

# Pathogenesis and prognostication in acute lymphoblastic leukemia

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*F1000Prime Reports* 2014, **6**:59 (doi:10.12703/P6-59)

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## Abstract

The process of lymphoid maturation is tightly controlled by the hierarchical activation of transcription factors and selection through functional signal transduction. Acute lymphoblastic leukemia (ALL) represents a group of B/T-precursor-stage lymphoid cell malignancies arising from genetic alterations that block lymphoid differentiation and drive aberrant cell proliferation and survival. With recent advances in next-generation sequencing, we are discovering new mutations affecting normal lymphopoiesis and the significance of cooperating mutations, as well as epigenetic alterations. The data obtained in this way aids in the evaluation of prognosis in the individual patient but, importantly, also in incorporating targeted therapy appropriate for the mutational abnormality.

## Introduction

ALL arises from hematopoietic precursors of the lymphoid lineage. It is the most common leukemia in pediatrics, accounting for up to 80% of leukemias in this group and 20% of leukemias in adults. With the advances in cytogenetic (and especially molecular) techniques over the last two decades, our understanding of the biology and pathogenesis of leukemia has progressed tremendously. This progression is now in the process of being translated into better biological prognostication, detection of residual disease and tailored/targeted therapy [1]. The following review will focus on recent findings in the cytogenetic and molecular alterations associated with ALL pathogenesis and its prognostic significance. However, despite the increasing awareness of cytogenetic and mutational factors for prognosis, it is important to remember that, on an individual basis, many of these favorable or unfavorable factors may be superseded by the detection or absence of minimal residual disease — a tool increasingly used to tailor patient therapy.

## Pathogenesis

Lymphoid cells are derived from pluripotent hematopoietic stem cells in the bone marrow, through stepwise

maturation. In the case of B cell development, this includes development initiated at the level of lymphoid-primed multipotent progenitors, common lymphoid progenitors, pro-B cells, pre-B cells, and mature B cells. This maturation process is tightly controlled by the hierarchical activation of transcription factors and selection through functional signal transduction [2].

ALL represents a group of B/T-precursor-stage lymphoid cell malignancies (arising from genetic insults) that blocks lymphoid differentiation and drives aberrant cell proliferation and survival. The clinical heterogeneity of the disease course and outcome, especially when comparing pediatric and adult populations, reflects different biological subtypes.

It has long been known that ALL is characterized by gross numerical and structural chromosomal abnormalities, including hyperdiploidy (>50 chromosomes), hypodiploidy (<44 chromosomes), translocations t{[12;21], [1;19], [9;22], [4;11]} and rearrangements (*MYC*, *MLL*). However, several observations indicate that these lesions alone are insufficient to induce leukemia and cooperating lesions are required. As an example, rearrangements such

as t(12;21), *ETV6-RUNX1*, comprising 22% of pediatric ALL, are present years before the development of leukemia [3,4]. Many of the genes involved encode proteins with key roles in lymphoid development. It is suggested that the initial event confers self-renewal coupled with mutation, leading to developmental arrest and a secondary cooperative event in cell cycle regulation, tumor suppression and chromatin modification, eventually leading to establishment of the leukemic clone [5,6].

The determination of prognosis was based until very recently on classical G banding cytogenetics. Several reports describe the outcome based on cytogenetic analysis, in large cohorts of pediatric and adult ALL patients, respectively [3,7-9].

Advances in genomic techniques over the last two decades have enabled better genetic profiling of the leukemic clone. The techniques used include three main categories. Firstly, cytogenetic studies with conventional G banding as well as fluorescence *in situ* hybridization (FISH) and polymerase chain reaction (PCR) analysis have identified structural chromosomal alterations. Secondly, genome-wide profiling uses array-based comparative genomic hybridization or single nucleotide polymorphism microarrays and gene expression profiling. Thirdly, sequencing studies use whole-genome sequencing, transcriptome sequencing and/or whole-exome sequencing to more comprehensively define the genomic landscape of these diseases.

ALL genomes typically harbor fewer structural genetic alterations than many solid tumors. More than 50 recurring regions of DNA copy number alterations have been identified, which are commonly focal deletions, limited to one or few genes involved in normal lymphoid development: transcriptional regulators of lymphoid development (*PAX5*, *IKZF1*, *EBF1*, *LEF1*), tumor suppressors (*CDKN2A*, *CDKN2B*, *RB1*, *TP53*), lymphoid signaling genes (*BTLA*, *CD200*, *TOX*), transcriptional regulators

and coactivators (*TBL1XR1*, *ERG*), as well as regulators of chromatin structure and epigenetic regulators (*CTCF*, *CREBBP*). T-lineage ALL is characterized by activating mutations of *NOTCH1* and rearrangements of transcription factors *TLX1* (*HOX11*), *TLX3* (*HOX11L2*), *LYL1*, *TAL1*, and *MLL* [10].

