of *IL7RA* mutations. TSLP induced preferential proliferation of *CRLF2* wild-type/*IL7RA* mutant cells in comparison to *CRLF2* wild-type/*IL7RA* wild-type cells. *CRLF2* wild-type/*IL7RA* mutant cells demonstrated constitutive pSTAT5 and pS6, which was further inducible with TSLP stimulation. Substitution of the mutant amino acid 185 cysteine residue with a glycine residue in the *IL7RA* constructs markedly diminished cytokine independent growth and constitutive pSTAT5. In addition, Shochat *et al.* observed marked homodimerization of Ba/F3-IL-7Rα InsPPCL cells under non-reducing vs. reducing conditions.⁴¹ These data suggest that, as observed with *CRLF2* F232C,²⁶ introduced cysteine residues likely facilitate IL-7Rα receptor homodimerization and are necessary for the gain-of-function phenotype.

III. ON THE HORIZON: DEVELOPMENT OF TARGETED THERAPIES FOR PATIENTS WITH *CRLF2*-OVEREXPRESSING ALL

Significant efforts have been made by cooperative research groups in a relatively short period of time to improve the biologic understanding of *CRLF2*-overexpressing ALL. *CRLF2* and other associated alterations occur in approximately 5–15% of B-precursor ALL, and children and adults with *CRLF2*-overexpressing ALL suffer unacceptably high rates of relapse with current therapies. These patients comprise an important newly-defined genetic subgroup of high risk ALL for whom new treatments are vital to improve RFS and OS.

Extensive preclinical data to date demonstrate aberrant activation of TSLPR-mediated signal transduction in these leukemias, particularly of the JAK/STAT and PI3K pathways. Additional preclinical and translational studies are needed to characterize the biochemical sequelae of *CRLF2* alterations and associated genetic lesions more fully, however. Early *in vitro* work with JAK inhibitors does suggest the promise of targeting aberrant signal transduction with small molecule inhibitors. Identification of other associated mutations, further delineation of hyperactive signaling networks and specific targets, and evaluation of relevant targeted signal transduction inhibitors (STIs) in *CRLF2*-overexpressing ALL will greatly facilitate these goals (Figure 1).

Precedent exists for the addition of STIs for treatment of pediatric leukemias, as evidenced by the dramatic improvement in long-term survival for children with *BCR-ABL*-positive ALL when imatinib is added to systemic chemotherapy.⁶ Currently, STIs targeting hyperactive signaling networks are also being tested in Phase II and III clinical trials for adults with *JAK2* V617F-associated MPNs,^{42–47} and the clinical, but not molecular, efficacy of the JAK inhibitor INCB018424 (Incyte) in adults with myelofibrosis was recently reported.⁴³ Determination of the maximum tolerated dose and dose-limiting toxicities of INCB018424 in pediatric patients with relapsed/refractory malignancies is further underway in a Children's Oncology Group Phase I trial. Simultaneous preclinical studies of JAK inhibitors and other relevant STIs in combination with cytotoxic chemotherapy are essential, however, and will provide additional rationale for development of such therapeutic approaches in subsequent clinical trials.

Modern genomic profiling techniques have provided important insights regarding the molecular pathogenesis of high risk ALL that will hopefully identify potential new therapeutic targets. Current data suggest that *CRLF2*-overexpressing ALL is a disorder of hyperactive JAK/STAT and PI3K signaling. Future development of STI-based therapies for adult and pediatric patients with these high risk leukemias will depend upon the successful identification of specific agents capable of abrogating hyperactive signaling networks shown to be perturbed in these leukemias, as well as our ability to test these agents in accurate pre-clinical systems, such as genetically engineered mice or xenograft models. Important questions remain, however, regarding why HR, but not SR or DS-ALL, patients fare poorly with current therapeutic strategies. While the first steps toward understanding the biological underpinnings of this subtype of leukemia have been made, much additional work is necessary to unravel fully the mechanisms that contribute to the pathogenesis of *CRLF2*-overexpressing ALL.

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Abbreviations

ALL	acute lymphoblastic leukemia
BFM	Berlin-Frankfurt-Münster
CNA	copy number alteration
COG	Children's Oncology Group
CRLF2	cytokine receptor-like factor 2
DS-ALL	Down Syndrome-associated acute lymphoblastic leukemia
EFS	event-free survival
GEP	gene expression profiling
HR	high risk
IKZF1	Ikaros
IL-7R	IL-7 receptor
IL7RA	IL-7 receptor alpha chain protein (IL-7R α) and gene
JAK	Janus kinase
JAK1	Janus kinase 1
JAK2	Janus kinase 2
MPNs	myeloproliferative neoplasms
MRC	Medical Research Council
NCI	National Cancer Institute

OHS	Oxford Hazard Score
pEIF4E	phospho-eIF4E
pERK	phospho-ERK 1/2
pJAK2	phospho-JAK2
pSTAT5	phospho-STAT5
pS6	phospho-S6
p4EBP1	phospho-4EBP1
PI3K	phosphatidylinositol 3-kinase
RFS	relapse-free survival
SR	standard risk
STAT	signal transduction activator of transcription
STIs	signal transduction inhibitors
TSLP	thymic stromal lymphopoietin
TSLPR	thymic stromal lymphopoietin receptor

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Figure 1.

