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

Charles G. Mullighan

Department of Pathology, St Jude Children’s Research Hospital, Memphis, TN

Address for Correspondence

Charles Mullighan MBBS(Hons) MSc MD
Assistant Member
St Jude Children’s Research Hospital
Department of Pathology
262 Danny Thomas Place
Mail Stop 342, Room D4047E
Memphis, TN 38105
T: 1-901-595-3387
F: 1-901-595-5947
E: charles.mullighan@stjude.org
Abstract

B-progenitor acute lymphoblastic leukemia (B-ALL) is the commonest childhood malignancy and remains a leading cause of death in children and young adults. Current therapeutic approaches involve intensive combination chemotherapy, which fail 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 \textit{IKZF1} (IKAROS) is associated with a high rate of leukemic relapse, and testing for \textit{IKZF1} alterations at diagnosis may aid risk stratification. A subset of B-ALL patients with \textit{IKZF1} alterations have a transcriptional profile similar to \textit{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 \textit{CRLF2} and activating mutations of Janus kinases (\textit{JAK1} and \textit{JAK2}). JAK inhibitor therapy is under investigation in children with relapsed and refractory malignancies, including leukemia.
Background

Acute lymphoblastic leukemia (ALL) is a neoplasm of lymphoid progenitors that may be of B- or T-lymphoid lineage, and is the commonest 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 commonest childhood tumors and the leading cause of cancer-related death in children and young adults. Current treatment regimens utilize 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. \textit{ETV6-RUNX1}, \textit{TCF3-PBX1}, \textit{BCR-ABL1} and rearrangement of \textit{MLL}), or dysregulate genes by juxtaposition to antigen receptor gene loci (4). Several of these, such as \textit{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 lack 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 US 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. While 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.

B-lineage ALL

Microarray profiling of structural genetic alterations, including deletions and gains of DNA (DNA copy number abnormalities, or CNA) and copy-neutral loss-of-heterozygosity (LOH) have 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 few genes (5, 9, 10). However, over 50 recurring genetic alterations have been identified, and these frequently involve genes with known or putative roles in lymphoid development and leukemogenesis. These include 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) (17). The frequency and nature of these alterations is 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 6-8 genetic alterations per case (5).

Genetic alterations targeting lymphoid development are key determinants of both the pathogenesis of B-ALL, and responsiveness to therapy. Over 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 while 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 (TKIs) such as imatinib, BCR-ABL1 positive ALL was associated with very poor outcome (24), and the reasons why BCR-ABL1 resulted in two such distinct diseases has been poorly understood. IKZF1 alterations are present in over 80% of Ph+ ALL cases in both adults and children, and at the progression of chronic myeloid leukemia (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). This suggests 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 thirty percent of B-ALL cases have deletion of IKZF1, and less commonly deleterious sequence mutations. Mutation of IKZF1 is associated with an up to three-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 count and cytogenetic subtype, IKZF1 status was independently associated with poor outcome. Consequently, there has been considerable interest in testing for IKZF1 alterations at the time of
diagnosis to assist with risk stratification. This is conceptually attractive, but prior to implementing *IKZF1* status testing in the clinical arena, these findings require confirmation in additional cohorts of patients treated with contemporary treatment regimens. Secondly, the assays used must be able to detect all types of *IKZF1* alteration. These include broad deletions of the gene that result in haploinsufficiency, focal intragenic deletions that result in expression of aberrant dominant negative IKAROS isoforms (most commonly deletions of coding exons 3-6) and sequence mutations. No single assay is capable of detecting all of these alterations. Routine diagnostic microarray profiling of DNA CNA in ALL is becoming increasingly widely used, either using oligonucleotide array-based comparative genomic hybridization (array-CGH) or single nucleotide polymorphism microarrays (SNP arrays). Data from these arrays must be analyzed and interpreted with great care, as several array platforms, even those with hundreds of thousands of features, lack sufficient resolution to detect very focal *IKZF1* alterations. Alternative approaches include customized arrays, genomic quantitative PCR (6), multiplex ligation-dependent probe amplification (MLPA) (28), and sequence mutation screening methods.

