Advances in the Biology of Acute Lymphoblastic Leukemia—From Genomics to the Clinic

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Despite impressive advances in cure rates for childhood acute lymphoblastic leukemia (ALL), ALL remains the leading cause of disease-related death in young people and new therapeutic approaches directed against rational therapeutic targets are urgently required to improve treatment outcomes. This is particularly true for ALL in older children, adolescents, and adults, in whom treatment outcomes are markedly inferior to those of young children. A major goal of current leukemia research is to use comprehensive genomic analysis of the leukemic cell genome, transcriptome, and epigenome to identify critical new genomic alterations that drive leukemogenesis and influence responsiveness to therapy. Genomic analyses in childhood ALL have been remarkably informative and have identified a number of new structural genetic alterations that play important roles in the establishment of the leukemic clone and determine risk of relapse. Notably, many high-risk ALL cases harbor loss-of-function and dominant mutations of genes that encode transcriptional regulators of lymphoid development coupled with mutations that result in activation of cytokine receptor and kinase signaling pathways. These advances have resulted in new diagnostic approaches and therapeutic trials in ALL. This review will discuss these advances and outline challenges for future studies, including the potential role of genome-wide sequencing approaches and the need for detailed studies of the genetics of ALL in the adolescent and young adult population.

The Problem of Childhood ALL

ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) is a neoplasm of immature lymphoid cells and is the commonest malignancy of childhood.¹ The last 50 years have witnessed spectacular advances in the success of ALL therapy, with combination chemotherapy regimens achieving cure in at least 80% of children.^{1–5} Despite these impressive advances, ALL remains a major challenge in oncology. Up to one-quarter of children with ALL relapse,^{6–11} and this proportion rises with increasing age.¹² While the genetics of ALL have long been studied in detail, the biologic determinants of treatment failure in ALL remain poorly understood.

ALL may be of B- or T-cell lineage and is characterized by recurring chromosomal alterations and sequence alterations that have until recently been identified from low resolution genetic approaches (karyotyping) and candidate gene sequencing of limited numbers of genes. These alterations include aneuploidy (e.g., high hyperdiploidy and hypodiploidy) and chromosomal rearrangements that commonly disrupt hematopoietic regulators or aberrantly active oncogenes and tyrosine kinases (e.g., *ETV6-RUNX1*, *TCF3-PBX1*, *BCR-ABL1*, and *MLL* rearrangement in B-ALL and rearrangement of *TAL1*, *TLX1*, and *TLX3* in T-ALL).^{13,14} While the identification of these rearrangements has provided critical insights into leukemogen-

esis and is central to accurate diagnosis and risk stratification, several observations indicate that additional genetic alterations not evident in conventional cytogenetic approaches must also influence leukemogenesis and treatment outcome. First, many of the chimeric fusions encoded by these alterations are insufficient to induce leukemia in experimental models and may be detected years prior to the clinical onset of leukemia,¹⁵ suggesting the acquisition of additional genetic alterations. Moreover, a substantial minority of children with ALL—including many that relapse—lack one of these sentinel chromosomal alterations.¹³ Second, the frequency of chromosomal alterations classically associated with favorable outcome (e.g., *ETV6-RUNX1* and high hyperdiploidy) falls with increasing age,¹³ and the genetic basis of the poor outcome in adolescents and adults with ALL is incompletely understood. Finally, while several genetic alterations are typically associated with a very high risk of treatment failure (e.g., *MLL* rearrangement,¹⁶ *BCR-ABL1*,¹⁷ and low hypodiploidy^{18–20}), many children that relapse lack one of these high-risk alterations.

