VOLUME 35 · NUMBER 9 · MARCH 20, 2017

JOURNAL OF CLINICAL ONCOLOGY

REVIEW ARTICLE

Genetic Basis of Acute Lymphoblastic Leukemia

Ilaria Iacobucci and Charles G. Mullighan

Author affiliations and support information (if applicable) appear at the end of this article.

Published at jco.org on February 13, 2017.

Corresponding author: Charles G. Mullighan, MD, St Jude Children's Research Hospital, 262 Danny Thomas Place, Mail Stop 342, Memphis, TN 38105; e-mail: charles.mullighan@stjude.org.

0732-183X/17/3509w-975w/$20.00

A B S T R A C T

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.

J Clin Oncol 35:975-983. © 2017 by American Society of Clinical Oncology

Acute lymphoblastic leukemia (ALL) is of B-cell precursor (BCP) lineage (BCP-ALL) or, less commonly, T-cell precursor lineage (T-ALL). Both comprise multiple subtypes commonly defined by structural chromosomal alterations that are initiating lesions, with secondary somatic (tumor-acquired) DNA copy number alterations and sequence mutations that contribute to leukemogenesis. Chromosomal alterations include aneuploidy and chromosomal rearrangements that result in oncogene deregulation or expression of chimeric fusion genes. The prevalence of these alterations varies according to age (Fig 1), and identification is important for diagnosis, risk classification, and, for some lesions, targeted therapy (Table 1).

BCP-ALL WITH RECURRING 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.[4,5] High hyperdiploidy with gain of at least five chromosomes and *ETV6-RUNX1* 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 composes 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.[6,7] 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.[8] A variant of the t(1;19) translocation, t(17;19)(q23;p13), results in the *TCF3-HLF* fusion[9] (< 1% of ALLs), which is associated with a poor prognosis.[10]

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.[11] Hypodiploidy with less than 44 chromosomes occurs in 2% to 3% of patients and is a negative prognostic factor.[12] 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.[13]

DOI: 10.1200/JCO.2016.70.7836

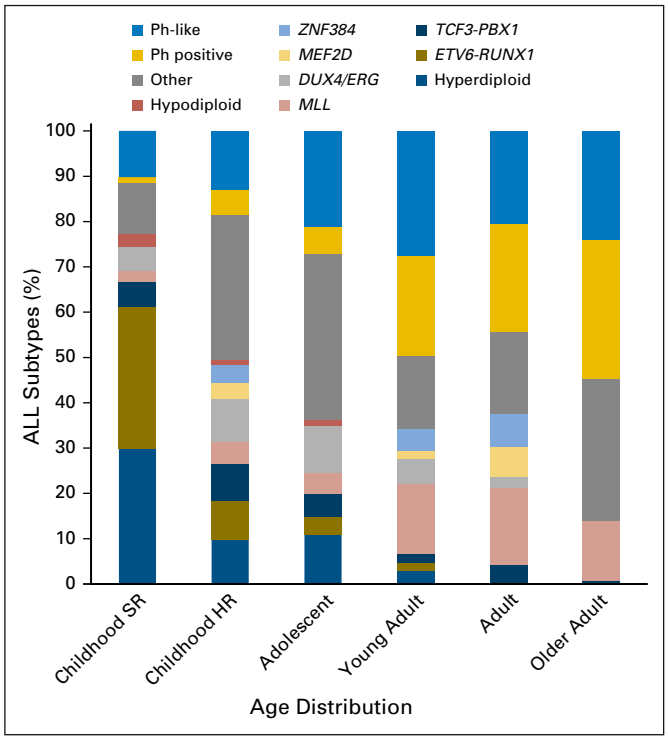

Fig 1. Age distribution of acute lymphoblastic leukemia (ALL) subtypes. The prevalence of ALL subtypes varies in children with standard-risk (SR) ALL (age 1 to 9 years and WBC count $< 50 \times 10^9$/L), children with high-risk (HR) ALL (age 10 to 15 years and/or WBC count $> 50 \times 10^9$/L), and adolescents (age 16 to 20 years), young adults (age 21 to 39 years), adults (age 40 to 59 years), and older adults (age 60 to 86 years) with ALL. Other, B-cell ALL lacking recurrent abnormalities; Ph, Philadelphia chromosome. Data adapted.[1-3]

