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*J Clin Invest.* 2012;122(10):3407-3415. <https://doi.org/10.1172/JCI61203>.

**Review Series**

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# Molecular genetics of B-precursor acute lymphoblastic leukemia

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**B-precursor acute lymphoblastic leukemia (B-ALL) is the most common childhood tumor and the leading cause of cancer-related death in children and young adults. The majority of B-ALL cases are aneuploid or harbor recurring structural chromosomal rearrangements that are important initiating events in leukemogenesis but are insufficient to explain the biology and heterogeneity of disease. Recent studies have used microarrays and sequencing to comprehensively identify all somatic genetic alterations in acute lymphoblastic leukemia (ALL). These studies have identified cryptic or submicroscopic genetic alterations that define new ALL subtypes, cooperate with known chromosomal rearrangements, and influence prognosis. This article reviews these advances, discusses results from ongoing second-generation sequencing studies of ALL, and highlights challenges and opportunities for future genetic profiling approaches.**

## Introduction

Acute lymphoblastic leukemia (ALL) is a neoplasm of immature lymphoid progenitors that is most commonly of B cell lineage. Most childhood cases of B-precursor ALL (B-ALL) may be subclassified by the presence of either gross or submicroscopic genetic alteration. Approximately 75% of B-ALL cases exhibit aneuploidy or a recurring gross chromosomal rearrangement (1, 2) (Table 1 and Figure 1). Hyperdiploidy, with gain of at least 5 chromosomes, is one of the most frequent alterations in childhood ALL and is associated with favorable outcome (3). However, the biologic basis of the acquisition of multiple whole chromosomal gains is poorly understood. Conversely, hypodiploidy, with fewer than 44 chromosomes, is associated with dismal prognosis (4). A number of recurring chromosomal rearrangements are common in B-ALL and are critical events in leukemogenesis. These rearrangements commonly perturb genes encoding regulators of hematopoiesis, tumor suppressors, oncogenes, or tyrosine kinases but commonly require additional genetic hits to establish the full leukemic phenotype.

In the last five years, genome-wide profiling using microarrays, candidate gene, and second-generation sequencing have provided a number of key insights into the genetic basis of ALL. These studies have identified new subtypes of ALL and have uncovered recurring submicroscopic genetic alterations in known ALL subtypes. These include loss-of-function mutations involving genes regulating lymphoid development that contribute to the arrest in maturation characteristic of B-ALL, mutations that inactivate tumor suppressor and cell cycle regulatory proteins, and mutations that drive cytokine receptor and/or kinase signaling. Thus, as in AML, concomitant lesions disrupting hematopoietic development and tumor suppression as well as driving signaling and proliferation are hallmarks of many ALL subtypes. Importantly, several of these alterations are associated with specific subtypes of ALL defined by recurring chromosomal alterations.

This Review describes the genetic landscape of ALL, focusing on important subtypes and recently discovered genetic alterations of biologic and therapeutic relevance.

## ETV6-RUNX1 (TEL-AML1) B-ALL

The most common rearrangement in B-ALL is the t(12;21) (p13;q22) rearrangement that encodes *ETV6-RUNX1* (5). This rearrangement is usually cryptic on cytogenetic analysis but is readily detected by fluorescent in situ hybridization and molecular techniques. *ETV6* is a member of the ETS family of transcription factors that are frequently targeted by rearrangements and mutations in leukemia and other malignancies (6). With CBFβ, *RUNX1* forms the core binding factor transcription complex and is commonly rearranged in acute myeloid leukemia (7), and harbors sequence mutations in myeloid and lymphoid disorders (8–10). Both *ETV6* and *RUNX1* are required for normal definitive hematopoiesis (7, 11), and the *ETV6-RUNX1* protein may perturb expression of *RUNX1*-regulated genes, converting *RUNX1* to a transcriptional repressor (12). *ETV6-RUNX1* also causes overexpression of the erythropoietin receptor (EPOR) and activation of downstream JAK-STAT signaling (13). Expression of *ETV6-RUNX1* promotes self-renewal in B cell progenitors but alone does not induce leukemia (14, 15). Furthermore, *ETV6-RUNX1* is commonly detectable at birth (16), years prior to the onset of leukemia, suggesting that secondary genetic events are required to induce leukemia. This model is supported by genome-wide profiling studies of genetic alterations in ALL that have identified additional recurring submicroscopic genetic alterations in *ETV6-RUNX1* ALL, including deletions of the B cell transcription factors *PAX5* and *EBF1* and deletion of the second copy of *ETV6* (17–19). Moreover, monozygotic twins concordant for *ETV6-RUNX1* ALL exhibit distinct submicroscopic DNA copy number alterations (CNAs), indicating that acquisition of the *ETV6-RUNX1* is an early event in leukemogenesis (20). Submicroscopic genetic alterations are more common in *ETV6-RUNX1* ALL than in many other ALL subtypes and include deletions targeting putative lymphoid signaling molecules (*BTLA*, *TOX*), transcriptional coactivators (*TBLIXR1*), the glucocorticoid receptor gene *NR3C1*, and the putative apoptosis regulatory gene *BTG1*. Although these alterations may directly contribute to leukemogenesis, the genomic breakpoints of many of these deletions bear the hallmark of aberrant activity of the recombinase activation genes. Thus, further work directly examining these alterations in leukemogenesis is required.