The completion of the Human Genome Project enabled the development of a variety of complementary approaches to survey genomic alterations at high resolution across the genome. These include microarray-based gene expression profiling and DNA copy number analysis using array-based comparative genomic hybridization (array-CGH) or single nucleotide

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polymorphism (SNP) microarrays.²¹ These have now been used extensively in childhood ALL and have identified a wealth of new genetic alterations that are of central importance in the pathogenesis of leukemia and influence outcome of therapy.^{22–25}

Genome-wide Profiling of Genetic Alterations in ALL

The first microarray-based approach to investigate genomic alterations in ALL was gene expression profiling, in which the level of expression of thousands of genes is profiled in leukemic cells. These studies demonstrated that known cytogenetic subtypes of ALL have distinct gene expression profiles, or signatures, which may then be interrogated in detail to examine pathways that are dysregulated in ALL and potentially contribute to leukemogenesis.^{26–28} While informative, it is often difficult to identify additional genetic alterations from transcriptional data alone. Notable exceptions include the identification of “outlier” genes that exhibit grossly perturbed expression that may highlight a novel target of chromosomal rearrangement or translocation.²⁹

Microarray-based analysis of structural genetic alterations has identified multiple new abnormalities in leukemia genomes. These studies have been reviewed in detail elsewhere,^{21,22,30} but are summarized here prior to discussing recent insights into the genetic basis of high-risk ALL.

In 2007, multiple groups reported analyses of structural alterations in the genomes of children with ALL using SNP microarrays.^{24,31,32} In contrast to many solid tumors,³³ genomic instability in ALL is uncommon, with an average of only six to eight DNA copy number alterations (CNA; deletions or gains) per case.^{24,34,35} Despite this low number of lesions per case, these studies identified more than 50 recurring CNAs,

with deletions more common than gains. Most deletions are focal (<1 Mb in size) and often involve only a single gene. There is also a remarkably strong correlation between the nature and frequency of genetic alterations and ALL subtype. For example, *MLL*-rearranged leukemias have very few additional genetic alterations, suggesting that few structural alterations are required in addition to *MLL* rearrangement to induce leukemia.³⁶ In contrast, *ETV6-RUNX1* and *BCR-ABL1* leukemias have more than eight lesions per case, with distinct genetic alterations between the two subtypes.^{24,34}

Many of these submicroscopic genetic alterations target genes and pathways with known or putative important roles in leukemogenesis. Genetic alterations disrupting the transcriptional regulation of normal B-cell development is a hallmark of B-lineage ALL, with deletions, translocations, and sequence mutations of *PAX5*, *IKZF1*, *EBF1*, and *LEF1* present in more than 60% of cases.³⁵ These genes encode transcription factors required for normal B-lineage commitment and maturation³⁷ and the mutations are usually loss-of-function or dominant negative alleles,²⁴ suggesting that these alterations contribute to the block in maturation characteristic of ALL. This is supported by recent studies modeling loss of *Pax5* or *Irf1* in retroviral and mutagenesis models of B-ALL, in which haploinsufficiency for these genes accelerates the onset of leukemia.^{38–41} Notably, the frequency of mutation of individual genes in this pathway and their relationship with outcome is variable. *PAX5* is most commonly mutated (>30% of cases),²⁴ but there is no association between *PAX5* alterations and outcome.³⁵ In contrast, *IKZF1* alterations are less frequent (approximately 15% of unselected B-ALL cases),³⁴ yet are a hallmark of multiple subtypes of high-risk ALL and strongly associated with adverse outcome (Table 1).^{34,42}

TABLE 1. GENETIC ALTERATIONS IN HIGH-RISK ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