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*, *PAX5*, *EBF1*), cell-cycle regulation and tumor suppression (*CDKN2A/CDKN2B*, *RB1*), regulation of apoptosis, transcriptional regulation and coactivation (*ETV6*, *ERG*), and epigenetic alterations.[14] The prevalence, type of alteration, and gene vary between subtypes. *KMT2A*-rearranged cases show low frequency of secondary somatic mutations, which are often subclonal, indicating that *KMT2A* rearrangement is sufficient to induce leukemia.[5] *IKZF1* alterations are a hallmark of *BCR-ABL1*–positive and Ph-like ALL and are associated with poor outcome[1,15-18]; in contrast, other members of the *IKAROS* transcription factor family, *IKZF2* and *IKZF3*, are selectively mutated in hypodiploid ALL.[13] In high hyperdiploid ALL, secondary events target genes in the Ras signaling pathway and in chromatin modifiers.[19]

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.[20] Ph-like ALLs harbor a gene-expression profile similar to *BCR-ABL1*–positive ALLs but lack *BCR-ABL1*.[17,21] 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).[1,2] 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.[22]

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 *ABL*-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*).[1,2,23] 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).[24] *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.[25,26] 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 *IL7R* sequence mutations, *SH2B3* deletions, *TSLP* rearrangements, and Ras mutations.[1,2,27] In most studies, *CRLF2* rearrangements are associated with poor prognosis, particularly in cases with concomitant *IKZF1* alteration.[28,29] Therapies targeting JAK-STAT, PI3K/mTOR, and BCL2 signaling alone or in combination have shown efficacy in preclinical models.[30,31]

Another major Ph-like ALL genetic subgroup involves ABL-class rearrangements, which include fusions to *ABL1*, *ABL2*, *CSF1R* (encoding the macrophage colony-stimulating factor receptor), *PDGFRA*, or *PDGFRB* that are all targetable by inhibitors of ABL1, such as imatinib and dasatinib.[1,2,32,33]

Table 1. Key Genetic Subtypes of ALL and Recurrent Genomic Features

Category	Age	Description
BCP-ALL		
Hyperdiploidy with > 50 chromosomes	Children > > adults	Excellent prognosis; mutations in Ras signaling pathway and histone modifiers
Near-haploid	Children and adults	24-31 chromosomes; poor prognosis; Ras-activating mutations and inactivation of *IKZF3*
Low hypodiploid	Children and adults	32-39 chromosomes; poor prognosis; *TP53* mutations, deletions of *CDKN2A/B* and *RB1*, and inactivation of *IKZF2*
High hypodiploid	Children and adults	40-43 chromosomes; rare; poor prognosis
Near-diploid	Children and adults	44-45 chromosomes; distinct entity frequently with *ETV6-RUNX1* fusion or rearrangements forming dicentric chromosomes
