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Childhood Acute Lymphoblastic Leukemia: Integrating Genomics into Therapy

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Abstract

Acute lymphoblastic leukemia (ALL), the most common malignancy of childhood, is a genetically complex entity that remains a major cause of childhood cancer-related mortality. Major advances in genomic and epigenomic profiling during the past decade have appreciably enhanced knowledge of the biology of *de novo* and relapsed ALL and have facilitated more precise risk stratification of patients. These achievements have also provided critical insights regarding potentially targetable lesions for development of new therapeutic approaches in the era of precision medicine. This review delineates the current genetic landscape of childhood ALL with emphasis upon patient outcomes with contemporary treatment regimens, as well as therapeutic implications of newly identified genomic alterations in specific subsets of ALL.

Keywords

acute lymphoblastic leukemia; cytogenetics; genomics; pediatrics; therapy

Introduction

Substantial advances have been made in the past five decades in the treatment of patients with acute lymphoblastic leukemia (ALL), the most common malignancy of childhood, which was universally fatal fifty years ago. Approximately 3,000 children are diagnosed with ALL each year in the United States, and long-term survival rates approach 85-90% with contemporary therapy.[1, 2] Improved cure rates have been largely attributable to recognition of the critical importance of central nervous system (CNS)-directed anti-leukemia therapy, implementation of multi-agent chemotherapy cycles with a prolonged

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maintenance phase, and improved supportive care measures. A key feature of contemporary ALL treatment regimens is stratification of patients into different risk groups based upon clinical and biological characteristics of the patient and the leukemia, as well as early treatment response. Therapies of different intensity are applied to the different risk groups with more intensive therapy needed to maximize the chance of cure for higher risk patients. This approach has provided a paradigm for the treatment of many pediatric and adult cancers.

Patient age and initial white blood cell (WBC) count are consistent predictors of outcome, as patients of older age and/or higher WBC counts fare less well than younger patients and/or those with lower WBC counts.[3] These continuous variables were dichotomized by the National Cancer Institute (NCI)-Rome criteria to define standard-risk (SR; age 1-9.99 years and WBC <50,000/microliter) and high-risk (HR; age ≥10 years and/or WBC ≥50,000/microliter) subgroups.[4] NCI-Rome classification criteria are used by many consortia for risk stratification of children with B-ALL, but have limited predictive power in T-ALL.

Improved understanding of the biologic heterogeneity of ALL and development of sensitive polymerase chain reaction- or flow cytometry-based minimal residual disease (MRD) response monitoring techniques have facilitated modern risk stratification for childhood ALL.[5-7] Implementation of risk-adapted algorithms with application of appropriately intensive therapy in subsets of patients with ALL has helped to improve relapse-free and overall survival (OS) and to minimize toxicities, although such algorithms remain imperfect. Approximately 75% of B-ALL cases have somatic aneuploidy or recurrent chromosomal translocations, many of which have prognostic significance. Conversely, the clinical significance of most recurrent genomic alterations in T-ALL is less clear, and current clinical trials stratify patients with T-ALL based primarily upon MRD responses to induction and/or post-induction chemotherapy. Despite excellent survival for most pediatric patients with ALL, relapse occurs in 15-20% of children and remains a significant source of childhood-cancer morbidity and mortality.[8]

Substantial genomic sequencing efforts now underway will better characterize B-ALL and T-ALL, define new prognostic factors, and identify genomic lesions and pathways suitable for molecularly-targeted therapies. As most children with *de novo* ALL diagnosed in North America and Western Europe are treated in large cooperative group clinical trials, correlation of leukemia genomics data with well-annotated clinical trial response and outcome data will continue to inform the development and prioritization of new therapeutic approaches for appropriate patient subsets. In this review, we discuss current knowledge concerning the genomic landscape of ALL and its relevance to treatment of children and adolescents/young adults (AYAs) with ALL.

B-Cell Acute Lymphoblastic Leukemia (B-All)

B-ALL comprises approximately 85% of pediatric ALL. The majority of childhood B-ALL cases are currently classified based upon the presence of specific recurrent genetic lesions delineated by the World Health Organization 2008 criteria (Figure 1).[9] Rare bone marrow failure and other constitutional leukemia predisposition syndromes, such as trisomy 21 and

TP53 mutations in Li-Fraumeni syndrome, are associated with higher incidence of childhood ALL and are discussed in detail elsewhere. Recent studies have also identified genomic polymorphisms in genes including *ARID5B, CEBPE, GATA3*, and *IKZF1* that increase the risk of developing ALL, and rare germline mutations in *PAX5* and *ETV6* have been linked to familial ALL occurrence.[10-15]

Aneuploidy

Leukemia-associated whole chromosome gains and losses are readily identified by conventional cytogenetics and/or fluorescence *in situ* hybridization (FISH), and many numerical alterations have prognostic and therapeutic significance (Table 1).[16] High hyperdiploidy with greater than 50 chromosomes per leukemia cell occurs in approximately 25% of childhood ALL, is most frequent in younger children (particularly those with SR ALL), and is associated with favorable chemotherapy responses and 5-year OS exceeding 90-95%.[17] Children with high hyperdiploid SR ALL who are rapid early responders to induction chemotherapy comprise a low-risk subgroup; therapy reduction for such patients can minimize therapy-associated toxicities without compromising cure rates.[18, 19]