Genetic feature	Comment
<i>BCR-ABL1</i>	Increased frequency in adolescence and adulthood; associated with poor outcome and <i>IKZF1</i> alteration
<i>IKZF1</i> alteration	<i>IKZF1</i> encodes IKZF1 (IKAROS), a key early lymphoid transcription factor. Alterations include loss-of-function deletion of the entire gene, focal deletions that result in expression of dominant negative alleles, and dominant negative point mutations. Associated with poor outcome in <i>BCR-ABL1</i> -positive and <i>BCR-ABL1</i> -negative disease. A subset of <i>IKZF1</i> -altered cases have a “ <i>BCR-ABL1</i> -like” phenotype and harbor novel rearrangements and mutations that activate kinase signaling pathways (<i>CRLF2</i> , <i>JAK2</i> , <i>ABL1</i> , <i>EPOR</i> , <i>PDGFRB</i>). Other more common alterations of lymphoid transcription factors (e.g., <i>PAX5</i> , <i>EBF1</i>) are not independently associated with poor outcome.
<i>CRLF2</i>	Encodes cytokine receptor-like factor 2. Receptor for thymic stromal lymphopoietin. Rearranged by translocation to immunoglobulin heavy chain locus (<i>IGH@-CRLF2</i>) or focal deletion of Xp/Yp 5' of the gene, resulting in <i>P2RY8-CRLF2</i> fusion. Both result in overexpression of full-length <i>CRLF2</i> . Up to 50% of cases have concomitant activating <i>JAK2</i> mutation. Present in more than 5–7% of ALL cases, but more than 50% of Down syndrome ALL cases. Variably associated with poor outcome in non-Down syndrome ALL.
<i>JAK</i> mutations	Observed in a wide range of acute leukemias (B-ALL, T-ALL, and acute megakaryoblastic leukemia). <i>JAK1</i> and <i>JAK3</i> mutations are observed in T-ALL. <i>JAK1/JAK2</i> mutations, most commonly mutations at/near <i>JAK2</i> R683, are associated with <i>CRLF2</i> rearrangement.
ETP ALL	Early T-cell precursor (ETP) ALL (CD1a-, CD8-, CD5 dim, aberrant myeloid/stem cell marker immunophenotype) is associated with a distinct gene expression profile and extremely poor outcome. No single unifying genetic lesion detected thus far, although alterations of <i>MEF2C</i> have been reported in a subset.
<i>CREBBP</i>	Mutations in this transcriptional coactivator are selected at relapse and are associated with resistance to glucocorticoids in cell lines. Suggests treatment with histone deacetylase inhibitors may represent a novel pathway for therapy in high-risk and relapsed ALL.

Additional genes mutated in ALL include tumor suppressors (*CDKN2A/CDKN2B*, *RB1*, *PTEN*), transcriptional regulators and coactivators (*ETV6*, *ERG*, *TBL1XR1*, *CREBBP*), lymphoid signaling molecules (*BTLA*, *CD200*, *TOX*), the glucocorticoid receptor *NR3C1*, and genes of unknown function in leukemia (e.g., *C20orf94* and *ADD3*).²⁴ It should also be noted that analysis of DNA sequence alteration has lagged behind that of copy number alteration. While several genes have been identified as targets of somatic sequence alteration in ALL, particularly in T-ALL (e.g., *NOTCH1*), these data have largely been derived from candidate gene sequencing studies rather than from genome- or exome-wide approaches.²⁴ Nonetheless, the frequency of copy number alteration is commonly higher than that of sequence mutation in genes for which both types of alteration have been studied, with several notable exceptions (e.g., the Ras signaling genes *KRAS* and *NRAS*, which are commonly mutated but rarely involved by CNA).⁴³

Genomic Alterations in High-risk ALL

A strength of ALL genomic profiling studies has been the availability of large cohorts of well-characterized ALL cases with detailed clinical information in which to examine associations between genomic alterations and clinical features, including outcome. These detailed studies have shown that several subtypes of high-risk B-ALL harbor distinct genetic alterations and transcriptional signatures. For example, *BCR-ABL1* lymphoid leukemia is characterized by the deletion of the early lymphoid transcription factor gene *IKZF1* (IKAROS), which is otherwise uncommon in many other subtypes of B-ALL and absent in *BCR-ABL1*-positive chronic myeloid leukemia at chronic phase.^{34,44} This suggests that perturbation of IKZF1 activity is central to the lineage of *BCR-ABL1* leukemia, and this has been borne out by recent experimental models of this disease.^{40,41,45} Interest in the role of *IKZF1* in leukemogenesis has been piqued by recent results from genome-wide association studies analyzing associations between inherited genetic variants and outcome, which have shown that *IKZF1* polymorphisms are associated with an increased risk of ALL.^{46,47}