t(12;21)(p13;q22) translocation encoding *ETV6-RUNX1*	Children > > adults	Excellent prognosis; cryptic rearrangement that is detectable by FISH or PCR
t(1;19)(q23;p13) translocation encoding *TCF3-PBX1*	Children and adults	Increased incidence in African Americans; generally excellent prognosis; association with CNS relapse
t(9;22)(q34;q11.2) translocation encoding *BCR-ABL1*	Children < < adults	Historically poor outcome, improved with tyrosine kinase inhibitors; common deletions of *IKZF1*, *CDKN2A/B*, and *PAX5*
Ph-like ALL	Children < adults	Multiple kinase-activating lesions; poor outcome; amenable to tyrosine kinase inhibitor therapy
CRLF2 rearrangement (*IGH-CRLF2*; *P2RY8-CRLF2*)	Children and adults	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
KMT2A (*MLL*) rearrangements	Infants > > > children and adults	Common in infant ALL; poor prognosis; low number of additional somatic mutations, commonly in kinase-PI3K-RAS signaling pathway
DUX4- and *ERG*-deregulated ALL	Children and adults	Distinct gene expression profile; majority have focal *ERG* deletions and favorable outcome despite *IKZF1* alterations
MEF2D-rearranged ALL	Children and adults	Distinct gene expression profile and aberrant immunophenotype (CD10 negative, CD38 positive); sensitivity to HDAC inhibitors
ZNF384-rearranged ALL	Children < AYA and adults	Fusions are associated with early pro-B-ALL, expression of myeloid markers, and activation of the JAK-STAT pathway
PAX5 rearrangements	Children and adults	Multiple partners, commonly from dic(7;9), dic(9;12), and dic(9;20)
iAMP21	Older children	Complex structural alterations of chromosome 21; rarely associated with a constitutional Robertsonian translocation rob(15;21)(q10;q10)c; poor prognosis
T-ALL		
TAL1 deregulation	Children < adults	t(1;7)(p32;q35) and t(1;14)(p32;q11) translocations and interstitial 1p32 deletion; generally favorable outcome
LMO2 deregulation	Children	(11;14)(p15;q11) translocation and 5′ *LMO2* deletion; generally favorable outcome
TLX1 (*HOX11*) deregulation	Children < adults	t(10;14)(q24;q11) and t(7;10)(q35;q24) translocations; good prognosis
TLX3 (*HOX11L2*) deregulation	Children and adults	t(5;14)(q35;q32) translocation; commonly fused to *BCL11B*, also a target of deletion and/or mutation; poor prognosis
MLL rearrangements	Children	Multiple partners; disruption of *HOX* gene expression and of self-renewing; poor outcome
9q34 amplification encoding *NUP214-ABL1*	Children	Amenable 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*
t(7;9)(q34;q34.3)	Children	Rearrangement of *NOTCH1*
NOTCH1 mutations	Children and adults	Impairment of differentiation and proliferation; overall favorable outcome
FBXW7 mutations	Children and adults	Impairment of differentiation and proliferation; usually evaluated in combination with *NOTCH1*
Early T-cell precursor ALL	Children and adults	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