**Conflict of interest:** The author has declared that no conflict of interest exists.

**Citation for this article:** *J Clin Invest.* 2012;122(10):3407–3415. doi:10.1172/JCI61203.



**Table 1**  
Key cytogenetic alterations and genetic subtypes in childhood B-ALL

Subtype	Frequency (%)	Comment	References
Hyperdiploidy (>50 chromosomes)	20–30	Excellent prognosis with antimetabolite-based therapy	3
Hypodiploidy (<44 chromosomes)	1–2	Poor prognosis, high frequency of RAS pathway and IKAROS gene family mutations	4
t(12;21)(p13;q22) <i>ETV6-RUNX1</i>	15–25	Expression of myeloid antigens; excellent outcome	5
t(1;19)(q23;p13) <i>TCF3-PBX1</i>	2–6	Increased incidence in African Americans; generally excellent prognosis; association with CNS relapse	121–123
t(9;22)(q34;q11.2) <i>BCR-ABL1</i>	2–4	Historically dismal outcome, improved with addition of imatinib to intensive chemotherapy	39, 42
<i>PAX5</i> rearrangement	~2%	Multiple partners, commonly from dic(7;9), dic/t(9;12) and dic(9;20); outcome unknown	17, 94
<i>ABL1</i> , <i>PDGFRB</i> , <i>JAK2</i> rearrangements	2–5	Identified in BCR-ABL1-like ALL; multiple rearrangements encoding chimeric proteins, fusing 5' partners with 3' kinase domains; associated with <i>IKZF1</i> alteration and very high leukocyte count; potentially amenable to tyrosine kinase inhibitor therapy	84
t(4;11)(q21;q23) <i>MLL-AF4</i>	1–2	Common in infant ALL (especially <6 months of age); poor prognosis	45
<i>MYC</i> rearrangement [t(8;14)(q24;q32), t(2;8)(q12;q24)]	2	Favorable prognosis with short-term, high-dose chemotherapy	70
<i>CRLF2</i> rearrangement ( <i>IGH@-CRLF2</i> , <i>PAR1</i> deletion, and <i>P2RY8-CRLF2</i> )	5–7	Extremely common in DS ALL (55%); association with <i>IKZF1</i> deletion/mutation and <i>JAK1/2</i> mutation; poor prognosis in non-DS ALL	71–75
<i>ERG</i> deletion	7	Subtype of B-ALL with a distinct gene expression profile; favorable outcome	124

### **TCF3-PBX1 (E2A-PBX1) and TCF3-HLF (E2A-HLF) B-ALL**

The t(1;19)(q23;p13) translocation is present in up to 6% of childhood B-ALL cases (21). The rearrangement is commonly unbalanced, with duplication of 1q distal to *PBX1*.

This translocation fuses the transactivation domains of *TCF3* with the C-terminal region of the homeobox gene *PBX1*. *TCF3* encodes the basic helix-loop-helix (bHLH) transcription factors E12 and E47 that are required for early lymphoid development, and loss of E12/E47 promotes the development of T cell lymphoma (22, 23). *PBX1*, which encodes a member of the three-amino acid loop extension family of homeodomain proteins, is required for the development of lymphoid precursors (24). *TCF3-PBX1* binds HOX proteins and likely interferes with hematopoietic differentiation by disrupting HOX-regulated gene expression (25).

The t(17;19)(q22;p13) translocation is a rare rearrangement that fuses the aminoterminal transactivation domains of *TCF3* to the C-terminal DNA-binding and dimerization domains of *HLF*, a member of the PAR family of basic leucine zipper transcription factors (26). *TCF3-HLF* aberrantly regulates genes that control cell death in lymphoid progenitors, including *LMO2* and *BCL2* (27). *TCF3-HLF*-positive leukemia is associated with older age and very poor outcome (28).