Conversely, hypodiploidy with fewer than 44 chromosomes accounts for 1-2% of childhood ALL and is associated with poor outcomes.[20, 21] A recent report from the Children's Oncology Group (COG) found that children with hypodiploid ALL had a 5-year event-free survival (EFS) rate of 47% versus 85% for children with non-hypodiploid ALL.[22] MRD response was highly predictive of outcome among the hypodiploid patients, and those with end-Induction MRD ≥0.01% had a 5-year EFS of <30%.[22] Based on these poor outcomes, hypodiploidy is often considered to be an indication for allogeneic hematopoietic stem cell transplant (HSCT) in first remission.[23-26]

Hypodiploid ALL can be further classified based upon the degree of hypodiploidy, such as low-hypodiploidy (32-39 chromosomes) and near-haploidy (24-31 chromosomes), which may correlate with incrementally inferior clinical outcomes.[24] The genomics of near-haploid and low-hypodiploid ALL are quite different. Remarkably, over 90% of pediatric low-hypodiploid ALL cases harbor *TP53* mutations, which are germline events indicative of Li-Fraumeni syndrome in half of patients.[27] The pattern of mutations present in hypodiploid ALL suggests that there may be clinical utility of phosphatidylinositol-3-kinase (PI3K) or mitogen/extracellular signal-regulated kinase (MEK) inhibitors in this high-risk ALL subset.[27]

Sentinel Chromosomal Translocations

Nearly half of childhood B-ALL cases harbor somatic chromosomal translocations, many of which may be cryptic on conventional cytogenetic analyses, but readily detectable by FISH or reverse-transcriptase polymerase chain reaction (RT-PCR) amplification of fusion genes created by these translocations. The most common is t(12;21)(p13;q22) resulting in *ETV6-RUNX1* (*TEL-AML1*) fusion that occurs in 20-25% of childhood NCI SR B-ALL. *ETV6-RUNX1* alterations are often accompanied by other submicroscopic alterations in lymphoid development and tumor suppressor genes.[28] Children with NCI SR *ETV6-RUNX1* ALL have an outstanding prognosis with >95% OS, and therapy de-escalation trials with lower-

intensity chemotherapy regimens for this patient population have been conducted by some cooperative groups.[18, 19] However, MRD response remains a critical predictor of outcome in childhood ALL, including *ETV6-RUNX1* ALL, and children with elevated end-Induction MRD remain at higher risk of treatment failure and/or relapse regardless of underlying leukemia-associated alterations.[5, 7] *ETV6-RUNX1* translocations have been detected at low levels in preserved neonatal blood spot specimens (Guthrie cards) from children who subsequently develop ALL, implicating a prenatal origin of this subtype of childhood ALL.[29] However, *ETV6-RUNX1* fusions are also detectable in blood spots from children who do not subsequently develop ALL, suggesting that additional cooperating mutations are necessary for leukemogenesis.[30]

Other recurrent chromosomal translocations in B-ALL include t(1;19)(q23;p13.3) resulting in *TCF3-PBX1* (*E2A-PBX1*) fusion, rearrangement of *KMT2A* (formerly *MLL*; 11q23), and t(9;22)(q34;q11.2) (the Philadelphia chromosome; Ph^+) resulting in *BCR-ABL1* fusion. While *TCF3-PBX1* ALL was previously associated with an intermediate or unfavorable prognosis, modern therapeutic regimens have improved outcome, and *TCF3-PBX1* fusion is no longer considered for risk stratification.[31-33] Children with *TCF3-PBX1* ALL appear to have higher risk of CNS relapse and may merit intensification of CNS-directed therapy.[34] *TCF3-HLF* fusion resulting from t(17;19)(q22;p13.3) occurs in less than 0.5% of patients with B-ALL and, despite its rarity, has been associated with extremely poor outcomes.[35, 36]

KMT2A is a promiscuous oncogene with rearrangements involving >75 fusion partners, and the incidence of *KMT2A* rearrangements in childhood B-ALL differs markedly by age.[37, 38] Approximately 75% of infants less than 1 year old with ALL harbor somatic *KMT2A* rearrangements, and these patients have high rates of hyperleukocytosis and CNS involvement with leukemia. The near-universal concordance rate of *KMT2A*-rearranged ALL in monozygotic twin infants suggests prenatal origin of leukemogenesis in this subtype of ALL.[39, 40] Infants with *KMT2A*-rearranged ALL have poor OS (<50% at 4 years) despite intensive multi-agent chemotherapy.[41, 42] Major adverse risk factors in infant ALL include age less than 90 days and WBC ≥300,000/microliter.[41, 43] Given concomitant overexpression of the fms-related tyrosine kinase 3 receptor (FLT3) in *KMT2A*-rearranged infant ALL and promising preclinical data with FLT3 inhibition,[44-46] clinical testing of FLT3 inhibitors (*e.g.*, lestaurtinib, quizartinib) with chemotherapy in infants with *KMT2A*-rearranged ALL is ongoing to determine if combination therapy can diminish relapse risk and/or improve EFS (www.clinicaltrials.gov NCT00557193 and NCT01411267[47]). Similarly, given frequent epigenetic dysregulation reported in *KMT2A*-rearranged infant ALL, demethylating/hypomethylating agents (*e.g.*, decitabine, 5-azacytidine, DOT1L inhibitors) and histone deacetylation inhibitors (*e.g.*, vorinostat, panobinostat, bromodomain inhibitors) are also under preclinical and early clinical evaluation.[48] The genomics of *KMT2A*-rearranged ALL differ between infants and older children. In infants, *KMT2A* rearrangement is accompanied by a remarkable paucity of other somatic mutations.[49, 50] Many mutations occur in genes associated with activated Ras or PI3K pathway signaling, although were often detected in subclonal populations and/or were diminished or lost at relapse. These data suggest that such alterations may be passenger mutations, and a potential role for PI3K pathway or MEK inhibition in infant ALL is not clear.[50] *KMT2A*-rearranged