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

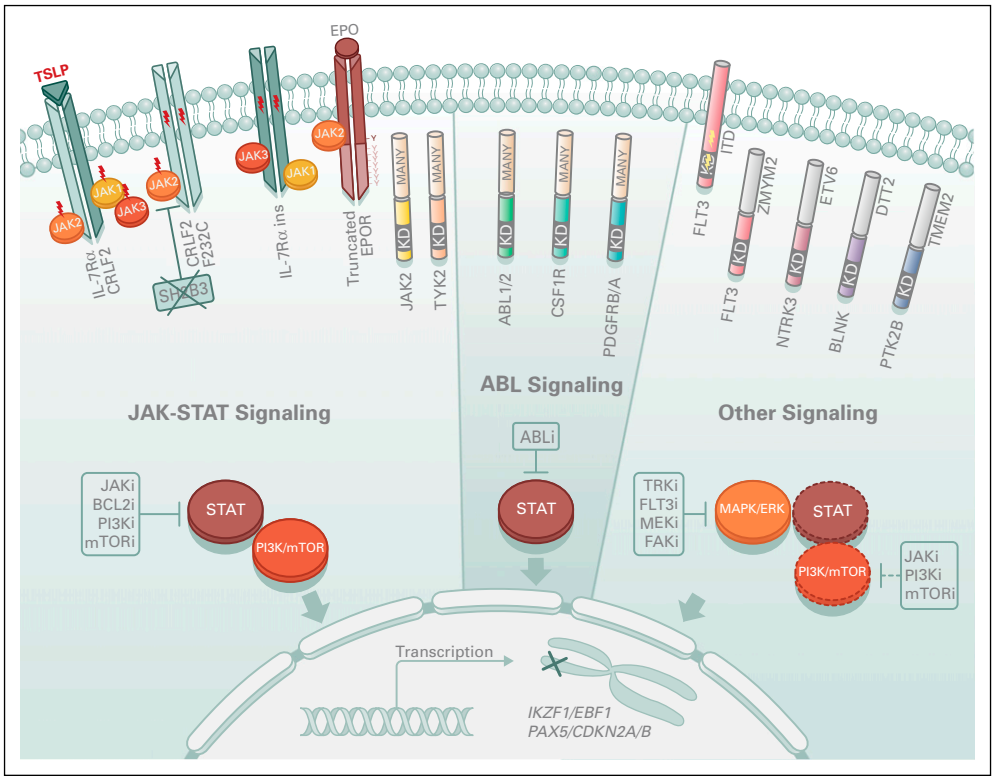

Fig 2. Signaling pathways in Philadelphia chromosome (Ph) –like acute lymphoblastic leukemia (ALL). Deregulation of JAK2, ABL, or other (FLT3, NTRK3, BLNK, ABL, PTK2B) signaling pathways in Ph-like ALL is caused by activating mutations (lightning bolts), fusion genes, and/or genomic deletions (X) that are responsible for overexpression of cytokine receptors (eg, CRLF2, IL-7, and EPOR), expression of truncated receptors missing regulatory domains (eg, EPOR), cell delocalization, and constitutive activation of tyrosine kinases. Some downstream signaling pathways are shown. Dashed circles and line represent likely pathways activated by the kinase alterations and amenable to inhibition by kinase inhibitors, respectively. ABLi, Abelson murine leukemia viral oncogene homolog 1 inhibitor; BCL2i, B-cell lymphoma 2 inhibitor; FAKi, focal adhesion kinase inhibitor; FLT3i, Fms-related tyrosine kinase 3 inhibitor; JAKi, JAK inhibitor; MAPK, mitogen-activated protein kinase; MEKi, MAPK/ERK kinase inhibitor; mTORi, mammalian target of rapamycin inhibitor; PI3Ki, phosphoinositide 3-kinase inhibitor; TRKi, tropomyosin receptor kinase inhibitor; Y, tyrosine residue.

expression and also truncate the cytoplasmic tail of the receptor, resulting in augmented JAK-STAT signaling.[1,2,23,32] 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*).[34-37] *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.[34-37] Less commonly, *ERG-DUX4* fusions have also been described.[37] 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.[36] 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.[38,39] *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.[3,34,35,40]

MEF2D is rearranged to *BCL9*, *HNRNPUL1*, *SS18*, *FOXJ2*, *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.[3,35,41] *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.[3]

ZNF384 rearrangements involve the fusion of a 5′ partner gene, usually a transcriptional regulator or chromatin modifier (*EP300*,