### **BCR-ABL1 ALL**

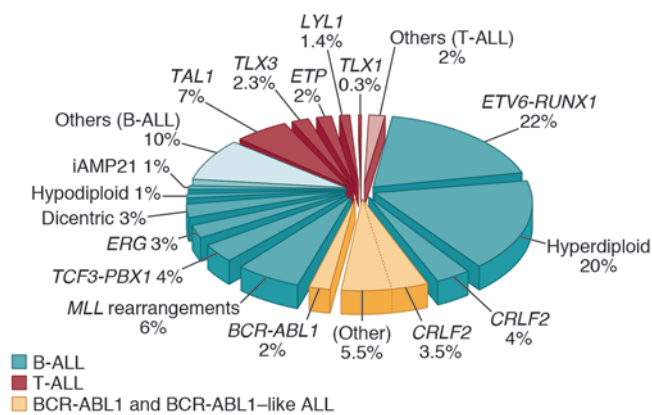
Breakpoint cluster region-*ABL1* (*BCR-ABL1*) is generated by the der(22) of the t(9;22)(q34;q11) translocation, or Philadelphia (Ph) chromosome, which is present in over 95% of chronic myelogenous leukemia (CML) cases, 25% of adult ALL cases, and 3% to 5% of pediatric ALL cases (29). The t(9;22) fuses 5' sequences of breakpoint cluster region (*BCR*) to 3' sequences from *ABL1*, which encodes a tyrosine kinase. The breakpoints on chromosome 9 are

scattered over a nearly 200-kb region within the first intron of *ABL1*, whereas the *BCR* breakpoints on chromosome 22 are clustered in two areas: a 5.8-kb major *BCR* (M-bcr) in CML and a minor *BCR* (m-bcr) in most cases of childhood Ph-positive ALL (30). Fusion genes created by breaks in M-bcr (CML-type break) encode a 210-kDa fusion protein (p210), whereas fusions that occur in m-bcr (ALL-type break) encode p190.

Both forms of *BCR-ABL1* can transform hematopoietic cells in vitro and induce a syndrome similar to CML in mice (31). *BCR-ABL1* activates multiple signaling pathways, increases cell proliferation, and deregulates differentiation and adhesion. Treatment of *BCR-ABL1*-positive leukemic cells with tyrosine kinase inhibitors results in the activation of the transcription factor *BCL6*, which may directly influence responsiveness to treatment with these agents (32).

Additional genetic alterations are critical determinants of the lineage and progression of *BCR-ABL1* leukemia. Deletion of the early lymphoid transcription factor gene *IKZF1* (IKAROS) is common in *BCR-ABL1* lymphoid leukemia (CML at progression to lymphoid blast crisis) but is rarely present in CML at chronic phase (33, 34). IKAROS is a zinc finger transcription factor required for the development of all lymphoid lineages (35, 36). The *IKZF1* deletions result either in haploinsufficiency or expression of dominant-negative isoforms. These alterations cooperate with *BCR-ABL1* in the induction of lymphoblastic leukemia and promote resistance to therapy in recent experimental models of *BCR-ABL1* ALL (37).

In childhood ALL, the presence of *BCR-ABL1* is associated with older age, higher leukocyte count, and more frequent CNS leukemia at diagnosis (38). Historically, *BCR-ABL1* ALL has been associated with extremely poor outcome (29, 39). Tyrosine kinase inhibitors

**Figure 1**

Frequency of cytogenetic subtypes of pediatric ALL. The pie chart includes all major B-ALLs and T-lineage subtypes of ALL, to illustrate the relative frequency of each. The recently described BCR-ABL1-like subtype and BCR-ABL1-positive ALL are shown in yellow to illustrate the high frequency of childhood B-ALL cases with genetic alterations activating tyrosine kinase and cytokine receptor signaling that may be amenable to targeted therapy. Data are derived from front-line studies of childhood ALL (121).

such as imatinib mesylate (Gleevec) have transformed the treatment and outcome of patients with BCR-ABL1-positive leukemia (40, 41). The addition of imatinib to intensive chemotherapy in childhood BCR-ABL1-positive ALL results in a 4-year event-free survival rate of 84%, more than double that of historical controls (42). A proportion of patients become resistant to these agents, typically through acquired mutations in the ABL1 kinase domain (43).