leukemias in older children and AYAs have a higher number of somatic mutations including mutations in epigenetic regulators in about half of cases.[50] The frequency of *KMT2A*-rearranged ALL diminishes markedly with increased age, occurring in approximately 5% of children with B-ALL older than one year.[43]

BCR-ABL1-rearranged (Ph^+) ALL occurs in 3-5% of children with B-ALL and conferred a particularly dismal prognosis in the pre-tyrosine kinase inhibitor (TKI) treatment era with 35% 3 year EFS.[51, 52] Sentinel studies conducted in the early 2000s demonstrated that ABL1-targeting TKIs (*e.g.*, imatinib; now also dasatinib, nilotinib, and ponatinib) could induce potent leukemia cytotoxicity in Ph+ ALL.[51, 53] Mature clinical trial data now demonstrate that combination therapy with imatinib and intensive cytotoxic chemotherapy has substantially improved EFS and OS in children with Ph^+ ALL and has minimized need for HSCT in first remission, one of the true precision medicine successes in pediatric oncology.[54, 55] As in chronic myelogenous leukemia, development of *ABL1* "gatekeeper" kinase domain mutations in patients treated chronically with TKIs remains a potential mechanism of drug resistance, although these mutations have not appeared to occur at high frequency in children with Ph^+ ALL to date.[25, 56] Most Ph^+ ALL cases also have deletions in the transcription factor *IKZF1*, which has been associated with poor prognosis in Ph^+ and other subtypes of ALL.[57-60]

Intrachromosomal Amplification of Chromosome 21

Intrachromosomal amplification of chromosome 21 (iAMP21) is a high-risk subset comprising approximately 2% of childhood B-ALL. This subtype was initially discovered via detection of multiple *RUNX1* copies on routine *ETV6-RUNX1* FISH testing. iAMP21 ALL generally occurs in older children with a median age of 9 years old. Although previously associated with poor outcomes, recognition of patients with iAMP21 ALL as high-risk/very high-risk with appropriate therapy intensification has improved their survival substantially without HSCT.[61-63] Although extremely rare, the constitutional Robertsonian translocation rob(15;21)(q10;q10)c is associated with a dramatically increased risk of developing ALL with iAMP21.[64]

Trisomy 21-associated ALL

Children with trisomy 21 (Down Syndrome) have increased risk of developing B-ALL, although the role of germline trisomy 21 in leukemogenesis remains incompletely understood.[65] Common childhood ALL-associated translocations occur less frequently in Down Syndrome-associated ALL (DS-ALL), and outcomes of children with DS-ALL are inferior to those without DS.[66-68] However, 50-60% of children with DS-ALL have rearrangements in the *CRLF2* (*cytokine receptor-like factor 2*) gene located in the pseudoautosomal region (PAR1) of chromosomes X and Y that most commonly result from interstitial PAR1 deletion causing *P2RY8-CRLF2* fusion.[69, 70] Concomitant JAK mutations (generally missense point mutations in *JAK2*) occur in half of *CRLF2*-rearranged DS-ALL cases, which demonstrate activated JAK/STAT and other cytokine receptor pathway signaling.[71, 72] Investigation of small molecule JAK inhibitors is thus of potential therapeutic interest given the mutational profile and potential for toxicity in patients with DS-ALL, but has not yet been studied in the clinic.

Philadelphia Chromosome-like ALL

Philadelphia chromosome-like (Ph-like) or *BCR-ABL1*-like ALL is a recently recognized subset of B-ALL defined by a gene expression profile similar to that of *BCR-ABL1*$^+$ ALL and suggestive of activated tyrosine kinase signaling.[73] Deletions of *IKZF1* and other genes encoding transcription factors that regulate B-cell differentiation commonly occur in Ph-like ALL.[74-76] *IKZF1*-deleted ALL has been associated with a high rate of treatment failure and/or relapse in HR patients, suggesting that new treatment approaches may be necessary to improve outcomes in some patients.[57-60, 74, 75, 77] Clinical outcomes of patients with Ph-like ALL are generally poor, although one small study reported better outcomes with MRD-based application of HSCT in first remission.[78-81] The prevalence of Ph-like ALL increases with age from approximately 10% in NCI SR ALL to 13% of NCI HR ALL, 21% of adolescents, and 27% of young adults with ALL.[82]