Genomic profiling has also identified potential therapeutic targets in high-risk ALL (Table 1). Many high-risk childhood B-ALL cases with alteration of *IKZF1* have a gene expression profile similar to that of Ph+ ALL. This suggests these cases may have alternative genetic alterations resulting in activation of kinase signaling pathways (Figure 1). Approximately one third of these “BCR-ABL1-like” (Ph-like) cases have rearrangements of *CRLF2*. This gene encodes cytokine receptor like factor 2, a lymphoid cytokine receptor which forms a heterodimer with interleukin 7 receptor alpha for the cytokine thymic stromal lymphopoietin (TSLP). These rearrangements are either a translocation of *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 *CRLF2* that results in a novel fusion *P2RY8-CRLF2* (8, 29, 30). Both rearrangements result in overexpression of full-length CRLF2 on the surface of leukemic cells. Less commonly, sequence mutations of *CRLF2* are present (30). Up to 60% of patients with *CRLF2* rearrangements have concomitant activating mutations in the kinase or pseudokinase domains of *JAK1* and *JAK2* (8, 29, 31). A range of different JAK mutations have been identified, most commonly at or near R683 in the pseudokinase domain of JAK2. Notably, the JAK2 V617F mutation commonly observed in myeloproliferative diseases (32) has not been identified in ALL.
These findings suggest that CRLF2 rearrangement and JAK mutations cooperate in lymphoid transformation, and expression of 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). CRLF2 rearrangements are present in up to 7% of B-ALL cases (8, 29), and strikingly, in over 50% of ALL cases associated with Down syndrome (DS-ALL) (8, 29). In non-DS-ALL cases, CRLF2 alterations and JAK mutations are associated with 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. CRLF2 overexpression may be conveniently detected by flow cytometry of leukemic cells, and CRLF2 rearrangement may be detected by fluorescent in situ hybridization, by genomic PCR for the PAR1 deletion, or by RT-PCR for the P2RY8-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 performed 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 rises, 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 resequencing (RNA-seq) was performed using the Illumina GAIIx platform. This 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 utilized 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 immunphenotype is being utilized 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.
References

17. Irving JA, Minto L, Bailey S, Hall AG. Loss of heterozygosity and somatic mutations of the glucocorticoid receptor gene are rarely found at relapse in pediatric acute lymphoblastic


Table 1.

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

<table>
<thead>
<tr>
<th>Alteration</th>
<th>Detection</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>IKZF1</em> deletions and sequence mutations</td>
<td>DNA microarrays, qPCR, sequencing</td>
<td>Range of deletions and sequence mutations. Associated with high risk of treatment failure in B-ALL</td>
</tr>
<tr>
<td><em>CRLF2</em> rearrangement</td>
<td>Flow cytometry, FISH, genomic PCR, RT-PCR</td>
<td>Concomitant JAK mutations in &gt;50% of cases</td>
</tr>
<tr>
<td><em>JAK1/2</em> mutations</td>
<td>Sequencing</td>
<td>Pseudokinase and kinase domains of JAK1/2. Phase I trial of JAK inhibitors in pediatric tumors under development</td>
</tr>
<tr>
<td>Other kinase gene rearrangements</td>
<td>RT-PCR</td>
<td>Preliminary data: <em>NUP214-ABL1</em> and <em>STRN3-JAK2</em> rearrangements in high-risk ALL</td>
</tr>
<tr>
<td>ETP immunophenotype in T-ALL</td>
<td>Immunophenotyping</td>
<td>Immature phenotype, very high risk of relapse, genetic basis unknown</td>
</tr>
</tbody>
</table>
Figure 1.

Schema of genetic alterations in high-risk “Ph-like” ALL. Many patients harbor alterations targeting normal lymphoid development, notably \(IKZF1\) 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.
Stem cell 

SPI1 (PU.1) 
IKZF1 (IKAROS) 

TCF3 
EBF1 

PAX5 
FOXP1 
LEF1 

Pre-B 
Pre-BI 
Pre-BII 

Immature B 
Mature B 
Plasma cell 

Lymphoid precursor 

IgM 
IgD 

CRLF2 rearrangement 
JAK 1/2 mutation 

Other rearrangements 
activating kinase signaling
New Strategies in Acute Lymphoblastic Leukemia: Translating Advances in Genomics into Clinical Practice

Charles G Mullighan

Clin Cancer Res Published OnlineFirst December 13, 2010.

Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-10-1203

Author Manuscript
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.