As stated above, many cases of childhood ALL that relapse lack known high-risk chromosomal rearrangements such as *BCR-ABL1*. A seminal study of the genetic determinants of *BCR-ABL1*-negative high-risk ALL was performed by the Children's Oncology Group (COG) as part of the National Cancer Institute's TARGET Initiative (Therapeutically Applicable Research to Generate Effective Treatments, www.target.cancer.gov). This integrated genomic study utilized SNP and gene expression profiling and extensive candidate gene resequencing to examine the genetic basis of high-risk B-ALL (HR B-ALL) in the COG P9906 study. Multiple reports from this initiative have shown that multimodality genomic analysis can identify novel subgroups of HR B-ALL characterized by distinct gene expression profiles and genomic alterations.^{35,48–52} These studies demonstrated that unsupervised hierarchical clustering of gene expression profiling data identifies multiple subtypes of ALL, several of which lack known chromosomal rearrangements⁴⁸ but are associated with specific submicroscopic genetic alterations.⁵² Importantly, these studies used gene expression profiling to identify genes with extremely high ("outlier") gene expression in HR B-ALL that might indicate the presence of novel

rearrangements, as was demonstrated for rearrangements of *ERG* and other *ETS* gene family members in carcinoma of the prostate.²⁹ This approach, together with complementary analysis of DNA copy number alterations, contributed to the identification of *CRLF2* as a target of novel rearrangements in ALL.⁵⁰

A goal of the TARGET project was to use unsupervised genome-wide analysis to discover structural genetic alterations associated with a high-risk of relapse in ALL. Strikingly, this identified deletions or sequence mutations of *IKZF1* in approximately one-third of HR B-ALL cases (all of which were *BCR-ABL1*-negative), and the presence of *IKZF1* alteration was strongly associated with an increased risk of treatment failure.³⁵ Notably, *IKZF1* alterations are also associated with poor outcome in *BCR-ABL1*-positive ALL.⁴² The association between *IKZF1* status and poor outcome in *BCR-ABL1*-negative ALL was independent of established prognostic markers in multivariable analysis, a finding that has been confirmed in multiple subsequent studies.^{53,54} Detection of *IKZF1* alterations at diagnosis to aid risk stratification and treatment assignment is consequently under investigation in ongoing prospective studies.

Identification of a Novel Subtype of "BCR-ABL1-like" ALL

An additional finding from these studies was that the gene expression profile of *IKZF1*-mutated high-risk *BCR-ABL1*-negative ALL was highly similar to that of *BCR-ABL1*-positive ALL,³⁵ a finding that has been confirmed by other groups.⁵⁵ This suggested that alteration of *IKZF1* is a key determinant of the gene expression profile of both *BCR-ABL1*-positive and -negative ALL, and/or that there are additional genetic alterations in *BCR-ABL1*-negative ALL that activate kinase signaling pathways. Subsequent analyses have shown both hypotheses to be correct. The gene expression profile of *IKZF1*-mutated, *BCR-ABL1*-negative ALL is enriched for hematopoietic stem cell genes and exhibits decreased expression of B-cell receptor signaling and differentiation genes,³⁵ compatible with a block in differentiation induced by alteration of *IKZF1*. In addition, subsequent analyses have shown that many *BCR-ABL1*-like ALL cases harbor previously cryptic alterations in cytokine receptor and kinase signaling genes.⁴⁹

Rearrangement of *CRLF2* and *JAK1/2* Mutations in ALL

Several avenues of investigation resulted in the identification of the *CRLF2* gene (encoding cytokine receptor-like factor 2, or thymic stromal lymphopoietin receptor, TSLPR) as a target of rearrangement and mutation in B-ALL. Christine Harrison *et al.* systematically searched for novel targets of rearrangement of the immunoglobulin heavy chain locus at 14q33 (*IGH@*) and identified rearrangement to the *CRLF2* locus at the pseudoautosomal region of chromosome Xp/Yp (*IGH@-CRLF2*).⁵⁶ Analysis of SNP microarray data in ALL identified a focal deletion immediately proximal to *CRLF2*⁵⁷ that was subsequently mapped and shown to result in a novel chimeric fusion, *P2RY8-CRLF2*, that juxtaposes the first non-coding exon of the purinergic receptor gene *P2RY8* to the entire coding region of *CRLF2*.⁵⁸ In general, the *PAR1* deletion resulting in *P2RY8-CRLF2* fusion is more common than *IGH@-CRLF2* (although this is somewhat cohort dependent).⁵⁰ Both rearrangements result in aberrant overexpression of *CRLF2* on the cell surface of leukemic