Sequencing of the full spectrum of ALL subtypes has shown that the alteration of multiple cellular pathways, including cytokine receptor and Ras signaling, tumor suppression, lymphoid development, and epigenetic regulation, are typical events in different ALL subtypes.

### Genomic prognostication in ALL

Not all genetic lesions have prognostic implications. Those relevant to prognosis will be reviewed here. Table 1 details major known abnormalities in B- and T-ALL and their prognostic significance.

Recent discoveries in the genomic landscape of ALL and its influence on prognosis include: the BCR-ABL1-like ALL, cytokine receptor-like factor 2 (*CRLF2*) over expression and Janus kinase (*JAK*) mutations, hypodiploid ALL and T-ALL [1,11].

#### BCR-ABL1-like B-ALL and IKZF1 alterations

BCR-ABL1-like ALL carries the gene expression signature similar to that of BCR-ABL1 ALL, while not harboring the *BCR-ABL1* translocation. More than 80% of patients with BCR-ABL1-like ALL have abnormalities in genes involved in B-cell development, such as *IKZF1*, *PAX5* and *VPREB1*. The prevalence of BCR-ABL1-like ALL is approximately 15% in pediatric B-cell ALLs and it was found to be associated with inferior outcome (5-year event-free-survival <60%), as is BCR-ABL1 ALL [12].

The lymphoid transcription factor gene *IKZF1* encodes IKAROS, the founding member of a family of zinc finger transcription factors that is required for the development of all lymphoid lineages [13]. The *IKZF1* alterations

**Table 1. Major abnormalities in B- and T-ALL and their prognostic significance**

Cytogenetic abnormality	Target gene	Frequency in pediatrics %	Frequency in adults %	Prognosis
t(1;19)(q23;p13)	<i>E2A-PBX1</i>	4-6	2-3	standard
t(9;22)(q34;q11)	<i>BCR-ABL1</i>	3-5	25-30	unfavorable
t(4;11)(q21;q23)	<i>MLL-AF4</i>	2-3	3-7	unfavorable
High hyperdiploid		20-30	7	favorable
Hypodiploid		5-6	3	unfavorable
t(12;21)	<i>ETV6-RUNX1</i>	25	–	favorable
T-ALL	<i>TCR</i>			
T(7;14)(14q;7q34 or 7p14)	Non-TCR( <i>NOTCH1</i> , <i>HOX11</i> , <i>JAK1</i> )	60%		favorable

Abbreviations: ALL, Acute lymphoblastic leukemia.

observed in ALL are mainly deletions that result in a loss of function. *IKZF1* alterations are present in more than 70% of cases of BCR-ABL1 ALL and are associated with a poor outcome both in BCR-ABL1<sup>+</sup> and BCR-ABL1<sup>-</sup> ALL [11,14].

In a recent large study of over 1000 pediatric patients from four collaborative groups, BCR-ABL1-like and *IKZF1* alterations were reported in 16% and 17% of patients, respectively, and in 53% and 40%, respectively, of B-ALL cases lacking known abnormalities, such as hyperdiploidy, *ETV6-RUNX1*, *TCF3* and *MLL* rearrangement. Patients with either BCR-ABL1-like or *IKZF1* alterations were found to have a statistically significantly higher relapse rate compared to other genetic groups, requiring treatment modification [15].

#### **CRLF2 over expression and JAK mutations**

CRLF2, also known as thymic stromal-derived lymphopoietin receptor, is a type I cytokine receptor. CRLF2 forms a heterodimeric complex with IL-7R $\alpha$  in a functional signaling unit. CRLF2 levels are markedly elevated in a subset of B-ALL cases caused by translocation or deletions. The frequency of CRLF2 alterations in B-ALL depends on the patient cohort. *P2RY8-CRLF2* fusion is 7% in pediatric patients with B-ALL, but occurs in up to 50% of B-ALL cases associated with Down syndrome.

More importantly, aberrant CRLF2 expression is frequent in B-ALL cases that lack recurrent B-ALL-associated translocations [16].