The genomics of Ph-like ALL are complex, but frequently cause activated kinase and cytokine receptor signaling.[73, 83] *CRLF2* rearrangements resulting in overexpression are present in approximately 50% of Ph-like ALL cases in the United States (US) and include *P2RY8-CRLF2* fusions and (often cryptic) *IGH-CRLF2* translocations.[69, 70] Interestingly, *IGH-CRLF2* translocations are more common in older adolescents and young adults and linked to Hispanic ethnicity and Native American genetic ancestry in the US, which may partially explain the difference in frequency of this abnormality between US and European reports.[84-86] Mutations of *JAK1* and particularly *JAK2* occur in half of *CRLF2*-rearranged ALL cases and are rarely detected in ALL cases without *CRLF2* rearrangements.[69, 70, 84]

Among the remaining half of Ph-like ALL cases, about one-third harbor often cryptic genomic rearrangements involving the *ABL1*, *ABL2*, *CSF1R*, or *PDGFRB* kinase genes.[82, 83] These rearrangements involve a diverse variety of translocation partners and encode fusion proteins (ABL-class fusions) that join the intact kinase domain of the carboxy terminal ABL-class kinase in frame to the amino terminus of the partner gene. The resultant fusion proteins phenocopy BCR-ABL1 in terms of their ability to confer growth factor independence in murine cell lines and, importantly, to respond to ABL-class TKIs imatinib and dasatinib.[82, 83]

A second major subgroup accounting for about 20% of *CRLF2*-intact Ph-like ALL is comprised of cases with genomic rearrangements that produce *JAK2* fusion genes analogous to ABL-class fusions or rearrangements targeting the erythropoietin receptor (*EPOR*). These lesions are also transforming in preclinical model systems and are highly sensitive to JAK2 inhibitors, including ruxolitinib.[81, 83, 87]

The observed *in vitro* sensitivity of ABL-class fusions to imatinib and dasatinib and *JAK2* and *EPOR* fusions to ruxolitinib strongly suggests that these TKIs might have clinical efficacy in genomically-defined subsets of Ph-like ALL. Numerous anecdotes have been reported of patients with ABL1-class fusions who had poor early responses to chemotherapy, then subsequently dramatic responses following addition of imatinib or dasatinib to chemotherapy.[81, 88, 89] It is now clear that *PDGFRB* fusions are present in a significant percentage of patients who fail to enter remission after one month of induction chemotherapy.[81] Because *PDGFRB* rearrangements can be detected readily via FISH with

commercially available probes, such testing should be performed on any cases of ALL with overt induction failure (>25% marrow blasts).[90]

Based upon these and other very promising preclinical and early clinical data, gene expression and sequencing assays for rapid identification of patients with Ph-like ALL and characterization of the driver genomic lesions are in development.[91] Such assays will facilitate incorporation of appropriate TKIs into treatment of subsets of patients with newly diagnosed Ph-like ALL.

Intragenic Deletion of ETS-Related Gene

Very recently, recurrent intragenic deletions of the transcription factor *ERG* (*ETS-related gene*) have been described in approximately 3% of children with B-ALL.[73, 92, 93] *ERG*-deleted ALL occurs in older children (median age 7 years), is associated with aberrant CD2 surface expression, and frequent *IKZF1* deletions. Despite the generally unfavorable prognosis of *IKZF1*-deleted ALL, patients with *ERG*-deleted ALL (even those with *IKZF1* deletions) appear to have excellent outcomes with standard therapy with >85% 8-year EFS and >95% 8-year OS in one study.[93]

T-Cell Acute Lymphoblastic Leukemia (T-All)

T-ALL comprises 10-15% of pediatric ALL and is associated with older age at diagnosis and greater incidence in males and in African Americans.[94] While outcomes of children with T-ALL were historically inferior to those with B-ALL, therapy intensification has recently resulted in excellent outcomes with >85% 5-year EFS, although T-cell immunophenotype remains an adverse risk factor in multivariate analyses of clinical trials.[2, 19, 95] Age and WBC, which are very powerful factors in B-ALL, have little prognostic impact in T-ALL.[96]

Genomic alterations in T-ALL are often cytogenetically cryptic, and the prognostic impact of the various T-ALL-associated lesions identified to date remain poorly understood (Figure 1). Consequently, genomic alterations are not currently used for risk stratification of T-ALL patients, which instead relies primarily upon CNS status at diagnosis and MRD response. Treatment of patients with relapsed T-ALL with the nucleoside analogue nelarabine demonstrated impressive early activity, but was associated with significant neurotoxicity.[97, 98] This toxicity delayed subsequent frontline testing efforts, but the COG has now shown that nelarabine can be safely added to treatment regimens for newly-diagnosed T-ALL patients.[99] A recently completed phase 3 clinical trial will determine, once outcome data are mature, whether addition of nelarabine improves outcomes for children and AYAs with T-ALL.[100] Clinical testing of γ-secretase inhibitors (GSIs) and other NOTCH1-targeted molecular therapeutic agents (discussed below) is also in progress.

