Genetic Basis of Acute Lymphoblastic Leukemia
Ilaria Iacobucci and Charles G. Mullighan

ABSTRACT

Acute lymphoblastic leukemia (ALL) is the most common childhood cancer, and despite cure rates exceeding 90% in children, it remains an important cause of morbidity and mortality in children and adults. The past decade has been marked by extraordinary advances into the genetic basis of leukemogenesis and treatment responsiveness in ALL. Both B-cell and T-cell ALL comprise multiple subtypes harboring distinct constellations of somatic structural DNA rearrangements and sequence mutations that commonly perturb lymphoid development, cytokine receptors, kinase and Ras signaling, tumor suppression, and chromatin modification. Recent studies have helped to understand the genetic basis of clonal evolution and relapse and the role of inherited genetic variants in leukemogenesis. Many of these findings are of clinical importance, and ongoing studies implementing clinical sequencing in the management of leukemia are expected to improve diagnosis, monitoring of residual disease, and early detection of relapse and to guide precise therapies. Here, we provide a concise review of genomic studies in ALL and discuss the role of genomic testing in clinical management.

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BCP-ALL WITH RECURRENT CHROMOSOMAL ALTERATIONS

KMT2A (MLL) rearrangements, particularly the t(4;11)(q21;q23) translocation, are most frequent in infants (< 1 year of age) and are associated with poor outcome. High hyperdiploidy with gain of at least five chromosomes and ETV6-RUNXI are each present in 25% to 30% of patients with childhood ALL but occur in less than 3% of young adults and are associated with favorable outcome. Conversely, BCR-ABL1 (Philadelphia [Ph] chromosome) –positive ALL comprises 2% to 5% of childhood and 25% of adult ALL, and although historically associated with poor prognosis, outcomes have been markedly improved with the use of tyrosine kinase inhibitors (TKIs). The translocation t(1;19)(q23;p13) resulting in the TCF3-PBX1 fusion occurs in approximately 5% to 6% of childhood and adult BCP-ALLs. It was originally considered to be a high-risk subtype of ALL, but with contemporary therapy, it is now associated with a favorable outcome, although some studies have reported that it has an independent risk factor for CNS relapse. A variant of the t(1;19) translocation, t(17;19)(q23;p13), results in the TCF3-HLF fusion (< 1% of ALLs), which is associated with a poor prognosis.

Complex intrachromosomal amplification of chromosome 21 (iAMP21) is most common in older children and is associated with poor prognosis, which is improved with intensive treatment. Hypodiploidy with less than 44 chromosomes occurs in 2% to 3% of patients and is a negative prognostic factor. Hypodiploid ALL itself comprises several subtypes with distinct transcriptional profiles and genetic alterations, including near-haploid cases (24 to 31 chromosomes) with Ras-activating mutations and IKZF3 alterations, and low hypodiploidy (32 to 39 chromosomes) with IKZF2 alterations and TP53 mutations that are frequently inherited.
Secondary DNA deletions, gains, and mutations are characteristic of BCP-ALL, are important cooperating lesions in leukemogenesis, and may be acquired or enriched during disease progression. These include alterations of lymphoid transcription factors (IKZF1, PAHX, EBF1), cell-cycle regulation and tumor suppression (CDKN2A/CDKN2B, RB1), regulation of apoptosis, transcriptional regulation and coactivation (ETV6, EGR), and epigenetic alterations. The prevalence, type of alteration, and gene vary between subtypes. KMT2A-rearranged cases show low frequency of secondary somatic mutations, which are often sub-clonal, indicating that KMT2A rearrangement is sufficient to induce leukemia. IKZF1 alterations are a hallmark of BCR-ABL1-positive and Ph-like ALL and are associated with poor outcome, in contrast, other members of the IKAROS transcription factor family, IKZF2 and IKZF3, are selectively mutated in hypodiploid ALL. In high hyperdiploid ALL, secondary events target genes in the Ras signaling pathway and in chromatin modifiers.

NEW SUBTYPES OF BCP-ALL

Approximately 25% of childhood ALLs and a higher proportion of adult BCP-ALLs lack a unifying chromosomal alteration on cytogenetic analysis (Fig 1). Several new subtypes of ALL have been recently described that exhibit distinct leukemic-cell gene expression profiles but diverse, often cytogenetically cryptic, founding alterations.

