New Strategies in Acute Lymphoblastic Leukemia: Translating Advances in Genomics into Clinical Practice

Charles G. Mullighan

Abstract

B-precursor acute lymphoblastic leukemia (B-ALL) is the most common childhood malignancy and remains a leading cause of death in children and young adults. Current therapeutic approaches involve intensive combination chemotherapy, which fails in up to one quarter of patients. New treatment approaches directed against rational therapeutic targets are required. Recent genomic profiling of ALL has identified several genetic alterations associated with a high risk of treatment failure. Deletion or sequence mutation of the lymphoid transcription factor gene IKZF1 (IKAROS) is associated with a high rate of leukemic relapse, and testing for IKZF1 alterations at diagnosis may aid risk stratification. A subset of B-ALL patients with IKZF1 alterations have a transcriptional profile similar to BCR-ABL1–positive ALL, and these patients commonly have novel rearrangements and mutations resulting in aberrant cytokine receptor signaling and activation of kinase signaling cascades, including rearrangement of CRLF2 and activating mutations of Janus kinases (JAK1 and JAK2). JAK inhibitor therapy is under investigation in children with relapsed and refractory malignancies, including leukemia. 

Introduction

Acute lymphoblastic leukemia (ALL) is a neoplasm of lymphoid progenitors that may be of B- or T-lymphoid lineage (B-ALL or T-ALL), and is the most common malignancy of childhood (1). The outcome of ALL therapy has improved dramatically in recent decades, with cure rates exceeding 80% (2). However, up to one quarter of patients relapse, which carries a poor prognosis (3). Consequently, relapsed ALL is one of the most common childhood tumors and the leading cause of cancer-related death in children and young adults. Current treatment regimens use intensive combination chemotherapy with little scope for significant intensification due to excessive short- and long-term side effects. Consequently, further improvements in the outcome of ALL therapy require the development of new, targeted, and less toxic therapies.

Moreover, our current understanding of the biologic determinants of treatment failure and relapse in ALL is limited. ALL is characterized by recurring genetic alterations, including aneuploidy (gains and losses of whole chromosomes) and structural rearrangements that commonly result in the expression of chimeric fusion genes (e.g., ETV6-RUNX1, TCF3-PBX1, BCR-ABL1, and rearrangement of MLL) or dysregulate genes by juxtaposition to antigen receptor gene loci (4). Several of these, such as MLL rearrangement and low hypodiploidy, are associated with a high risk of relapse, but the majority of patients that fail therapy lack one of these very high-risk alterations. Moreover, a substantial minority of patients lacks a known, recurring gross chromosomal alteration. Consequently, there has been intensive effort in recent years to use high-resolution genomic profiling to identify novel genetic alterations that contribute to leukemogenesis, influence treatment responsiveness, and ultimately, may be translated to the clinic as new prognostic tools and therapeutic targets. These approaches have been highly informative in B-progenitor ALL and have already resulted in potential new diagnostic tests and treatment approaches (Table 1).

On the Horizon

Several centers in the United States and Europe have performed genomic profiling of B-progenitor ALL and identified novel genetic alterations associated with high-risk disease (5–12). In general, these studies have profiled leukemic cells obtained at diagnosis. Most of these studies have used microarray-based approaches to profile structural genetic alterations, gene expression profiling, as well as selective sequencing of individual genes. Although informative, it should be emphasized that microarray approaches do not directly identify chromosomal rearrangements or tumor-acquired sequence mutations. Consequently, there is currently intense interest in the use of next-generation sequencing technology to comprehensively identify sequence alterations (13) and rearrangements (14), but at present, these have not been widely deployed in ALL.

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Table 1. Genomic alterations associated with outcome and/or are targets for therapy in ALL

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B-lineage ALL

Microarray profiling of structural genetic alterations, including deletions and gains of DNA (DNA copy-number abnormalities or CNA) and copy-neutral LOH, has identified multiple recurring submicroscopic genetic alterations in ALL (reviewed in ref. 15). In contrast to the genomes of many solid tumors, which commonly harbor multiple large genetic alterations (16), most ALL samples have relatively few alterations, and those observed are commonly focal and limited to one or a few genes (5, 9, 10). However, more than 50 recurring genetic alterations have been identified, and these frequently involve genes with known or putative roles in lymphoid development and leukemogenesis. These genes encode regulators of lymphoid development (e.g., PAX5, IKZF1, and EBF1), transcription factors (ETV6, ERG), lymphoid signaling molecules (BTLA, CD200, BLNK, VPREB1), regulators of cell cycle and tumor suppressors (CDKN2A/CDKN2B, ATM, RB1, PTEN), and less commonly, regulators of drug responsiveness (e.g., the glucocorticoid receptor NR3C1; ref. 17). The frequency and nature of these alterations are significantly associated with the presence of known chromosomal alteration, suggesting that specific genetic changes cooperate in leukemogenesis. For example, few genetic changes are observed in MLL-rearranged leukemia (18), whereas ETV6-RUNX1 and BCR-ABL1-positive (Ph+) ALL both harbor 6 to 8 genetic alterations per case (5).