### MLL-rearranged B-ALL

Mixed-lineage leukemia-rearranged (*MLL*-rearranged) leukemia is a unique entity notable for initiation in utero, for myeloid and lymphoid features, and for poor responsiveness to therapy (44, 45). Rearrangement of the *MLL* gene at 11q23 occurs in at least two-thirds of infants with ALL, 5% of AML, and 85% of secondary leukemias that occur in patients treated with topoisomerase II inhibitors (46, 47) but is less frequent in older individuals (48, 49). Over 80 partners of *MLL* rearrangement have been identified (50).

The most common *MLL* rearrangements are t(4;11)(q21;q23)/*MLL-AFF1*(*AF4*), found in approximately 50% of cases, followed by t(9;11)(p22;q23)/*MLL-MLLT3*(*AF9*), *MLL-ENL*, and t(10;11)(p13-15;q14-21)/*MLL-MLLT10*(*AF10*) (51, 52). The translocation breakpoints are located in an 8.5-kb cluster between exons 5 and 11 and juxtapose the A-T hook and methyltransferase domains to partner proteins. *MLL* regulates hematopoiesis through maintenance of normal homeotic gene expression (53), in part through transcriptional activation of *HOX* genes, mediated through the histone H3 lysine 4 (H3K4) methyltransferase activity of the SET domain (54). In contrast to other subtypes of B-ALL, additional genetic alterations are uncommon in *MLL*-rearranged leukemia (17, 55, 56).

*MLL*-rearranged ALL exhibits a distinct gene expression profile characterized by the upregulation of class I *HOX* genes (*HOXA5*, *-A7*, and *-A9* and the *HOX* cofactor gene *MEIS1*) (57). Co-expression of *HOXA* genes and *MEIS1* cooperates with *MLL* fusions in induction of leukemia and maintenance of a “stem cell-like” state of maturation (58). *fms*-related tyrosine kinase 3 (*FLT3*) is overexpressed in *MLL*-rearranged ALL and harbors point mutations in 10%–20% of cases (59). In animal models, *FLT3* kinase inhibitors have shown potential as therapeutic agents for *MLL*-rearranged leukemias, and *FLT3* inhibitors are currently being tested in clinical trials (59, 60). *MLL*-rearranged leukemias also exhibit a distinct epigenetic profile, with signatures of cytosine (61), microRNA (62) and H3K79 methylation (63) that differ from non-*MLL* leukemias.

Many of the *MLL* fusion proteins are located in protein complexes that regulate transcriptional elongation, which may be in part responsible for transcriptional dysregulation and leukemogenesis. These include polymerase-associated factor complex, which associates with the N terminus of *MLL* and regulates RNA polymerase II (Pol II), the pTEFb (CDK9/cyclin T) complex, which associates with the fusion partners *MLLT1* (*ENL*), *ELL*, and *AFF1* (*AF4*) to release stalled Pol II and stimulate transcriptional elongation, and the H3K79 histone methyltransferase *DOT1L*, which binds *MLL* fusion partners including *AFF1*, *AF9*, *AF10*, and *ENL* (64). H3K79 methylation is a mark of actively transcribed genes, and suppression or inactivation of *DOT1L* in human or murine leukemia cells leads to suppression of the *MLL* fusion protein-induced gene expression program (63), differentiation and/or apoptosis of leukemic cell lines, and suppression of leukemogenesis (65). *DOT1L* inhibition is thus being pursued as a therapeutic approach in *MLL*-rearranged ALL (66).

These findings highlight the potential importance of epigenetic dysregulation in other subtypes of ALL. Several studies profiling cytosine methylation have shown that different ALL subgroups exhibit distinct methylation profiles and that promoter methylation is associated with gene expression and outcome (67, 68). Additional studies are needed to comprehensively determine the relative importance of genetic and epigenetic alteration in the pathogenesis of ALL as well as the potential therapeutic role of epigenetic modifying drugs.

### Rearrangement of *CRLF2* and other immunoglobulin heavy locus rearrangements in B-ALL

Rearrangements of the immunoglobulin heavy locus (*IGH@*) at 14q32.33, resulting in juxtaposition of *IGH@* enhancer elements with transcription factor and cytokine receptor genes, are observed in both B-ALL and T-lineage ALL (69). B-lineage leukemia/lymphoma with rearrangement of *MYC* is reviewed elsewhere (70).

Cytokine receptor-like factor 2 (*CRLF2*; also known as thymic stromal-derived lymphopoietin [TSLP] receptor) is located at the pseudoautosomal region (PAR1) at Xp22.3/Yp11.3 and is rearranged or mutated in up to 7% of B-ALL cases (71, 72). With the IL-7R $\alpha$  chain, *CRLF2* forms a heterodimeric receptor for TSLP. The rearrangements are either rearrangement into *IGH@-CRLF2* or focal deletion immediately upstream of *CRLF2*, resulting in a chimeric fusion of the purinergic receptor gene *P2RY8* to the entire coding region of *CRLF2*. Both rearrangements result in overexpression of *CRLF2* on the cell surface (71). Less commonly,



a p.Phe232Cys mutation in the transmembrane domain of CRLF2 results in receptor dimerization and overexpression (73).