Ph-Like ALL

The 2016 revision to the WHO classification of myeloid neoplasms and acute leukemia recognized BCR-ABL1-like or Ph-like ALL as a new leukemia entity of clinical importance due to its association with an adverse prognosis and responsiveness to TKIs. Ph-like ALLs harbor a gene-expression profile similar to BCR-ABL1–positive ALLs but lack BCR-ABL1. The prevalence of Ph-like ALL increases with age and varies from 10% in standard-risk childhood ALL to greater than 20% in adult ALL, with a peak prevalence of 27.9% in young adults (age 21 to 39 years). In both children and adults, Ph-like ALL is associated with high-risk clinical features, a poor response to induction chemotherapy, elevated minimal residual disease (MRD) levels, and/or poor survival.

Common genomic features of BCR-ABL1-like ALL are alterations of B-lymphoid transcription factor genes (particularly IKZF1 deletions) and genetic alterations deregulating cytokine receptor and tyrosine kinase signaling. These include rearrangements and mutation of CRLF2 (approximately 50%), rearrangements of ABL1-class tyrosine kinase genes (12%), rearrangements of JAK2 (7%) and the erythropoietin receptor gene (EPOR; 3% to 10%), mutations activating JAK-STAT signaling (11%) and Ras signaling (NRAS, KRAS, PTPN11, and NF1; 6%), and less common kinase alterations (FLT3, NTRK3, BLNK, TYK2, and PTK2B). All kinase fusions retain an intact tyrosine kinase domain and typically exhibit constitutive kinase activation (Fig 2). With the exception of EPOR and JAK2 rearrangements, which are increased in adult Ph-like ALL, there are no significant differences in the frequency of kinase subtypes across different age groups (Fig 3).

CRLF2 encodes cytokine receptor-like factor 2, also known as the thymic stromal-derived lymphopoietin receptor (TSLPR) that forms a heterodimeric receptor with the interleukin-7 receptor α chain (IL7Rα) for thymic stromal lymphopoietin (TSLP). CRLF2 is deregulated by translocation into the immunoglobulin heavy chain locus (IGH-CRLF2); focal deletion upstream of CRLF2, resulting in formation of a P2RY8-CRLF2 fusion; and less commonly, CRLF2 point mutations (F232C). CRLF2 rearrangements are most common in Ph-like and Down syndrome–associated ALL and are age dependent, with P2RY8–CRLF2 associated with young age and IGH-CRLF2 associated with older age and Hispanic ancestry. CRLF2 is overexpressed on the cell surface of leukemic lymphoblasts and detectable by flow cytometric immunophenotyping. The majority of CRLF2-rearranged ALLs have additional alterations driving JAK-STAT or Ras signaling, particularly activating JAK1 or JAK2 mutations, FLT3 and ILG sequence mutations, SH2B3 deletions, TSLP rearrangements, and Ras mutations. In most studies, CRLF2 rearrangements are associated with poor prognosis, particularly in cases with concomitant IKZF1 alteration. Therapies targeting JAK-STAT, PI3K/mTOR, and BCL2 signaling alone or in combination have shown efficacy in preclinical models.

Another major Ph-like ALL genetic subgroup involves ABL-class rearrangements, which include fusions to ABL1, ABL2, CSFIR (encoding the macrophage colony-stimulating factor receptor), PDGFRα, or PDGFRβ that are all targetable by inhibitors of ABL1, such as imatinib and dasatinib.
### Genetics of ALL