Genetic alterations targeting lymphoid development are key determinants of both the pathogenesis of B-ALL and responsiveness to therapy. More than two thirds of B-ALL cases harbor deleterious mutations targeting transcription factors that target early stages of B-lineage commitment and differentiation, most commonly PAX5 and IKZF1 (7, 10). These alterations often involve only a single copy of the gene and are predicted to result in haploinsufficiency. Accordingly, experimental mouse models of ALL have shown that haploinsufficiency of Pax5 or Ikzf1 increases the penetrance and reduces the latency of B-ALL (19–22).

An important observation from these studies is that different alterations targeting lymphoid development exhibit markedly variable associations with treatment outcome. PAX5 is the most common target of genetic alteration in B-ALL and is involved by a remarkable range of deleterious mutations, including focal and broad deletions, intragenic amplifications, multiple translocations (23), and sequence mutations that affect key residues in the DNA-binding and transcriptional regulatory domains of PAX5. However, multiple studies have failed to observe any association between PAX5 alterations and outcome (at least in the context of current therapeutic regimens) suggesting that, although these changes may be important in establishing the leukemic clone, they do not influence responsiveness to therapy.

In contrast, deletion or sequence mutation of the early lymphoid transcription factor gene IKZF1 (encoding IKAROS) is associated with very poor outcome in ALL. Expression of the constitutively active tyrosine kinase BCR-ABL1 is a hallmark of chronic myeloid leukemia (CML) and a subset of ALL (Ph+ ALL). Prior to the advent of tyrosine kinase inhibitors (TKI) such as imatinib, Ph+ ALL was associated with very poor outcome (24), and the reasons why BCR-ABL1 resulted in two such distinct diseases have been poorly understood. IKZF1 alterations are present in more than 80% of Ph+ ALL cases in both adults and children and at the progression of CML to lymphoid blast crisis, but not in CML at chronic phase (7, 25). Moreover, alterations of IKZF1 are associated with poor outcome in adult Ph+ ALL (26). IKZF1 alterations are uncommon in other subtypes of ALL that otherwise harbor multiple DNA copy-number alterations, such as ETV6-RUNX1 ALL (6, 27). These findings suggest that IKZF1 alteration is a key determinant of the lineage and progression of Ph+ leukemia.

IKZF1 deletion or sequence mutation is also associated with poor outcome in Ph− ALL, at least in children (7, 11, 12). Fifteen to 30 percent of B-ALL cases have deletion of IKZF1 and, less commonly, deleterious sequence mutations. Mutation of IKZF1 is associated with an up to 3-fold increased risk of treatment failure in ALL (7, 11). In a multivariable analysis of established prognostic factors including age, sex, presentation peripheral blood leukocyte...
Figure 1. Schema of genetic alterations in high-risk “Ph-like” ALL. Many patients harbor alterations targeting normal lymphoid development, notably \textit{IKZF1}, which are associated with very poor outcome. These alterations are associated with a block in lymphoid development and the pro–to pre-B-cell stage. Many cases have concomitant rearrangements and mutations targeting cytokine receptor and kinase signaling.

This gene encodes cytokine receptor like factor 2, a lymphoid cytokine receptor that forms a heterodimer with interleukin 7 receptor alpha for the cytokine thymic stromal lymphopoietin. These rearrangements are either a translocation of \textit{CRLF2}, which is located at the pseudoautosomal region 1 (PAR1) of chromosome Xp/Yp, into the immunoglobulin heavy chain locus at chromosome 14q or a focal PAR1 deletion proximal of \textit{CRLF2} that results in a novel fusion \textit{P2RY8-CRLF2} (8, 29, 30). Both rearrangements result in overexpression of full-length \textit{CRLF2} on the surface of leukemic cells. Less commonly, sequence mutations of \textit{CRLF2} are present (30). Up to 60% of patients with \textit{CRLF2} rearrangements have concomitant activating mutations in the kinase or pseudokinase domains of \textit{JAK1} and \textit{JAK2} (8, 29, 31). A range of different Janus kinase (JAK) mutations have been identified, most commonly at or near R683 in the pseudokinase domain of \textit{JAK2}. Notably, the \textit{JAK2} V617F mutation commonly observed in myeloproliferative diseases (32) has not been identified in ALL.