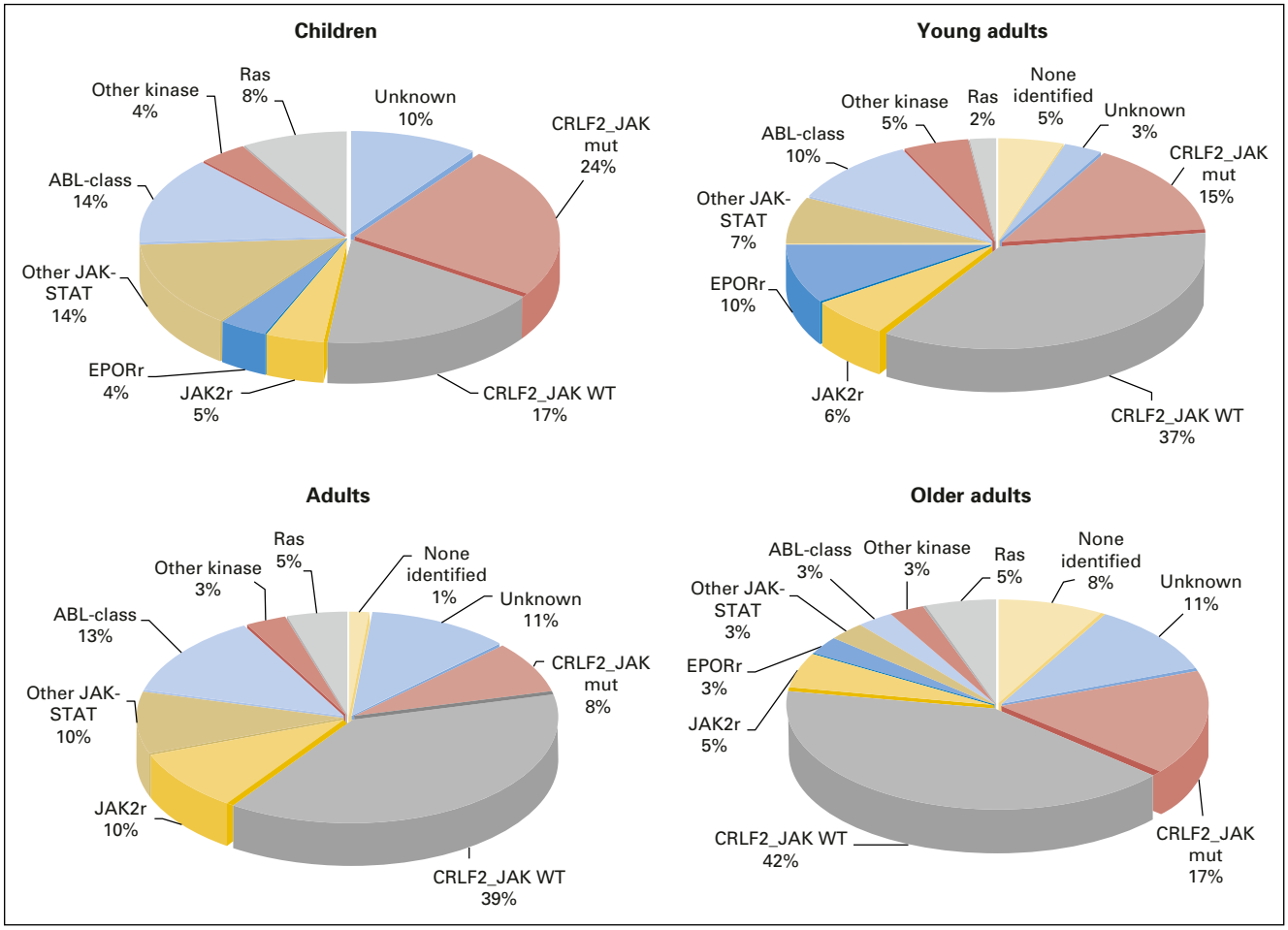

Fig 3. Frequency of Philadelphia chromosome (Ph) –like acute lymphoblastic leukemia (ALL) subtypes across age. Prevalence of *CRFL2*-rearranged *JAK* mutant (mut), *CRFL2*-rearranged *JAK* wild-type (WT), *JAK2* rearrangements (JAK2r), *EPOR* rearrangements (EPORr), other JAK-STAT alterations, *ABL1*-class fusions, all other kinase lesions, and unknown subtype in children, young adults, adults, and older adults. Data adapted.[1,2,23]

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

ALL Subtype	Target	Therapy	Clinicaltrials.gov Identifier	Study Phase	Age of Patients
Ph-like	TKI-sensitive mutations	Dasatinib + chemotherapy	NCT02883049	Phase III	1-30 years
Ph-like	TKI-sensitive mutations	Dasatinib + chemotherapy	NCT02420717	Phase II	≥ 10 years
Ph-like	*JAK2* activation mutations	Ruxolitinib + chemotherapy	NCT02420717	Phase II	≥ 10 years
Ph-like	*JAK2* activation mutations	Ruxolitinib + chemotherapy	NCT02723994	Phase II	1-21 years
MLL rearranged	DNA methylation	Azacitidine + chemotherapy	NCT02828358	Not provided	≤ 364 days
MLL rearranged	DOT1L	EPZ-5676	NCT02141828	Phase I/completed	> 3 months to < 18 years

Abbreviations: ALL, acute lymphoblastic leukemia; DOT1L, DOT1 like histone lysine methyltransferase; TKI, tyrosine kinase inhibitor.