<table>
<thead>
<tr>
<th>Category</th>
<th>Age</th>
<th>Description</th>
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<tbody>
<tr>
<td><strong>BCP-ALL</strong></td>
<td></td>
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<tr>
<td>Hyperdiploidy with &gt; 50 chromosomes</td>
<td>Children &gt; &gt; adults</td>
<td>Excellent prognosis; mutations in Ras signaling pathway and histone modifiers</td>
</tr>
<tr>
<td>Near-haploid</td>
<td>Children and adults</td>
<td>24-31 chromosomes; poor prognosis; Ras-activating mutations and inactivation of IKZF3</td>
</tr>
<tr>
<td>Low hypodiploid</td>
<td>Children and adults</td>
<td>32-39 chromosomes; poor prognosis; TP53 mutations, deletions of CDKN2A/B and RB1, and inactivation of IKZF2</td>
</tr>
<tr>
<td>High hypodiploid</td>
<td>Children and adults</td>
<td>40-43 chromosomes; rare; poor prognosis</td>
</tr>
<tr>
<td>Near-diploid</td>
<td>Children and adults</td>
<td>44-45 chromosomes; distinct entity frequently with ETV6-RUNX1 fusion or rearrangements forming dicentric chromosomes</td>
</tr>
<tr>
<td><strong>t(12;21)(p13;q22)</strong> translocation encoding ETV6-RUNX1</td>
<td>Children &gt; &gt; adults</td>
<td>Excellent prognosis; cryptic rearrangement that is detectable by FISH or PCR</td>
</tr>
<tr>
<td><strong>t(11;19)(q23;p13)</strong> translocation encoding TCF3-PBX1</td>
<td>Children and adults</td>
<td>Increased incidence in African Americans; generally excellent prognosis; association with CNS relapse</td>
</tr>
<tr>
<td><strong>t(9;22)(q34;q11.2)</strong> translocation encoding BCR-ABL1</td>
<td>Children &lt; &lt; adults</td>
<td>Historically poor outcome, improved with tyrosine kinase inhibitors; common deletions of IKZF1, CDKN2A/B, and PAX5</td>
</tr>
<tr>
<td><strong>Ph-like ALL</strong></td>
<td>Children &lt; adults</td>
<td>Multiple kinase-activating lesions; poor outcome, amenable to tyrosine kinase inhibitor therapy</td>
</tr>
<tr>
<td><strong>CRLF2 rearrangement (IGH-CRLF2, P2RY8-CRLF2)</strong></td>
<td>Children and adults</td>
<td>Common in Down syndrome–associated and Ph-like ALL (approximately 50%); associated with IKZF1 deletion and/or mutation and JAK1/2 mutation and poor prognosis in non-Down syndrome–associated ALL</td>
</tr>
<tr>
<td><strong>KMT2A (MLL) rearrangements</strong></td>
<td>Infants &gt; &gt; &gt; children and adults</td>
<td>Common in infant ALL; poor prognosis; low number of additional somatic mutations, commonly in kinase-PI3K-RAS signaling pathway</td>
</tr>
<tr>
<td><strong>DUX4- and ERG-deregulated ALL</strong></td>
<td>Children and adults</td>
<td>Distinct gene expression profile; majority have focal ERG deletions and favorable outcome despite IKZF1 alterations</td>
</tr>
<tr>
<td><strong>MEF2D-rearranged ALL</strong></td>
<td>Children and adults</td>
<td>Distinct gene expression profile and aberrant immunophenotype (CD10 negative, CD38 positive); sensitivity to HDAC inhibitors</td>
</tr>
<tr>
<td><strong>ZNF384-rearranged ALL</strong></td>
<td>Children &lt; AYA and adults</td>
<td>Fusions are associated with early pro-B-ALL, expression of myeloid markers, and activation of the JAK-STAT pathway</td>
</tr>
<tr>
<td><strong>PAX5 rearrangements</strong></td>
<td>Children and adults</td>
<td>Multiple partners, commonly from dic(7;9), dic(9;12), and dic(9;20)</td>
</tr>
<tr>
<td><strong>iAMP21</strong></td>
<td>Older children</td>
<td>Complex structural alterations of chromosome 21; rarely associated with a constitutional Robertsonian translocation rob(15;21)(q10;q10); poor prognosis</td>
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<td><strong>T-ALL</strong></td>
<td></td>
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<tr>
<td><strong>TEL1 deregulation</strong></td>
<td>Children &lt; adults</td>
<td>t(11;14)(p32;q35) and t(11;14)(p32;q11) translocations and interstitial t(13;12) deletion; generally favorable outcome</td>
</tr>
<tr>
<td><strong>LMO2 deregulation</strong></td>
<td>Children</td>
<td>t(11;14)(p15;q11) translocation and 5‘ LMO2 deletion; generally favorable outcome</td>
</tr>
<tr>
<td><strong>TLX1 (HOX11) deregulation</strong></td>
<td>Children &lt; adults</td>
<td>t(10;14)(q24;q11) and t(7;10)(q35;q24) translocations; good prognosis</td>
</tr>
<tr>
<td><strong>TLX3 (HOX11L2) deregulation</strong></td>
<td>Children and adults</td>
<td>t(5;14)(q35;q32) translocation; commonly fused to BCL11B, also a target of deletion and/or mutation; poor prognosis</td>
</tr>
<tr>
<td><strong>MLL rearrangements</strong></td>
<td>Children</td>
<td>Multiple partners; disruption of HOX gene expression and of self-renewing; poor outcome</td>
</tr>
<tr>
<td><strong>9q34 amplification encoding NUP214-ABL1</strong></td>
<td>Children</td>
<td>Aminable to tyrosine kinase inhibitors, also identified in high-risk B-ALL; other kinase fusions identified in T-ALL include EML1-ABL1, ETV6-JAK2, and ETV6-ABL1</td>
</tr>
<tr>
<td><strong>t(7;9)(q34;q34.3)</strong></td>
<td>Children</td>
<td>Rearrangement of NOTCH1</td>
</tr>
<tr>
<td><strong>NOTCH1 mutations</strong></td>
<td>Children and adults</td>
<td>Impairment of differentiation and proliferation; overall favorable outcome</td>
</tr>
<tr>
<td><strong>FBXW7 mutations</strong></td>
<td>Children and adults</td>
<td>Impairment of differentiation and proliferation; usually evaluated in combination with NOTCH1</td>
</tr>
<tr>
<td><strong>Early T-cell precursor ALL</strong></td>
<td>Children and adults</td>
<td>Immature immunophenotype; expression of myeloid and/or stem-cell markers; poor outcome; genetically heterogeneous with mutations in hematopoietic regulators, cytokine and Ras signaling, and epigenetic modifiers</td>
</tr>
</tbody>
</table>