*CRLF2* rearrangements are present in at least 50% of Down syndrome-associated (DS-associated) B-ALL cases (71, 72) and are also present in up to 50% of a novel subtype of high-risk B-ALL, BCR-ABL1-like ALL (see below) (74). *CRLF2* alterations are associated with the presence of activating mutations in the Janus kinase genes *JAK1* and *JAK2* (71–73, 75), which are otherwise uncommon in B-ALL. The JAK mutations in B-ALL most commonly disrupt p.Arg683 in the pseudokinase domain of *JAK2*, in contrast to the *JAK2* p.Val617Phe mutations that are a hallmark of myeloproliferative diseases. Like the *JAK2* p.Val617Phe mutation, the *JAK1/2* mutant alleles observed in ALL confer cytokine-independent proliferation in cultured cells, particularly in the presence of a cytokine receptor such as the EPOR or CRLF2, suggesting that a receptor scaffold is required for mutant JAK alleles to drive proliferation and lymphoid transformation (76, 77). In non-DS ALL, *CRLF2* rearrangements are associated with *IKZF1* alteration, *JAK* mutation, and poor outcome (74, 78). Recently, activating mutations in IL-7R have been identified in both B-ALL and T-lineage ALL, including cases of *CRLF2* rearrangement (10, 79, 80). Thus, the use of JAK inhibitors is actively being pursued to target leukemic cells harboring *CRLF2/JAK* alterations and leukemic cells with IL-7R mutations that also activate JAK/STAT signaling. Several other *IGH@* translocation partners have been identified in B-ALL, including EPOR, the CEBP transcription factor gene family, the inhibitor of DNA binding 4 (*ID4*) gene, the LIM domain-containing protein LHX4, and rarely, IL-3 (69).

### BCR-ABL1-like ALL

Up to 15% of childhood B-ALL cases exhibit a gene expression profile similar to that of BCR-ABL1-positive ALL, often have deletion/mutation of *IKZF1*, and have a very poor outcome (81–83). *CRLF2* is rearranged in up to 50% of BCR-ABL1-like ALL cases, but until recently the lesions responsible for kinase activation in the remaining cases were unknown. Second-generation mRNA sequencing and whole genome sequencing of 15 BCR-ABL1-like ALL cases identified rearrangements, sequence mutations, and DNA CNAs activating kinase signaling in all cases (84), including rearrangements of *PDGFRB*, *ABL1*, *JAK2*, and EPOR as well as deletion/mutation of *SH2B3* and *IL7R*. Several of these alterations have been shown to result in activation of downstream (e.g., JAK/STAT) signaling pathways, and cellular transformation was attenuated with JAK or ABL1/PDGFRB inhibitors. Thus, defining kinase-activating alterations in this subtype of ALL is critical to identifying and treating patients with this subtype of leukemia.

### Intrachromosomal amplification of chromosome 21

Intrachromosomal amplification of chromosome 21 (iAMP21) occurs in up to 2% of B-progenitor ALL cases (85). The region of amplification is typically large but variable, but includes gain of at least three copies of *RUNX1*, commonly with deletion of the subtelomeric regions of chromosome 21. iAMP21 is characterized by commonly complex patterns of chromosomal rearrangement suggestive of a breakage-fusion-bridge cycle (86). iAMP21 frequently occurs in older children or adolescents and has been reported to be associated with poor outcome (87), although this association may not be independent of other established prognostic variables such as levels of minimal residual disease (88).

### Hypodiploid ALL

Hypodiploidy, with fewer than 44 chromosomes, is present in up to 3% of childhood ALL cases and is associated with a high risk of treatment failure (89). Hypodiploid ALL may be subclassified by severity of aneuploidy into near-haploid (NH; 24–31 chromosomes) and low-hypodiploid (LH; 32–44 chromosomes) cases, but until recently the concomitant submicroscopic genetic alterations were poorly characterized. NH ALL has a very high frequency of deletions and sequence mutations that activate RAS signaling, and NH and LH ALL have a high frequency of inactivating alterations of IKAROS genes *IKZF2* (HELIOS) and *IKZF3* (AIOLOS) that are otherwise rare in ALL (90). Moreover, LH cases have a very high frequency of loss-of-function mutations in *TP53* (encoding p53) and RB1, which are otherwise uncommon at diagnosis in B-ALL.