Proposed schema of aberrant signal transduction mediated by the TSLPR heterodimer in *CRLF2*-overexpressing ALL. JAK/STAT, PI3K, and possibly MAPK signaling networks are hyperactive. Targeting hyperactive signaling nodes with STIs (such as JAK, PI3K pathway, and MEK inhibitors) is a rational therapeutic strategy for preclinical and clinical testing.

Table 1

Details of studies reporting alterations of *JAK1*, *JAK2*, *CRLF2*, and *IL7RA* in acute lymphoblastic leukemia.

<i>JAK</i> mutations	Cohort	Findings
Malinge 2007 (16)	ALL and AML (N=90)	Identification of a transforming <i>JAK2</i> pseudokinase domain mutation: <i>JAK2</i> p.I682_D686del in a single DS-ALL case.
Flex 2008 (45)	Adult B-ALL (N=88) and T-ALL (N=38), pediatric B-ALL (N=85) and T-ALL (N=49)	<i>JAK1</i> FERM, SH2, JH2, and JH1 mutations in 18% adult T-ALL. Mutations transforming <i>in vitro</i> , associated with distinct GEP, older age, poor outcome.
Bercovich 2008 (12)	DS-ALL (N=88), non-DS B-ALL (N=109), DS-AMKL (N=11), ET (N=96)	Missense mutations at/near R683 in the <i>JAK2</i> pseudokinase domain in 18% DS-ALL. Mutations transforming in Ba/F3-EpoR cells.
Kearney 2008 (15)	DS-ALL (N=42)	<i>JAK2</i> R683 mutations in 28% cases.
Mullighan 2009 (13)	High-risk B- progenitor ALL (N=187)	<i>JAK1</i> (pseudokinase), <i>JAK2</i> (pseudokinase and kinase) and <i>JAK3</i> mutations in 20 (10.7%) cases. 18 of 20 cases non-DS B-ALL. Mutations associated with <i>IKZF1</i> deletion, deletions adjacent to <i>CRLF2</i> , <i>BCR-ABL1</i> -like gene expression profile, and very poor outcome. Mutations transform Ba/F3-EpoR cells <i>in vitro</i> , and transformation abrogated by pharmacologic JAK inhibitors.
Gaikwad 2009 (14)	Pediatric DS-ALL (N=53)	<i>JAK2</i> pseudokinase (R683) mutations in 18.9% DS-ALL cases.
<i>CRLF2</i> alterations	Cohort	Findings
Russell 2009 (19)	Pediatric ALL (N>1000)	97 B-ALL cases with <i>CRLF2</i> rearrangement; 33 t(X;14)(p22;q32) or t(Y;14)(p11;q32) and 64 with PAR1 deletion (del(X)(p22.33p22.33) or del(Y)(p11.32p11.32)). Translocation 0.8% and deletion 4.2% BCP-ALL. 52% of 68 DS-ALL cases have PAR1 deletion.
Mullighan 2009 (20)	Pediatric B-ALL (N=272) and T-ALL (N=57)	<i>IGH@-CRLF2</i> translocation or Xp/Yp PAR1 deletion in 7% B-ALL and 53% DS-ALL. PAR1 deletion mapped and definitively shown to result in <i>P2RY8-CRLF2</i> fusion. Rearrangements result in overexpression of <i>CRLF2</i> . Strong association between <i>CRLF2</i> rearrangement and <i>JAK</i> mutations; lesions co-transforming in Ba/F3 cells.
Hertzberg 2010 (18)	Pediatric DS-ALL (N=53) and non-DS-ALL	Confirmatory study identifying <i>CRLF2</i> rearrangement in 53% DS-ALL samples.
Yoda 2010 (22)	Adult and pediatric ALL	Overexpression of <i>CRLF2</i> in 15% adult and pediatric ALL. Confirmation of <i>CRLF2</i> rearrangement and association with JAK mutations. Identification of <i>CRLF2</i> F232C mutation.
Harvey 2010 (21)	Pediatric high-risk ALL (N=207)	Overexpression of <i>CRLF2</i> in 14% cases due to <i>IGH@</i> translocation (62%) and/or <i>P2RY8-CRLF2</i> (34%). <i>CRLF2</i> rearrangement associated with JAK mutation, Hispanic/Latino ethnicity, <i>IKZF1</i> alteration, and poor outcome.
Cario 2010 (25)	Pediatric ALL (ALL-BFM-2000; N=555)	<i>CRLF2</i> overexpression due to rearrangement and associated with poor outcome.
<i>IL7RA</i> mutations	Cohort	Findings
Shochat 2011 (35)	Pediatric B-ALL (BFM AIEOP 2000; N=286, of which 83 DS-ALL) and T-ALL (BFM; N= 285)	Identification of <i>IL7RA</i> S185C and insertional/deletional mutations in 3.1% cases of B-ALL (8 of 9 cases also <i>CRLF2</i> -overexpressing) and in 10.5 % T-ALL. Mutations transforming in Ba/F3- <i>CRLF2</i> cells.

Abbreviations: AIEOP, Associazione Italiana Ematologia Oncologia Pediatrica; ALL, acute lymphoblastic leukemia; AMKL, acute megakaryoblastic leukemia; BFM, Berlin-Frankfurt-Münster; DS, Down syndrome; ET, essential thrombocytosis.

Modified from JR Collins-Underwood and CG Mullighan, Genomic profiling of high-risk acute lymphoblastic leukemia. *Leukemia* 24, 1676–1685 (October 2010). Used with permission.