NOTE: The frequency of some alterations in the adult cohort may be underestimated as a result of lack of studies. Abbreviations: ALL, acute lymphoblastic leukemia; AYA, adolescents and young adults; B-ALL, B-cell acute lymphoblastic leukemia; BCP-ALL, B-cell precursor acute lymphoblastic leukemia; FISH, fluorescence in situ hybridization; HDAC, histone deacetylase; PCR, polymerase chain reaction; Ph-like, Philadelphia chromosome–like; T-ALL, T-cell acute lymphoblastic leukemia; >, high; >>, very high; >>>, considerably high; <, low; <<<, very low.

Genomic rearrangements that produce JAK2 fusion genes or rearrangements targeting EPOR are highly sensitive to JAK2 inhibitors, including ruxolitinib, in preclinical models. JAK2 is rearranged to at least 14 different partner genes in Ph-like ALL. EPOR rearrangements include reciprocal or cryptic translocations with immunoglobulin and other loci that deregulate receptor
expression and also truncate the cytoplasmic tail of the receptor, resulting in augmented JAK-STAT signaling. The extensive preclinical data showing activation of signaling pathways, inhibition with JAK-STAT or ABL inhibitors, synergy with conventional chemotherapy, and anecdotal responsiveness to TKI therapy in patients with Ph-like ALL have led to the multiple prospective studies examining the efficacy of TKIs in Ph-like ALL (Table 2).

**DUX4- and ERG-Deregulated ALL**

Several studies recently identified a subtype of BCP-ALL (up to 7% of BCP-ALLs) with a distinct immunophenotype and gene expression profile characterized by deregulation of the double homeobox 4 gene (DUX4) and the ETS transcription factor gene (ERG). DUX4 encodes a double homeobox transcription factor located in a macrosatellite D4Z4 repeat in the subtelomeric region of the long arm of chromosome 4.

DUX4 is not expressed in normal B cells, and translocation to IGH results in expression of a truncated DUX4 isoform in the B-cell lineage. Less commonly, ERG-DUX4 fusions have also been described. Prior studies had reported intragenic deletions of the ERG gene in approximately 5% of childhood ALLs, which are now known to be restricted to DUX4-rearranged cases. Notably DUX4-rearranged ALLs commonly express aberrant ERG transcripts and truncated C-terminal ERG proteins irrespective of the presence of ERG deletions. The basis for this association has now been elucidated (Fig 4). DUX4 rearrangement is an early initiating event in leukemogenesis, and aberrantly expressed DUX4 binds to an intragenic region of ERG, resulting in expression of a non-canonical first exon and transcript, ERGalt. This encodes a truncated C-terminal ERG protein that retains the DNA-binding and transactivating domains of ERG, inhibits wild-type ERG transcriptional activity, and is transforming. Notably, DUX4/ERG-deregulated ALL is associated with a favorable outcome, despite the presence of concomitant genetic alterations otherwise associated with a poor outcome, such as IKZF1 deletions, which are present in approximately 40% of patients. DUX4 rearrangement is not evident on karyotypic analysis but is important to identify by gene expression or sequencing approaches to accurately assign risk and guide therapy.