Up to half of BCR-ABL1-like cases harbor rearrangement of CRLF2 resulting in over-expression of CRLF2 on the surface of lymphoid blasts that may be detected by immunophenotyping. Additionally, approximately half of CRLF2-rearranged cases harbor concomitant activating mutations of the JAK genes *JAK1* and *JAK2* [17]. The JAK/signal transducers and activators of transcription (STAT) pathway mediates signaling from cytokine, chemokine, and growth factor receptors via the JAK non-receptor tyrosine kinases and the STAT family of transcription factors [18]. These alterations result in activation of JAK-STAT signaling that may be amenable to therapy with JAK inhibitors such as ruxolitinib, and this is currently being explored as a therapeutic strategy. Ongoing next-generation sequencing studies in childhood and adult ALL are designed to define the repertoire of kinase-activating alterations in BCR-ABL1-like ALL and to develop clinical trials aimed at directing patients with BCR-ABL1-like ALL to appropriate tyrosine-kinase inhibitor (TKI) therapy [11].

#### **Hypodiploid ALL**

Two subtypes of hypodiploid ALL have been described according to the severity of aneuploidy: near-haploid cases

with 24 to 31 chromosomes and low-hypodiploid cases with 32 to 39 chromosomes.

Recently, an analysis of a large cohort of more than 120 hypodiploid pediatric ALL patients has demonstrated that near-haploid and low-hypodiploid ALL have distinct transcriptomic signatures and submicroscopic genetic alterations [19]. The majority of near-haploid cases harbor alterations targeting receptor tyrosine kinase signaling and Ras signaling (71%) and the lymphoid transcription factor gene *IKZF3* (13%). In contrast, low-hypodiploid ALLs with 32–39 chromosomes are characterized by alterations in *TP53* (91.2%), *IKZF2* (53%) and *RB1* (41%). Both near-haploid and low-hypodiploid leukemic cells show activation of Ras-signaling and phosphoinositide 3-kinase (PI3K)-signaling pathways and are sensitive to PI3K inhibitors, indicating that these drugs might be used for this aggressive form of leukemia [11].

#### **T-ALL**

T-lineage ALL is characterized by an older age of onset, male sex preponderance, and inferior outcome in comparison with B-ALL [20]. Recently, next-generation sequencing identified sequence mutations and, less commonly, deletion of *PHF6* in 16% and 38% of childhood and adult T-ALL cases, respectively [9]. The role of *PHF6* in leukemogenesis is poorly understood, but the loss-of-function alterations suggest that *PHF6* is a tumor suppressor.

Early T-cell precursor ALL is an aggressive subtype of immature leukemia that accounts for a high proportion of T-ALL treatment failures. Recent studies found this entity to be associated with loss-of-function mutations in hematopoietic regulators (*GATA3*, *IKZF1*, *RUNX1*, *ETV6*), gain-of-function mutations in Ras, *FLT3*, *JAK*, and *IL7R*, and also inactivating mutations in epigenetic regulators (*EZH2*, *SUZ12*, *EED*, *SETD2*, *DNMT3A*) [21]. The mutational spectrum of this ALL subtype is similar to that observed in myeloid leukemias, and comparison of its transcriptional profile with those of normal human hematopoietic progenitors showed significant similarity to hematopoietic stem and early myeloid progenitors. Thus, the T-cell precursor ALL is likely to represent part of a spectrum of immature, stem cell-like leukemias. Epigenetic modifiers and agents targeting JAK-STAT signaling are currently being explored [22,23].

In addition to acquired mutation, an inherited susceptibility to develop ALL was found to be associated with a specific genome composition. *ARID5B*, *IKZF1*, *CEBPE*, and *BMI1-PIP4K2A* variants cumulatively conferred a strong predisposition to ALL, with children carrying six

to eight copies of risk alleles at a nine-fold higher ALL risk relative to those carrying zero to one risk allele at these four single nucleotide polymorphisms [24].

## Conclusion

Progress in sequencing methods has advanced our understanding of the molecular basis of lymphoid neoplasms by implicating new genes and core pathways, refining classification schema, and identifying new targets for therapeutic intervention. It is important to comprehend that this picture is continually evolving and is expected to change as better molecular techniques and more data accumulate.












## Abbreviations

ALL, acute lymphoblastic leukemia; CRLF2, cytokine receptor-like factor 2; JAK, Janus kinase; PI3K, phosphoinositide 3-kinase; STAT, signal transducers and activators of transcription.

## Disclosures

The authors declare that they have no disclosures.

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