Chromosomal Translocations

Approximately 50% of T-ALL cases harbor chromosomal translocations that most commonly involve fusion of T-cell receptor genes to various oncogenes (*e.g.*, *TLX1-TCRδ*) or interstitial deletions resulting in juxtaposition of two genes (*e.g.*, *STIL-TAL1*). Gene expression profiling studies have classified T-ALL into four molecular subtypes based upon

underlying genetic alterations and resulting oncogenic signaling pathway activation: (1) *TLX1/HOX11*, (2) *LYL1*, (3) *TAL1/LMO2*, and (4) *TLX3/HOX11L2*.[101-104] The prognostic significance of these alterations in childhood T-ALL remains largely unknown, although patients with *TLX1* alterations appear to have more favorable outcomes (Table 1).[103, 105]

Mutations in X Chromosome Genes

The *TLX1/HOX11*, *TAL1/LMO2*, and *TLX3/HOX11L2* subsets are associated with inactivating mutations and deletions in *PHF6* (*plant homeodomain finger 6*) located on the X chromosome, which may partially help to explain the greater frequency of T-ALL in males.[106] *PHF6* is hypothesized to function as a tumor suppressor given its role as an RNA-interacting protein and component of the nucleosome remodeling and deacetylation (NuRD) complex.[106] Somatic loss-of-function mutations in the X-linked histone H3K27me3 demethylase ubiquitously transcribed X (UTX) chromosome also occur frequently in T-ALL cases, are enriched in males, and appear sensitive *in vitro* to histone methyltransferase EZH2 inhibition.[107]

NOTCH1 Mutations

Somatic mutations in *NOTCH1*, which encodes a transmembrane receptor responsible for T-cell lineage commitment and survival, result in constitutive PI3K pathway signaling activation. *NOTCH1* mutations occur in greater than 50% of T-ALL cases and are generally associated with more favorable therapeutic responses and outcomes.[108, 109] Common cooperating lesions such as deletion of tumor suppressor *CDKN2A* or mutation in ubiquitin protein ligase *FBXW7* are also hypothesized to enhance aberrant PI3K signaling via attenuation of NOTCH1 protein degradation. Various NOTCH1-directed strategies are under preclinical and clinical study, such as anti-NOTCH1 antibodies and small molecule GSIs to block NOTCH1 pathway signaling and proteolytic degradation of NOTCH1, respectively. To date, first-generation GSIs have not been efficacious in patients with T-ALL at least in part due to on target/off tumor gastrointestinal toxicity.[110] Development of newer GSIs with more acceptable toxicity profiles is in progress.

Early Thymic Precursor ALL

A fifth subtype of T-ALL called early thymic precursor or early T-cell precursor (ETP) accounts for 10-15% of T-ALL and is diagnosed by its characteristic immature immunophenotype ($CD1a^-$, $CD8^-$, $CD5^-$ or $CD5^{dim}$ with co-expression of myeloid or stem cell markers).[111] Patients with ETP ALL tend to respond more slowly to induction chemotherapy, although most patients appear to achieve molecular remission with negative MRD by the end of consolidation therapy.[95] An initial study of patients with ETP ALL reported extremely poor outcomes with high rates of primary chemotherapy-refractory disease and/or relapse and 19% 10-year EFS.[111] More recent data from the United Kingdom Medical Research Council and the COG suggest that outcomes of children with ETP ALL are comparable to those of patients with non-ETP ALL when stratified by MRD responses with overall 80-85% 5-year EFS.[95, 112]

Whole-genome sequencing of ETP ALL cases demonstrated high frequency of activating mutations in Ras pathway and cytokine receptor signaling (*e.g.*, *FLT3*, *IL7RA*, *JAK1* and

JAK3, *KRAS* and *NRAS*, *SH2B3*) in comparison to non-ETP ALL cases.[113] Inactivating mutations in hematopoiesis development and histone modification genes also were common in ETP ALL. These mutational profiles appear similar to those of myeloid leukemias, suggesting that ETP ALL may be part of a spectrum of leukemias derived from very early progenitor cells and/or hematopoietic stem cells. Recent preclinical studies also suggest potential therapeutic efficacy of molecularly-targeted agents such as JAK inhibitors to target aberrant cytokine receptor signaling.[114-116]

Other recurrent alterations in T-ALL include *KMT2A* rearrangements (10-15% of T-ALL), t(9;14)(q34;q32) resulting in *NUP214-ABL1* fusion (10%), and t(8;14)(q24;q11) resulting in *TRA-MYC* or *TRD-MYC* fusion (1%).[103] The *PICALM-MLLT10* rearrangement in particular has been associated with very poor outcomes, although more recent studies suggest intermediate outcomes with intensive therapy.[103, 117]

Relapsed All

Prognostic factors are imperfect, and relapse occurs across the genomic spectrum of childhood ALL. Some subtypes are associated with particularly high risk of relapse, such as Ph^+ ALL in the pre-TKI era, hypodiploid ALL, and the newly-recognized Ph-like ALL subset. Relapsed ALL is often associated with greater resistance to chemotherapy, which may result from emergence of a pre-existent resistant subclone and/or from mutation acquisition during chemotherapy exposure that promote drug resistance (Figure 2).[118, 119] Genomic profiling of matched diagnosis, remission, and relapsed ALL specimens has helped to elucidate various features of leukemia clonal evolution, mechanisms of chemoresistance, and emergence of new mutations.[119-122] The majority of relapsed ALL cases maintain key genetic features from diagnosis, particularly sentinel chromosome translocations which are almost always retained and are likely very early or even initiating events in leukemogenesis. Almost all relapsed ALL cases also exhibit new genetic alterations, suggesting dynamic evolution of leukemogenesis.[120, 121] Interestingly, the bulk leukemia clone present at diagnosis is typically eradicated, while rare subclones persist and acquire new mutations to become the predominant clone at relapse.[121] Genome-wide association studies have also recently identified specific germline single nucleotide polymorphisms that occur more frequently in patients with relapsed ALL.[123]