lymphoblasts that may be detected by diagnostic immunophenotyping.⁵⁶

The rearrangements of *CRLF2* are notable for several reasons. First, they are present in at least 50% of Down syndrome-associated B-ALL,^{56,58,59} and are also present in up to 50% of *BCR-ABL1*-like ALL cases.⁵⁰ Second, *CRLF2* alterations are associated with the presence of activating mutations in the Janus kinase genes *JAK1* and *JAK2*, which with the exception of T-lineage ALL⁶⁰ are otherwise uncommon in ALL.^{56,58,59,61} The Janus kinase family also includes *JAK3*, which is mutated in acute megakaryoblastic leukemia,^{62,63} and *TYK2*. The *JAK1/2* mutations are most commonly missense or deleterious insertion-deletion (indel) mutations at R683 in the pseudokinase domain of *JAK2*, and are distinct from the *JAK2* V617F mutations that are a hallmark of myeloproliferative diseases.⁶⁴ Like the *JAK2* V617F mutation, the *JAK1/2* mutant alleles observed in ALL are transforming *in vitro*.^{51,65,66} Up to 50% of *CRLF2*-rearranged cases harbor activating *JAK1/2* mutations,^{50,56,58,59} and conversely almost all cases of B-ALL with *JAK1/2* mutations harbor concomitant rearrangements of *CRLF2*.^{56,58} Together with the observation that coexpression of *CRLF2* and *JAK1/2* mutant alleles is transforming *in vitro*, this suggests these two lesions are central in lymphoid transformation. Importantly, however, many cases with *CRLF2* rearrangement lack a *JAK1/2* mutation, and the nature of alternative kinase signaling mutations in these cases is incompletely understood, although recent data indicate that a proportion of these *JAK1/2* wild-type cases harbor activating mutations in the transmembrane domain of the interleukin-7 receptor alpha chain, which heterodimerizes with *CRLF2*.⁶⁷ Recent Sanger sequencing of the tyrosine kinome in *JAK1/2*-wild type *BCR-ABL1*-like ALL cases has failed to identify additional activating kinase sequence mutations.⁶⁸

CRLF2 alterations are relatively uncommon in non-Down syndrome ALL cases (5–7% of B-ALL^{56,58,59}) but are enriched in *BCR-ABL1*-like ALL cases.⁵⁰ Up to 50% of *BCR-ABL1*-like ALL cases harbor *CRLF2* alterations,⁵⁰ where they are also significantly associated with *JAK1/2* mutations (identical in spectrum to those observed in Down syndrome-associated ALL), as well as alterations of *IKZF1* (which are less frequent in Down syndrome ALL^{58,59,66,69}). Multiple studies have examined associations between *CRLF2* alterations and outcome with conflicting results, which may in part reflect the methodology used (and the resulting ability to identify all *CRLF2*, *IKZF1*, and *JAK* mutations) and treatment regimen.^{50,70,71} Specifically, some studies have utilized only *P2RY8-CRLF2* fusion screening, but not *IGH@-CRLF2* FISH (required to detect this rearrangement), and/or have not performed comprehensive analysis capable of detecting all *IKZF1* deletions and sequence variants. Nonetheless, in the COG P9906 HR B-ALL study in which cases were analyzed in detail for *CLRF2* rearrangement, *IKZF1* mutation and deletion, and *JAK1/2/3* mutational status, the presence of *CRLF2* alterations was associated with *JAK* mutations, *IKZF1* alteration, and very poor outcome.^{50,51} These findings have stimulated ongoing studies evaluating the feasibility of diagnostic testing for *CRLF2* overexpression, *IKZF1* alterations, and *JAK* mutations at diagnosis in ALL, and the development of a phase I trial of the Novartis *JAK1/2* inhibitor INCB18424 (ruxolitinib) in relapsed and refractory childhood solid tumors, leukemia, or myeloproliferative disease (the COG ADVL1101 study, ClinicalTrials.gov Identifier NCT01164163).⁷²