### Submicroscopic genetic alterations in B-ALL

The observation that up to 25% of children with ALL lack a recurring chromosomal alteration (2) and the identification of translocation-encoded fusions at birth or in years prior to the onset of leukemia (16) indicates that additional genetic alterations cooperate in leukemogenesis. The advent of array-comparative genomic hybridization and SNP microarrays has enabled interrogation of structural genetic alterations at very high resolution (91). These approaches, coupled with candidate gene sequencing and integrated gene expression profiling (92), have identified a number of critical new targets of mutation in B-ALL that often involve genes that regulate lymphoid development, cell cycle, tumor suppression, and a variety of other key cellular pathways (Table 2). The mean number of DNA CNAs is lower than in many solid tumors (an average of 6–8 lesions per case) (17, 19, 33), but over 50 recurring CNAs have been identified. The nature and frequency of these CNAs are significantly associated with ALL subtype, with few lesions in MLL-rearranged ALL (17, 55) but 6–8 lesions per case in *BCR-ABL1* and *ETV6-RUNX1* ALL (33, 34).

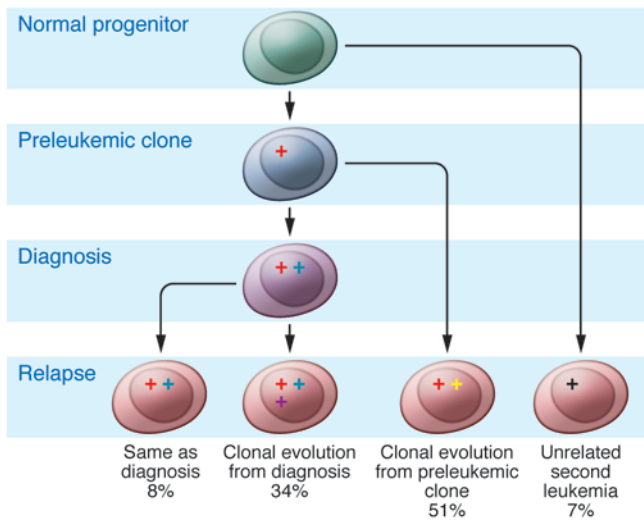
Most notably, genes encoding transcriptional regulators of B-lineage commitment and differentiation are mutated in over two-thirds of B-ALL. These include *PAX5* (encoding paired box 5, mutated in over 30% of cases), EBF1 (early B cell factor 1), *IKZF1-3*, and lymphoid enhancer factor 1. *PAX5* harbors broad deletions predicted to result in haploinsufficiency, focal internal deletions that result in the expression of truncated *PAX5* transcripts, sequence mutations that disrupt DNA binding or truncate the transactivating domain (17, 93), and translocations that create chimeric fusion proteins (17, 93, 94). These lesions usually only involve a single copy of *PAX5*, and expression of key *PAX5* targets, such as CD19, in ALL cells is frequently normal, suggesting that *PAX5* haploinsufficiency contributes to leukemogenesis.

Despite their high frequency of occurrence, *PAX5* alterations are not associated with outcome in ALL (81, 95). In contrast, *IKZF1* alterations are less frequent (15% of B-ALLs) but are associated with adverse outcome in both BCR-ABL1-positive and negative ALL cases. *IKZF1* alterations are present in up to one-third of high-risk B-ALL cases and triple the risk of treatment failure (81, 96). *IKZF1*, which is required for lymphoid development, binds DNA and forms homodimers or heterodimers with other IKAROS family members. *IKZF1* has multiple functions including transcriptional activation, repression, and chromatin remodeling, in part mediated via the nucleosome remodeling and deacetylase complex that also contains *IKZF3*. The alterations observed in ALL include broad and focal deletions of *IKZF1*, which result in loss of func-