CREBBP (*CREB-binding protein*) mutations have been reported in approximately 20% of patients with relapsed ALL.[124, 125] The CREBBP (also known as CBP) protein is involved in glucocorticoid-mediated transcription and histone deacetylation; preclinical studies demonstrate activity of histone deacetylase inhibition and other small molecule inhibitor treatment of steroid-resistant ALL, which may merit further study in the clinic.[124, 126] Co-occurrence of *CREBBP* and *KRAS* mutations was also recently reported in a large proportion of early relapse ALL cases, providing additional rationale for exploration of small molecule inhibitor therapies (*e.g.*, MEK inhibitors, PI3K pathway inhibitors) in this subset.[125] More recently, gain-of-function *NT5C2* mutations were identified in nearly 20% of relapsed B- and T-ALL cases.[127, 128] NT5C2 is a 5′-nucleotidase catalytic enzyme that metabolizes and inactivates nucleoside analogues, such as mercaptopurine and thioguanine, which are critical components of anti-ALL therapy. Acquisition of *NT5C2* mutations thus likely confers

resistance to anti-metabolite drugs, and *NT5C2* mutations occur predominantly in patients that relapse during therapy, particularly during maintenance chemotherapy.[126, 127] Other recurrent somatic mutations identified in relapsed ALL include deletions in the DNA mismatch repair gene *MSH6* and the glucocorticoid receptor *NR3C1* and mutations in the H3K36 trimethyltransferase *SETD2*, the lysine-specific demethylase *KDM6*, and the epigenetic regulator *MLL2*. [121, 129-131] Activating somatic mutations in Ras pathway-associated genes (*e.g*., *KRAS*, *NRAS*, *FLT3*, and *PTPN11*) have also been identified in one-third to one-half of patients with relapsed ALL, and inhibition of leukemia proliferation with MEK inhibitor treatment of Ras pathway-mutant human ALL xenograft models was recently reported.[130, 132]

Summary and Clinical Implications

Despite similar morphological features, ALL is a genetically heterogeneous group of diseases. To date, therapeutic success for children and AYAs with ALL has largely been driven by implementation of biology- and response-based risk stratification with appropriate chemotherapy intensification for high-risk patients. Depth and timing of leukemia remission after initial chemotherapy, as measured by MRD testing, remain critical determinants of long-term outcomes.[133-135] Advances in next-generation sequencing technologies have facilitated the identification of additional high-risk subtypes of *de novo* ALL, as well as alterations enriched at relapse. Efforts are ongoing to integrate genomic features with biochemically- and epigenetically-informed molecular treatment approaches. TKI addition to cytotoxic chemotherapy for children with Ph^+ ALL has dramatically improved EFS and OS, and implementation of similar strategies for patients with other high-risk ALL genetic subgroups may also improve outcomes. However, testing of precision medicine therapies is currently in its early stages for most potentially “targetable” subtypes of ALL, and the long-term success of such approaches remains unknown. In addition, presence of inherited leukemia predisposition gene variants and detection of chemoresistance-associated mutations in sizable proportions of patients who relapse demonstrates the dynamic biology of ALL that remains incompletely understood.

In vitro and *in vivo* evaluation of new agents in ALL model systems with swift bench-to-bedside translation of preclinically promising therapies remains a lengthy and often imperfect system. Single-agent biologic activity in murine models does not always translate into clinical activity in patients, and improper drug sequencing can result in untoward clinical outcomes.[136] Access to biologically relevant new agents for children with ALL and other cancers often appreciably lags behind that of adult hematology and oncology. Initial evaluation of new anti-cancer drugs in children generally occurs in the multiply relapsed/chemorefractory setting where lack of response may not necessarily mirror activity in newly-diagnosed patients or predict activity when combined with chemotherapy. Distinct drug dose intensity and tolerability factors for children also exist that must be carefully considered, particularly as new drugs are incorporated into multi-agent regimens.[137] Drug development may be halted when deleterious side effects occur in patients that were not previously observed or predicted by preclinical modeling, as well as for economic reasons despite evidence of anti-cancer activity in patients.[137] It is becoming increasingly apparent that combination of two or more targeted agents (*e.g*., growth factor receptor inhibitors,

TKIs) for some cancers will be necessary to evoke robust anti-tumor efficacy while minimizing development of resistance mutations or upregulation of “escape” pathways. Improved collaboration among cancer researchers, clinical oncologists, government agencies, and pharmaceutical industry partners is thus likely necessary to accelerate the pipeline of preclinical drug discovery and early clinical testing of promising new drugs. Clinical trial design for evaluation of new agents in increasingly smaller “boutique” subsets of genomically-defined patients also presents new challenges, and development of robust biomarkers of response (pharmacokinetic, pharmacodynamic, others) will be necessary to define clinical activity and to prioritize further drug development for children with ALL.