Future Directions in the Genetics of High-risk B-ALL

These studies have demonstrated the utility of detailed genomic analysis in identifying critical new genetic alterations in HR B-ALL. It should be noted, however, that the genetic lesions in many HR B-ALL cases remain to be identified. For example, many *BCR-ABL1*-like cases lack *CRLF2*/*JAK* alterations and other high-risk cases do not exhibit the *BCR-ABL1*-like gene expression profile. Alternative approaches that comprehensively identify all sequence and structural genetic variations will be required to identify these lesions. These include transcriptomic sequencing, exome sequencing, and whole genome sequencing, and these approaches are being pursued actively in cancer and leukemia genetic research.⁷³ For example, a pilot study of transcriptome sequencing (RNA-seq) performed by the TARGET initiative identified a range of novel rearrangements that result in activation of cytokine receptor and tyrosine kinase signaling in *BCR-ABL1*-like HR B-ALL cases that lack *CRLF2* rearrangements.⁴⁹ Moreover, extended candidate gene sequencing has identified a range of new somatic sequence mutations in HR and relapsed ALL.⁴³ These results strongly suggest that next-generation sequencing approaches will provide additional key insights into the genetic basis of ALL subtypes that are at present poorly characterized. Current areas of interest include novel subgroups of childhood B-ALL defined by clustering of gene expression profiling data,⁵² hypodiploid ALL,⁷⁴ adolescent and young adult ALL, and ALL cases lacking known chromosomal rearrangements.

Genomics of T-lineage ALL

T-ALL is less common than B-progenitor childhood ALL, but has an inferior outcome to B-ALL.¹⁴ Cytogenetic and genomic analyses have been used to identify subgroups of T-ALL cases using gene expression profiling, as well as to identify a range of genetic alterations that target transcription factors, tumor suppressors, and oncogenes. T-ALL leukemic cells commonly have rearrangements (particularly involving T-cell antigen receptor gene loci) that dysregulate oncogenes, including *TLX1* (*HOX11*), *TLX3* (*HOX11L2*), *LYL1*, *TAL1*, and *MLL* genes.^{13,75–78} T-ALL exhibits a very high frequency of homozygous deletion of *CDKN2A/CDKN2B*^{79,80} (encoding the *INK4/ARF* tumor suppressors) and activating mutations of *NOTCH1*.⁸¹ Genome-wide analyses have recently identified deletions dysregulating *LMO2*,⁸² amplification of *MYB*,^{24,83,84} amplification associated with the *NUP214-ABL1* rearrangement,⁸⁵ fusion of *SET* to *NUP214*,⁸⁶ and deletion and sequence mutation of *BCL11B*,⁸⁷ *FBXW7*,^{88,89} *PHF6*,⁹⁰ *PTEN*,⁹¹ *PTPN2*,⁹² and *WT1*.⁹³ Unfortunately, few of these alterations have been shown to influence outcome in this disease.

Recently, a subtype of T-ALL has been described with an immature immunophenotype similar to that of early (DN1) thymic progenitors. These cases have absent CD1a or CD8 expression, diminished CD5 expression, aberrant expression of myeloid and stem cell markers, and a distinct gene expression signature.^{94,95} These early T-cell precursor (ETP) cases have poor responsiveness to initial therapy with frequent induction failure, high levels of minimal residual disease, and very poor outcome.^{94,95} Detection of the ETP immunophenotype is being utilized to identify these patients and intervene with aggressive therapies, such as bone marrow transplantation. ETP ALL leukemic cells commonly have

a high burden of genomic alterations, but at present the underlying genetic lesion(s) have not been identified.⁹⁵ ETP ALL has a distinct gene expression profile, including overexpression of *MEF2C*, that has been identified as a target of rearrangement in a fraction of ETP ALL cases.⁹⁶ The identification of the genetic basis of this ALL subtype awaits comprehensive analysis of genetic alterations.