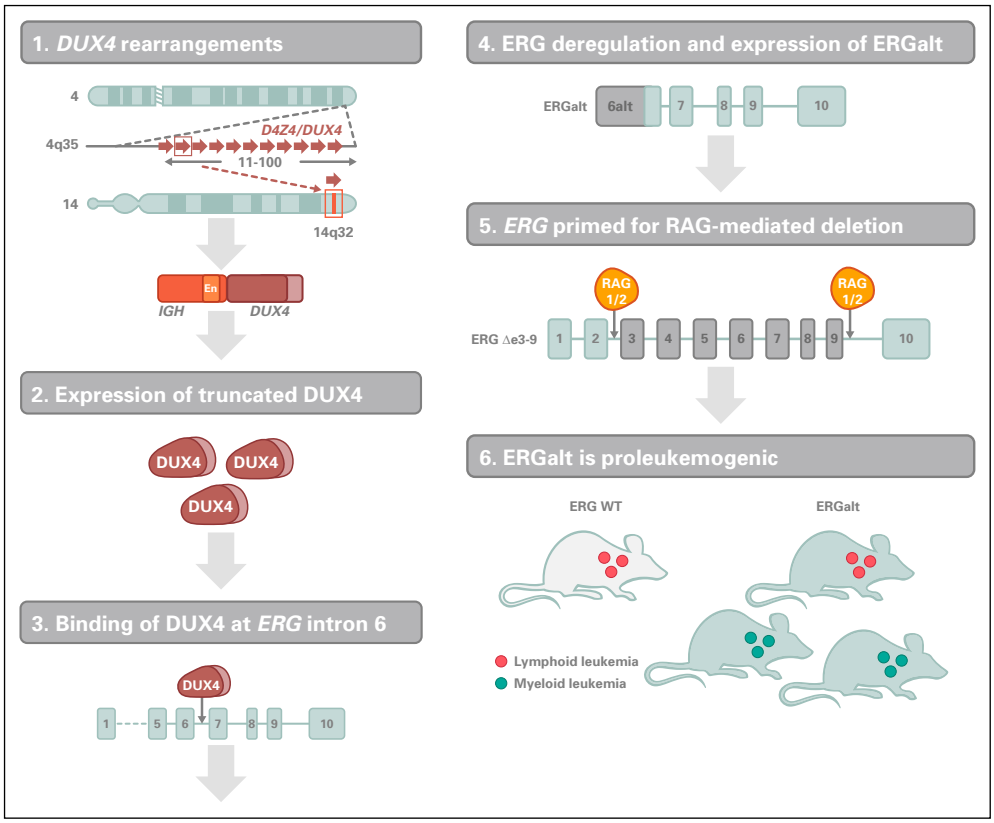

Fig 4. Mechanism of leukemogenesis mediated by *DUX4* and *ERG* deregulation. *DUX4* rearrangements result in profound transcriptional deregulation of *ERG* and expression of a novel *ERG* isoform, ERGalt, and frequent RAG-mediated *ERG* deletions. ERGalt uses a noncanonical first exon whose transcription is initiated by DUX4 binding. It inhibits wild-type (WT) ERG transcriptional activity and is proleukemogenic.

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*[43] 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*, *LYL1*, *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 (*LEF1*, *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 is genetically heterogeneous, with mutation of multiple cellular pathways including hematopoietic and lymphoid development (*RUNX1*, *IKZF1*, *ETV6*, *GATA3*, and *EP300*); Ras, cytokine receptor, and kinase signaling (*NRAS*, *IL7R*, *KRAS*, *JAK1*, *JAK3*, *NF1*, *PTPN11*, and *SH2B3*); and loss-of-function mutations targeting epigenetic regulators (*EZH2*, *SUZ12*, *EED*, and *SETD2*).[52] The gene expression profile of ETP-ALL is similar to that of hematopoietic stem cells, suggesting that ETP-ALL may represent one of

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]

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]

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.

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)c, 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]

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 frame[68] 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]

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at jco.org.

AUTHOR CONTRIBUTIONS

Conception and design: All authors
Collection and assembly of data: All authors
Data analysis and interpretation: All authors
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

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Affiliations

All authors: St Jude Children's Research Hospital, Memphis, TN.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Genetic Basis of Acute Lymphoblastic Leukemia

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Ilaria Iacobucci
No relationship to disclose

Charles G. Mullighan
Honoraria: Incyte, Amgen
Consulting or Advisory Role: Incyte
Speakers' Bureau: Amgen
Research Funding: Loxo
Patents, Royalties, Other Intellectual Property: Inventor on a pending patent application related to gene expression signatures for detection of underlying Philadelphia chromosome–like events and therapeutic targeting in leukemia (PCT/US2012/069228).
Travel, Accommodations, Expenses: Incyte, Amgen

Acknowledgment

We thank our 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) Consortium (https://ocg.cancer.gov/programs/target). Several of the studies described were supported by the American Lebanese Syrian Associated Charities of St Jude Children's Research Hospital, the National Cancer Institute of the US National Institutes of Health, Alex's Lemonade Stand Foundation, the American Association for Cancer Research, the American Society of Hematology, the Henry Schueler 41 & 9 Foundation, the Leukemia and Lymphoma Society, the National Health and Medical Research Council (Australia), the Pew Charitable Trusts, Stand Up To Cancer, the St Baldrick's Foundation, and the Lady Tata Memorial Trust. We thank B. Stelter of Biomedical Communications at St Jude Children's Research Hospital for assistance with figure preparation and apologize to the authors of the many articles that could not be cited as a result of space considerations.