**MEF2D and ZNF384 Gene Fusions**

Myocyte enhancer factor 2D (MEF2D) and zinc finger 384 (ZNF384) rearrangements characterize distinct B-ALL subtypes, accounting for approximately 3% to 4% and 3% of pediatric patients and approximately 6% and 7% of adult patients, respectively. MEF2D is rearranged to BCL9, HNRNPUL1, SS18, FOXXJ2, CSF1R, and DAZAP1, with the most common target of rearrangement being BCL9. All fusions preserve the MEF2D MADS-box domain that mediates DNA binding, result in enhanced MEF2D transcriptional activity, and are transforming and leukemogenic in vitro and in vivo. MEF2D ALL is associated with older age of onset, an aberrant immunophenotype (CD10 negative, CD38 positive), and poor outcome. The deregulation of MEF2D target genes results in a therapeutic vulnerability, because one such gene is histone deacetylase 9 (HDAC9), and human xenografts of MEF2D ALL are exquisitely sensitive to histone deacetylase inhibitors, such as panobinostat.

ZNF384 rearrangements involve the fusion of a 5' partner gene, usually a transcriptional regulator or chromatin modifier (EP300,
CREBBP, TAF15, SYNRG, EWSR1, TCF3, and ARID1B), to the entire coding region of ZNF384. ZNF384-rearranged ALLs are often diagnosed as B-ALLs with expression of myeloid antigens or as B/myeloid mixed-phenotype acute leukemias, suggesting transformation of a hematopoietic progenitor with B/myeloid potential. ZNF384-rearranged B-ALL has an intermediate prognosis and is characterized by upregulation of the JAK-STAT pathway, suggesting a potential benefit from treatment with inhibitors of this pathway.35

Other Rearrangements

Additional recurrent alterations in BCP-ALL include IGH translocations, including CRLF2 and EPOR in Ph-like ALL, CEBP gene family members, and ID4. Their frequency is low among children younger than 10 years old (<3%) but considerably higher (10%) among adolescents and young adults (age 15 to 24 years), and prognosis is poor.42

PAX5 is also rearranged to a diverse range of fusion partners in approximately 2% of B-ALLs. These commonly result in the

Table 2. Clinical Trials in ALL Targeting Specific Alterations and/or Deregulated Pathways

<table>
<thead>
<tr>
<th>ALL Subtype</th>
<th>Target</th>
<th>Therapy</th>
<th>Clinicaltrials.gov Identifier</th>
<th>Study Phase</th>
<th>Age of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ph-like TKI-sensitive mutations</td>
<td>Dasatinib + chemotherapy</td>
<td>NCT02883049 Phase III 1-30 years</td>
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<tr>
<td>Ph-like TKI-sensitive mutations</td>
<td>Dasatinib + chemotherapy</td>
<td>NCT02420717 Phase II 10 years</td>
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<tr>
<td>Ph-like JAK2 activation mutations</td>
<td>Ruxolitinib + chemotherapy</td>
<td>NCT02420717 Phase II 10 years</td>
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<tr>
<td>Ph-like JAK2 activation mutations</td>
<td>Ruxolitinib + chemotherapy</td>
<td>NCT02739094 Phase II 1-21 years</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>MLL rearranged DNA methylation</td>
<td>Azacitidine + chemotherapy</td>
<td>NCT02929388 Not provided 3-364 days</td>
<td></td>
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<tr>
<td>MLL rearranged DOT1L</td>
<td>EPZ-05676</td>
<td>NCT02141828 Phase I 1-21 years</td>
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</table>

Abbreviations: ALL, acute lymphoblastic leukemia; DOT1L, DOT1 like histone lyase methyltransferase; TKI, tyrosine kinase inhibitor.
5’ N-terminal DNA-binding domain of PAX5 fused to the 3’ C terminus of the partner gene, resulting in loss of the transactivating domain of PAX5. Several of these fusions inhibit the normal transcriptional activity of PAX5, although it remains to be directly shown whether these fusions promote leukemogenesis through haploinsufficiency of wild-type PAX5 or whether they are exerting an oncogenic effect.