Evolution of Genetic Changes at Relapse in ALL

The studies described above have focused on genomic profiling of bulk populations of leukemic cells and have identified important alterations that influence the risk of relapse. However, for many years it has been known that leukemia genomes frequently acquire secondary karyotypic alterations during disease progression and subsequent relapse.⁹⁷ Consequently, there has been considerable interest in performing detailed genomic analyses of sequential leukemic samples in order to address several questions, including the nature of secondary genetic alterations that are acquired with disease progression, the genetic relationship between the diagnosis and relapsed leukemias, and the degree of clonal heterogeneity present at the time of diagnosis.

Several groups have reported SNP microarray profiling of matched samples obtained at diagnosis and relapse.^{57,98–100} These studies demonstrated that the leukemia genome is not static, but that the samples profiled at relapse exhibit striking differences from the matched diagnosis samples, with loss of at least one of the diagnosis DNA gains or deletions and the acquisition of new genetic alterations in the majority of cases. Rather than the simple emergence of an unrelated secondary leukemia, most matched diagnosis–relapse pairs have a common clonal origin as indicated by profiling of copy number alterations at antigen receptor gene loci and commonality of specific CNAs at both time points. Notably, many of the deletions that emerged in the predominant clone at relapse were lesions previously identified at diagnosis, including deletions of *CDKN2A/B*, *ETV6*, and *IKZF1*, strengthening support for these alterations having a role in treatment resistance. Moreover, lesions detected for the first time at relapse are commonly detectable by molecular methods at diagnosis, indicating that the predominant relapse clone(s) are present at low levels at diagnosis. Detailed profiling of CNAs in more than 60 diagnosis–relapse pairs suggested that relapse with truly unrelated second leukemias or identical leukemias is uncommon, although second leukemias may be more common in late relapse.¹⁰¹ In contrast, 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 more than 50% show a complex picture with both loss of diagnosis CNA and the acquisition of new lesions, and evidence of relapse clones at low levels at diagnosis.^{57,99}

These observational data suggest that in many children with ALL, 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 several lines of genetic and experimental data, such as twin studies that have shown that monozygotic twins concordant for ALL and harboring the identical translocation harbor

different secondary CNA.¹⁰² Recent elegant studies have modeled clonal evolution and heterogeneity in xenotransplantation studies in which *ETV6-RUNX1*, *BCR-ABL1*, and T-lineage ALL were transplanted into immunocompromised mice, and coupled the transplantation assays with comparative genomic analysis of primary and engrafted tumors.^{103–105} These studies have clearly demonstrated clonal heterogeneity in the diagnosis samples and that specific genetic alterations (e.g., deletion of *CDKN2A/CDKN2B*) influence the efficiency and tempo of engraftment.¹⁰³

Sequence Alterations in Relapsed ALL

A major challenge in the field now is to identify comprehensively all structural and sequence alterations in leukemia genomes. Notably, most studies thus far have performed detailed profiling of CNA, but with rather limited analysis of sequence variation due to the high cost of these approaches using Sanger sequencing technologies. However, recent detailed candidate gene sequencing studies suggest that comprehensive profiling of DNA sequence alterations will be informative. Sequencing of 120 genes in HR B-ALL cases identified a high frequency of sequence mutations in key pathways, including Ras signaling, JAK-STAT signaling, TP53/tumor suppression, and lymphoid development. Akin to the pattern of CNAs, the frequency and type of somatic sequence mutations is associated with known ALL subtypes and novel ALL subgroups defined by gene expression profiling.⁴³