**Table 2**  
Key novel genetic alterations in B-ALL

Gene	Alteration	Frequency	Consequences of alteration	Clinical relevance	References
<i>PAX5</i>	Focal deletions, translocations, sequence mutations	31.7% of B-ALL cases	Transcription factor required for B-lymphoid development; mutations impair DNA binding and transcriptional activation		17, 19, 33
<i>IKZF1</i>	Focal deletions or sequence mutations	15% of pediatric B-ALL cases  Over 80% of BCR-ABL1 ALL cases and 66% of CML cases in lymphoid blast crisis  Approximately 30% of high-risk BCR-ABL1-negative ALL cases	Transcription factor required for development of HSCs to lymphoid precursors; deletions and mutations result in loss of function or dominant-negative isoforms	Associated with poor outcome	17
<i>JAK1/2</i>	Inherited variants  Pseudokinase and kinase domain mutations	18%–35% of DS-ALL  10.7% of high-risk BCR-ABL1 ALL cases	Constitutive JAK/STAT activation; transforms hematopoietic cell lines to be growth factor independent	Tripled relapse rate  Increased risk of ALL	81, 82, 96  118, 119 76, 77  77
<i>CRLF2</i>	<i>IGH@-CRLF2</i> or <i>P2RY8-CRLF2</i> resulting in <i>CRLF2</i> overexpression	5%–16% of pediatric and adult B-ALL cases; >50% of DS ALL	Associated with mutant JAK in up to 50% of cases; <i>CRLF2</i> mutations and JAK mutations co-transform in Ba/F3 cells and result in constitutive JAK/STAT activation		71–73, 75
<i>CREBBP</i>	Focal deletion and sequence mutations	14% of pediatric high-risk ALL cases  19% of relapsed ALL cases, especially ALL with high hyperploidy; also mutated in non-Hodgkin lymphoma	Associated with IKZF1 alteration and JAK mutations  Mutations result in impaired histone acetylation and transcriptional regulation	Associated with poor outcome  Mutations selected for at relapse and associated with glucocorticoid resistance	74, 78  112, 114, 115



**Figure 2**

Patterns of genomic evolution from diagnosis to relapse in ALL. Leukemic clones at relapse are frequently related to leukemic clones present at diagnosis. In more than half of cases, the relapse clone arises from a clone present prior to diagnosis, retaining some but not all of the lesions found at diagnosis and containing some additional mutations. The relapse clone is often present as a rare subclone within the diagnostic sample. Alternatively, cases of relapse can stem from the diagnosis clone, acquiring additional mutations. Less commonly, the relapse clone may be identical to the diagnosis clone or appear to be an unrelated second leukemia.

tion, focal internal deletions of exons 4–7, resulting in expression of the dominant-negative isoform IK6, and less commonly, loss-of-function and dominant-negative sequence mutations. How these IKZF1 alterations contribute to leukemogenesis remains poorly understood. IKZF1 inactivation likely contributes to the arrest in maturation characteristic of ALL, and loss of *Ikzf1*, like that of *Pax5*, accelerates BCR-ABL1 lymphoid leukemia development in murine models (37). However, the striking association of *IKZF1* alterations with poor outcome suggests that other mechanisms may be involved. Detection of *IKZF1* alterations at diagnosis to aid risk stratification and treatment assignment is consequently under investigation in ongoing prospective clinical trials.

Additional recurring genetic alterations in B-ALL target cell cycle regulation, transcriptional activation, tumor suppression, lymphoid signaling, apoptosis, and drug responsiveness. These include *CDKN2A/CDKN2B*, *RB1*, *CD200*, *BTLA/TOX*, *BTG1*, the glucocorticoid receptor *NR3C1*, *TBL1XR1*, *ETV6*, and *ERG* (17–19, 97). Candidate gene sequencing approaches have identified additional sequence mutations in B-ALL, most commonly mutations resulting in activation in RAS signaling, which are enriched in *MLL* and hyperdiploid ALL (98, 99). Additional pathways targeted include JAK/STAT signaling, the TP53/RB1 pathway, and lymphoid development and additional mutations have been found in transcriptional coactivators, including *ERG*, *TBL1XR1*, *CTCF*, *CREBBP*, and *EP300* (100).

**Genetics of relapse in ALL**

ALL genomes are not static, frequently acquiring secondary karyotypic and genetic alterations during disease progression (101–103). Genome-wide profiling has enabled key questions in the genetic basis of treatment failure to be investigated, including the nature of genetic alterations that are acquired with disease progression, the genetic relationship between the diagnosis and relapsed leukemic clones, and the degree of clonal heterogeneity present at the time of diagnosis.

Microarray profiling has shown that relapse samples exhibit striking differences in the patterns of genomic alterations from the matched diagnosis samples (104–108), with the majority of relapse samples exhibiting the loss of CNAs present at diagnosis and the acquisition of new genetic alterations. Importantly, most

paired diagnosis-relapse samples share a common clonal origin. Moreover, lesions detected at relapse are often detectable in diagnostic samples, indicating that the predominant relapse clone(s) are present at low levels at diagnosis. Genetically unrelated second leukemias (at least at the level of DNA CNA analysis by SNP array) are infrequent but may be more common in late relapse (109). Approximately one-third of cases show a pattern of linear clonal evolution (with the acquisition of new genetic changes by the relapse clone in addition to those seen at relapse), and over 50% show a complex picture with both loss of diagnosis CNAs and the acquisition of new lesions, as well as evidence of low levels of relapse clones at diagnosis (Figure 2).