Treatment of children and AYAs with years of multi-agent chemotherapy is well-associated with untoward short- and long-term sequelae, and it is likely that a proportion of children with favorable-risk ALL (*e.g.*, rapid early responders with *ETV6-RUNX1* translocation or with hyperdiploidy) can achieve similarly outstanding cure rates with less intensive therapy. Therapy reduction for strictly defined low-risk subsets of ALL has been investigated or is under current evaluation by several cooperative groups, and several studies have demonstrated excellent feasibility of this approach.[18, 19, 116, 138] The major challenges for the next decade are to improve cure rates through improved risk stratification and more widespread application of molecularly-targeted therapies, including TKIs and antibody- and T-cell-based immunotherapies, as well as to minimize late effects by identifying the least toxic regimens that can cure low-risk patients.

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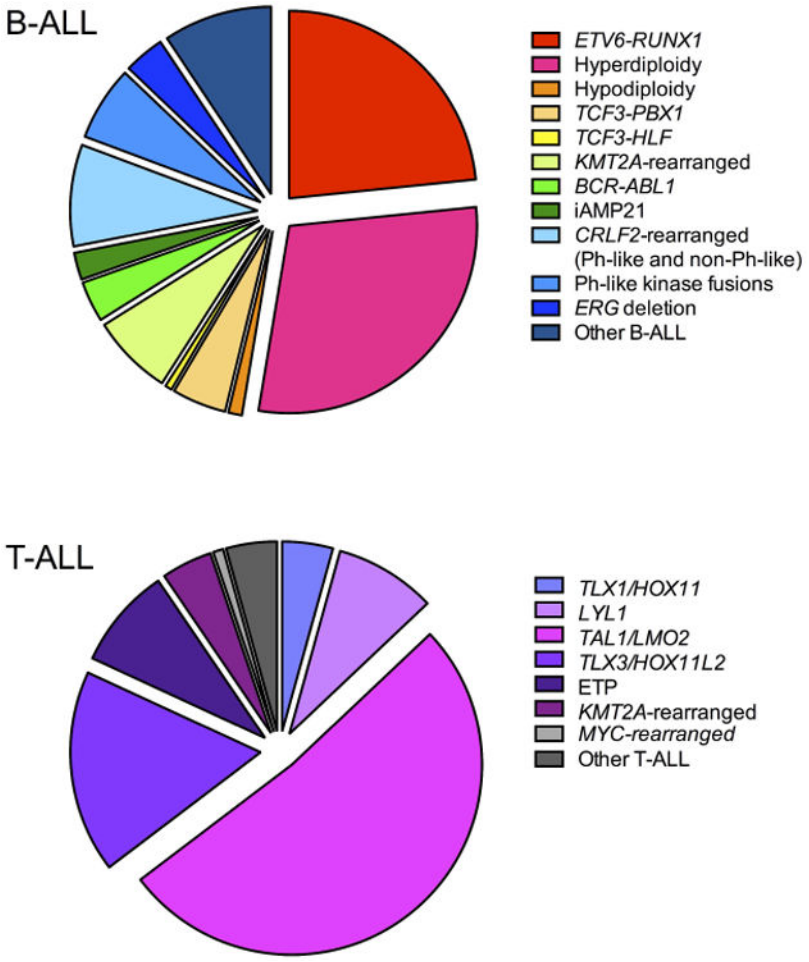

Figure 1. Frequency of cytogenetic alterations in childhood B-ALL and T-ALL

Data regarding submicroscopic genetic alterations are not included.