**GENETICS OF T-ALL**

T-ALL is an aggressive and heterogeneous disease that accounts for approximately 15% and 25% of pediatric and adult ALLs, respectively. Approximately 50% of patients with T-ALL harbor chromosomal translocations that most commonly involve the 14q11 (T-cell receptor α and δ [TRA and TRD]) and 7q34 (TRB) regions, juxtaposing the T-cell receptor genes to pivotal transcription factor genes, such as TAL1, TAL2, ILY1, OLIG2, LMO1, LMO2, TLX1 (HOX11), TLX3 (HOX11L2), NKX2-1, NKX2-2, NKX2-5, HOXA genes, MYC, and MYB. In addition, T-ALLs may harbor cryptic rearrangements of ABL1 (NUP214-ABL1, EML1-ABL1, and ETV6-ABL1) that may be amenable to TKI therapy. Moreover, gene expression profiling studies have helped in the classification of T-ALL into molecular subgroups that are characterized by unique gene expression signatures and aberrant activation of specific T-ALL transcription factor oncogenes, including MEF2C, HOXA, TLX1, NKX2.1, TLX3, TAL1, LMO1, and LMO2.44,45

Sequence mutations and DNA copy number alterations include those in NOTCH1, FBXW7, and MYB; in genes involved in the JAK-STAT (IL7R, JAK1, JAK3, and STAT5B) and Ras/PI3K/AKT (NRAS/KRAS and PTEN) pathways; in epigenetic regulators (PHF6, SUZ12, EZH2, TET2, H3F3A, and KDM6A); in transcription regulators (LEFI1, WT1, BCL11B, and ZEB2); and in genes involved in mRNA maturation and ribosome activity (CNOT3, RPL5, and RPL10). Activating NOTCH1 mutations and loss-of-function mutations of FBXW7, leading to inhibition of ubiquitin-mediated degradation of the activated form of NOTCH1, occur in more than 60% and 15% of T-ALLs, respectively.46 Despite promising preclinical studies inhibiting NOTCH signaling by γ-secretase inhibitors, severe GI toxicities and lack of cytotoxic antitumor responses still limit their direct translation into patient benefit.47 Given the role of MYC, a known FBXW7 substrate, in T-ALL leukemia initiation, inhibitors of the bromodomain and extraterminal (BET) family of proteins have shown antileukemic activities in in vitro and in vivo models of T-ALL.48

Early T-cell precursor (ETP) ALL is a distinct form of leukemia characterized by reduced expression of T-cell markers (CD1a, CD8, and CD5) and aberrant expression of myeloid or stem-cell markers.49 ETP-ALL has a poor outcome, although this is mitigated by contemporary risk-adapted therapy.50,51 ETP-ALL leukemia initiation, inhibitors of the bromodomain and extraterminal (BET) family of proteins have shown antileukemic activities in in vitro and in vivo models of T-ALL.48
a spectrum of immature leukemias, rather than a true T-ALL. The involvement of JAK-STAT and PRC2 pathways in ETP-ALL suggests that JAK inhibition and/or chromatin-modifying agents may be therapeutically useful.53

Recent studies have identified pathogenic noncoding mutations in T-ALL, notably mutations upstream of the oncogene TAL1. These generate a binding site for the MYB transcription factor, thereby recruiting a protein complex including TAL1 and the H3K27 acetylator CREBBP, resulting in formation of an oncogenic superenhancer region with high levels of H3K27 acetylation.54

TP53 alterations occur in 91% of low-hypodiploid ALLs in children, 43% of which are found in nontumor cells, suggesting that low-hypodiploid ALL represents a manifestation of Li-Fraumeni syndrome.13 Risk of developing ALL is increased by 20-fold in patients with Down syndrome, whereas the rare constitutional Robertsonian translocation, rob(15;21)(q10;q10), is associated with an approximately 2,700-fold increased risk of developing iAMP21-ALL compared with the general population.