Together, these data suggest that a pre-diagnosis “ancestral” clone harboring one or more genetic alterations (such as a founding translocation) undergoes divergent evolution into multiple clones that acquire different genetic alterations and emerge as the predominant clones at diagnosis and relapse. This is supported by studies of monozygotic twins concordant for ALL, showing different secondary CNAs in each twin (20), and recent xenotransplantation studies that have modeled clonal evolution and heterogeneity by engrafting *ETV6-RUNX1* and *BCR-ABL1* samples into immunocompromised mice. These studies have confirmed clonal heterogeneity in the majority of cases at the time of diagnosis and have shown that specific genetic alterations (e.g., deletion of *CDKN2A/CDKN2B*) influence the efficiency and tempo of engraftment (110, 111).

Genome-wide sequencing is required to comprehensively identify all sequence and structural variants contributing to the pathogenesis of ALL, and a number of sequencing efforts are ongoing. However, a recent study performed Sanger sequencing of 300 genes in 23 matched diagnosis-relapse samples, with recurrence testing of over 300 paired and unpaired ALL samples (112). This study identified a number of uncharacterized mutations in ALL, most notably deleterious mutations in *CREBBP* (encoding CREB-binding protein) in over 20% of relapse ALL samples (112). The mutations were present at relapse and were detected in either the predominant clone or a subclone at diagnosis. *CREBBP* is a transcriptional coactivator, histone and non-histone acetylase, and ubiquitin ligase (113). Mutations were common in the histone acetyl transferase (HAT) domain and resulted in impaired acetylation of histone targets (112). *CREBBP* mutations are highly enriched



in hyperdiploid ALL (114) and are common at diagnosis in B cell lymphoma samples (115). Importantly, the HAT mutations impair the normal CREBBP-mediated transcriptional response to glucocorticoids, which are widely used in ALL therapy. Thus, CREBBP mutations may impair the response of leukemic cells to glucocorticoids and influence treatment responsiveness, and therapeutic approaches modifying acetylation may reverse glucocorticoid resistance. Mutations in *TP53*, which are otherwise infrequent in ALL, are also enriched in relapsed ALL (116).

### Inherited genetic variation and risk of ALL

Several candidate gene studies have implicated inherited genetic variation, for example in the folate metabolic pathway, in the risk of developing leukemia (117). Multiple genome-wide association studies have reproducibly identified variations at several genomic loci that are associated with ALL risk, including *IKZF1*, *ARID5B* (encoding the AT-rich interactive domain 5B transcription factor), and *CEBPE* (encoding the transcription factor CCAAT/enhancer-binding protein, epsilon) (118, 119). All three genes encode proteins considered important for normal lymphoid development and/or lymphoid leukemogenesis. *CEBPE* is a target of translocations in ALL, and *Arid5b* knockout mice exhibit defects in the B lymphoid compartment (120). Although the functional effects of these inherited variants are poorly understood, the *IKZF1* variants may influence level of *IKZF1* gene expression (119), suggesting a direct role for these variants in disease susceptibility.

### Conclusions

The use of contemporary technologies to identify genetic alterations in B-ALL has been tremendously informative, but clearly much work

remains to be done. Microarray and candidate gene sequencing approaches are incapable of identifying mutations at nucleotide-level resolution, and initial second-generation sequencing of ALL genomes has identified multiple new genes targeted in ALL. An additional high-priority area of investigation is ALL in older children, adolescents, and adults, who typically have a substantially inferior prognosis. Profiling of ALL in these age groups, as well as in additional uncommon but high-risk ALL subtypes, is ongoing and likely to yield additional insights and therapeutic approaches.

### Acknowledgments

The author thanks members of his laboratory and colleagues at St. Jude Children's Research Hospital, the Children's Oncology Group, and the National Cancer Institute Therapeutically Applicable Research to Generate Effective Treatments (TARGET) Initiative, who have contributed to the studies described in this Review, and apologizes to the many investigators whose important studies could not be cited due to space restrictions. The author is supported by the American Lebanese Syrian Associated Charities of St. Jude Children's Research Hospital and by funding from the National Cancer Institute, the American Society of Hematology, the American Association of Cancer Research, and Stand Up to Cancer. The author is a Pew Scholar in the Biomedical Sciences and a St. Baldrick's Scholar.

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