These findings suggest that \textit{CRLF2} rearrangement and JAK mutations cooperate in lymphoid transformation, and expression of \textit{CRLF2} and mutant JAK alleles in murine Ba/F3 cells results in transformation to growth factor–independent growth and constitutive Jak-Stat activation (8, 30). \textit{CRLF2} rearrangements are present in up to 7% of B-ALL cases (8, 29) and, strikingly, in more than 50% of ALL cases associated with Down syndrome (DS-ALL; refs. 8, 29). In non–DS-ALL cases, \textit{CRLF2} alterations and JAK mutations are associated with \textit{IKZF1} alterations and very poor outcome (33, 34).

These findings have identified a novel pathway of transformation in B-ALL and are also of great clinical interest. Detection of these alterations in ALL samples at diagnosis is being actively pursued. \textit{CRLF2} overexpression may be conveniently detected by flow cytometry of leukemic cells.
and CRLF2 rearrangement may be detected by FISH, by genomic PCR for the PAR1 deletion, or by reverse transcriptase PCR (RT PCR) for the F2RY8-CRLF2 transcript (8). JAK mutations may be detected by a variety of well-established methods for sequence mutation detection, such as genomic PCR and sequencing. As in the case of IKZF1 mutations, these approaches are being investigated in large prospective clinical trials, including those done by the Children’s Oncology Group.

Non–DS-ALL patients with CRLF2/JAK alterations commonly have very poor outcome (33, 34), even with current maximal intensive therapy. Several selective JAK1/2 inhibitors are being investigated for the treatment of myeloproliferative diseases (35), and the potential utility of these agents in JAK-mutated ALL will be of great interest. The Children’s Oncology Group is in the process of establishing a phase I clinical trial of JAK inhibitor therapy in relapsed and refractory pediatric tumors. It remains to be determined if JAK inhibitors will show activity as single agents or if combination chemotherapy will be required in patients with these alterations.

Microarray-based profiling of high-risk B-progenitor ALL has proven exceptionally informative in identifying novel genetic alterations associated with poor outcome and in defining new subtypes of ALL harboring previously unrecognized genetic alterations. Continued efforts to identify additional genetic alterations, however, are required. Up to one half of patients with CRLF2 alterations lack a JAK mutation, and the nature of the additional mutation(s) in these cases is unknown. Moreover, many Ph-like cases lack a CRLF2 alteration; many IKZF1-mutated cases are not Ph-like but fail therapy; and a substantial proportion of ALL cases lack IKZF1 alteration and/or a known chromosomal rearrangement. Importantly, the frequency of cases lacking known genetic alterations increases, and outcome declines with increasing age (4, 36, 37). Thus, detailed analysis of these cases is required to identify additional prognostic markers and targets for therapy. It is also likely that the limits of microarray-based technology are being reached and that next-generation sequencing-based approaches will be required to comprehensively identify all sequence variations and structural rearrangements in ALL. The potential utility of these approaches has been identified in both B-ALL and T-lineage ALL.

In a study of high-risk B-ALL cases, transcriptomic sequencing (RNA-seq) was done using the Illumina GAIIx platform. RNA-seq identified rearrangements not previously identified in B-ALL, including the NUP214-ABL1 fusion previously described in T-lineage ALL and a novel rearrangement, STRN3-JAK2 (14). These findings are clinically relevant, as both these rearrangements are known or predicted to result in constitutive kinase activation and are potentially amenable to TKI therapy.

T-lineage ALL

T-ALL is less common than B-progenitor childhood ALL but has an inferior outcome to B-ALL (38). Genomic profiling has also been successfully used to identify new structural and sequence alterations in T-ALL, including deletions dysregulating LMO2 (39), amplification of MYB (40, 41), amplification associated with the NUP214-ABL1 rearrangement (42), and deletion and sequence mutation of PTEN (43) and WT1 (44). A notable recent study used exon capture and next-generation sequencing of X chromosome genes and identified common mutations in PHF6 in childhood and adult T-ALL (13). However, few associations between individual genetic alterations and outcome in T-ALL have been identified.

Recently, a subtype of T-ALL has been described with an immature immunophenotype similar to that of early thymic progenitors (ETP). These cases have absent CD1a or CD8 expression, weak or absent CD5 expression, aberrant expression of myeloid and stem cell markers, and a distinct gene expression signature (45, 46). These “ETP T-ALL” cases have poor responsiveness to initial therapy (as evidenced by high levels of minimal residual disease) and very poor outcome, and detection of the ETP immunophenotype is being used to identify these patients and intervene with aggressive therapies, such as bone marrow transplantation. ETP T-ALL leukemic cells commonly have a high burden of genomic alterations, but at present the underlying genetic lesion(s) have not been identified.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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