There are recent reports of several families with deleterious inherited mutations in the ETS domain of ETV6, which affect DNA binding efficiency and altered intracellular localization of the protein,65,66 and with a mutation in PAX5 (p.Gly183Ser), which attenuates the transcriptional activity of PAX5.67

**RELAPSED ALL**

Relapsed ALL has a poor outcome with conventional therapy and is more common with increasing age, so there is great interest in characterizing genetic drivers of relapse. Genomic studies have shown that leukemia evolution leading to relapse usually does not proceed in a sequential linear fashion but, instead, follows a complex branched pathway. Although primary chromosome translocations are retained, the majority of patients who experience relapse also exhibit new secondary genetic alterations or, commonly, relapse-acquired lesions frequently arising from a minor clone at diagnosis. Genetic lesions driving clonal evolution may arise from cooperation between recombination-activating genes (RAG1 and RAG2) and activation-induced cytidine deaminase (AID).15,55,56 Mutations influencing drug sensitivity and proliferation in particular stroma or environments will outgrow and become dominant.

Common relapse-acquired lesions include mutations in the transcriptional coactivator and acetyl transferase CREBBP (CREB-binding protein [CBP]), which occur in up to 20% of relapsed ALLs and impair sensitivity to glucocorticoid therapy,57-59 and mutations in the 5'-nucleotidase catalytic enzyme II (NT5C2) gene, which confer increased resistance to purine analogs.60,61 Other recurrent somatic mutations in relapsed ALL include deletions in the DNA mismatch repair gene mut-S homolog 6 (MSH6) and the glucocorticoid receptor NR3C1 and mutations in the H3K36 trimethyltransferase SETD2, the lysine-specific demethylase KDM6, and the epigenetic regulator MLL2.62,63

Ras pathway mutations (eg, KRAS, NRAS, FLT3, and PTPN11) are often selected for or acquired during treatment and thus predominate in the relapsed leukemic clone. They are associated with high-risk features and poor prognosis, but treatment with MEK inhibitors has been reported to offer clinical benefit in vitro and in xenograft models.64

**CLINICAL IMPLICATIONS**

Accurate, comprehensive identification of the full range of genetic alterations in ALL is important for diagnosis, risk stratification, implementation of targeted therapy, and sensitive monitoring of treatment response. This is now possible but poses logistic, financial, and ethical challenges.

Ideally, diagnostic testing should detect all types of alterations of clinical relevance, including nucleotide substitutions, insertion/deletion mutations, DNA copy number alterations, and chromosomal rearrangements. The choice of the optimal detection method will depend on the number of genes to be screened and the desire to detect genomic rearrangements, as well as the desired sensitivity. Sequencing-based assays that use DNA or RNA of sets of genes are able to accurately detect mutations and rearrangements in a clinically relevant time frame68 but may not detect all focal deletions characteristic of ALL. It is likely that unbiased approaches such as transcriptome, exome, and whole-genome sequencing will be increasingly used. Sequencing-based approaches have also been used successfully to analyze antigen receptor rearrangements and quantitate MRD more sensitively than flow cytometric or conventional polymerase chain reaction–based approaches.69 Active areas of investigation include the use of similar approaches to quantitate specific mutations and rearrangements that facilitate resistance to therapy (eg, IKZF1, CREBBP, and NT5C2, and ABL in BCR-ABL1–positive ALL) and to incorporate these into clinical trials and management and the use of such results to change therapy when a mutation that confers resistance to a specific agent emerges.

These approaches are also important to identify mutated genes and deregulated pathways amenable to inhibition by targeted therapies, either at initial diagnosis or at relapse, particularly for high-risk ALL subtypes. This includes treatment of Ph-like ALL with kinase-activating mutations that have shown evidence of activity in case reports and are now are being tested in clinical trials (Table 2). It is likely that genomic data will also inform the use of immunotherapeutic approaches. For example, T cells engineered with a chimeric antigen receptor targeting the thymic stromal lymphopoietin receptor encoded by CRLF2 have demonstrated potent activity in preclinical models of Ph-like ALL.70

**INHERITED VARIANTS AND RISK FOR ALL DEVELOPMENT**

Inherited variants and rare deleterious mutations have been shown to play a role in the risk of developing ALL. Some of these variants are in IKZF1 (7p12.2), CDKN2A/CDKN2B (9p21), ARID5B (10q21.2), CEBPE (14q11.2), Pip4k2A (10p12.2), and GATA3 (10p14). ARID5B and Pip4k2A genotypes are associated with risk of hyperdiploid ALL, whereas the risk allele in GATA3 has been associated with Ph-like ALL.
Disclosures provided by the authors are available with this article at jco.org.

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AUTHORS’ DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Genetic Basis of Acute Lymphoblastic Leukemia

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