A recent study extended profiling of CNA alterations in matched diagnosis and relapse ALL samples by sequencing 300 genes in 23 matched diagnosis–relapse paired samples, with recurrence testing in a panel of more than 70 pairs and 270 diagnosis ALL samples.¹⁰⁶ A key finding from this study was that the *CREBBP* gene, encoding CREB-binding protein, was targeted by loss-of-function mutations in almost 20% of relapse ALL samples.¹⁰⁶ The mutations were present at diagnosis and persisted or were duplicated at relapse, or were detected in the predominant clone at relapse but were also detectable in minor clones at diagnosis. CREBBP is a transcriptional coactivator, histone and non-histone acetylase, and ubiquitin ligase,¹⁰⁷ and the mutations were commonly in the histone acetyl transferase domain and impaired acetylation function of the encoded protein. Notably, mutations in *CREBBP* and its homolog *EP300* (p300) are also common in diffuse large B-cell lymphoma and follicular lymphoma.¹⁰⁸ These findings are of potential clinical relevance as CREBBP is involved in regulation of the transcriptional response of lymphoid cells to glucocorticoids and the mutations identified impaired this transcriptional response. These findings suggest that the mutations may impair the response of leukemic cells to glucocorticoids and influence treatment responsiveness. Furthermore, if impaired acetylation is indeed a mechanism underlying steroid resistance, this suggests that therapeutic approaches modifying acetylation may reverse glucocorticoid resistance. Accordingly, several T-ALL cell lines that harbor *CREBBP* mutations and that are highly steroid-resistant are killed by the histone deacetylase inhibitor vorinostat.¹⁰⁶ Together, these findings provide a compelling rationale for comprehensive identification of all sequence variations in ALL in order to fully identify the genetic alterations that contribute to treatment resistance.

Implications for ALL in Adolescents and Young Adults

Adolescents and young adults (AYAs) with ALL typically have an inferior outcome to that of children.^{109,110} The reasons for this discrepancy are incompletely understood, and likely reflect both biologic and therapeutic factors.^{13,111,112} For example, the use of less intensive regimens,^{110,113,114} increased toxicity, and poor compliance may in part be responsible for the inferior outcome of AYA ALL.^{110,113–115} Indeed, treatment outcomes for older adolescents and young adults with ALL are more favorable with the use of more intensive treatment regimens.¹¹⁶ AYA patients have a lower frequency of favorable genetic alterations commonly seen in younger children.^{112,117} Hyperdiploidy occurs in up to 20% of children but is less common in AYAs.¹² *ETV6-RUNX1* (*TEL-AML1*) is the commonest chromosomal rearrangement in childhood ALL and is associated with favorable outcome, but is rarely seen in AYAs and older adults.^{118,119} The *t*(9;22) encoding *BCR-ABL1* is associated with unfavorable outcome and is present in less than 5% of young children, approximately 10% of older children, and up to one-third of older adults.^{17,120} AYA patients also have a high frequency of T-lineage immunophenotype than younger children. ETP ALL comprises up to 15% of childhood T-ALL cases and may be more frequent in the AYA and older adult population.⁹⁵ Moreover, the outcome of several of these subgroups is worse with increasing age, including *BCR-ABL1* ALL¹⁷ and *MLL*-rearranged ALL.¹²¹ Together, however, these differences in underlying genetic lesions are likely insufficient to explain the inferior outcome of adolescent compared to young childhood ALL, and there is considerable interest in performing detailed genome-wide profiling of large AYA cohorts to better characterize genetic features responsible for differences in outcome. Currently, there are limited data. Previous studies of small patient cohorts have shown that many of the submicroscopic genetic alterations identified in childhood ALL are also recurrent in AYA and older adult ALL. However, these studies were not powered to examine associations with outcome.¹²² The genome-wide profiling studies performed in the HR B-ALL COG P9906 cohort included approximately 70 patients in the AYA age range, and *post-hoc* analysis in this sub-cohort also identified associations between *IKZF1*, *CRLF2*, and *JAK2* alteration and poor outcome.^{50,51} The authors are involved in an ongoing study performing genome-wide profiling of genomic alterations in AYA ALL, and it is likely that these will provide important insights into the biology of the ALL in this age group.

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