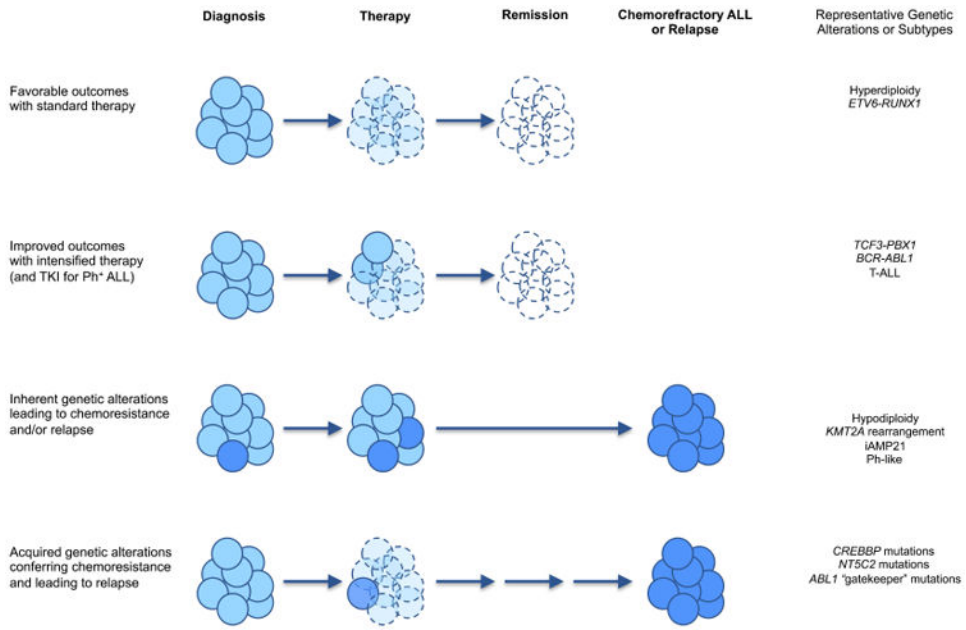

Figure 2. Impact of genetic features of ALL upon therapy response

Chemosensitive ALL cells (pale blue) respond to appropriately intensive chemotherapy based upon underlying leukemic genetics and risk stratification, often allowing patients to achieve sustained remission. Chemoresistant ALL cells (dark blue) may result from inherently drug-resistant subclones present at diagnosis or from selective pressure during chemotherapy that leads to development of acquired mutations and facilitates relapse. Very rarely, secondary malignancies may arise from genetically distinct subclones than those present at diagnosis.

Table 1
Common genetic alterations in childhood ALL and associated clinical outcomes

Genetic subtype	Common alterations	Frequency in ALL	Prognosis	Comment
B-ALL				
Aneuploidy				
Hyperdiploidy (>50 chromosomes)		25%	Favorable	
Hypodiploidy (<44 chromosomes)	Near-haploidy (24-31 chromosomes), low-hypodiploidy (32-39 chromosomes)	1-2%	Unfavorable	Association with *TP53* mutations, *IKZF2* and *IKZF3* deletions, and Ras and PI3K pathway mutations
Chromosomal Translocations				
t(12;21)(p13;q22)	*ETV6-RUNX1* (*TEL-AML1*)	20%	Favorable	
t(1;19)(q23;p13.1)	*TCF3-PBX1* (*E2A-PBX1*)	4%	Intermediate	
t(17;19)(q22;p13)	*TCF3-HLF*	<0.5%	Unfavorable	
KMT2A (*MLL*) rearrangements		5-6%	Unfavorable (infants), intermediate (non-infants)	Highest frequency in younger infants (80%); associated with FLT3 overexpression and epigenetic dysregulation
t(1;11)(q21;q23)	*KMT2A-MLLT11*		Less unfavorable	Rare
t(4;11)(q21;q23)	*KMT2A-AFF1(AF4)*		Particularly unfavorable	Comprises 50% of infant *KMT2A*-rearranged ALL
t(9;11)(p22;q23)	*KMT2A-MLLT3(AF9)*			Comprises 15% of infant *KMT2A*-rearranged ALL
t(10;11)(p12;q23)	*KMT2A-AF10*			Comprises 5% of infant *KMT2A*-rearranged ALL
t(11;19)(q23;p13.3)	*KMT2A-ENL*			Comprises 20-25% of infant *KMT2A*-rearranged ALL
Other fusion partners				
t(9;22)(q34;q11.2)	*BCR-ABL1*	3-5%	Unfavorable prior to TKI therapy, intermediate with TKI therapy?	Associated with *IKZF1* deletions
Other				
iAMP21	Multiple copies of *RUNX1*	2%	Unfavorable	Rare rob(15;21)(q10;q10)c associated with greatly increased risk of iAMP21 ALL
Trisomy 21-associated ALL	*P2RY8-CRLF2*, *JAK2* mutations		Intermediate	
Philadelphia chromosome-like (Ph-like)	*IGH-CRLF2*, *P2RY8-CRLF2*	7-8%	Unfavorable	50% of Ph-like; associated with *JAK1* and *JAK2* mutations, *CDKN2A/B* deletions, *IKZF1* deletions;

Genetic subtype	Common alterations	Frequency in ALL	Prognosis	Comment
				increasing incidence with older age; possibly targetable with TKIs
	ABL1, *ABL2*, *CSF1R*, *PDGFRB* rearrangements	5-6%	Unfavorable	10-20% of Ph-like; potentially targetable with TKIs
	EPOR, *JAK2* rearrangements	2%	Unfavorable	10% of Ph-like; potentially targetable with TKIs
ERG deletion		3%	Favorable	Associated with *IKZF1* deletions and aberrant CD2 expression
T-ALL*				
Transcription factor oncogenes				
t(10;14)(q24;q11)	*TLX1 (HOX11)* fusions	5-10% of T-ALL	Favorable	Associated with *PHF6* mutations
t(7;19)(q34;p13)	*LYL1* fusions	10% of T-ALL	Unfavorable	
t(1;14)(p32;q11), t(1;7)(p32;q34), t(11;14)(p15;q11), t(11;14)(p13;q11)	*TAL1*, *LMO1*, *LMO2* fusions	50-60% of T-ALL	Unfavorable	Associated with *PHF6* mutations
t(11;14)(p15;q11), t(5;14)(q35;q32)	*TLX3* (*HOX11L2*) fusions	20-25% of T-ALL	Unfavorable (some studies), intermediate (some studies), favorable (some studies)	Associated with *PHF6* mutations
7p15 translocations	*HOXA10*, *HOXA9* overexpression	3% of T-ALL	Unfavorable	
KMT2A rearrangements				
	KMT2A-AFF1, *KMT2A-MLLT1*	5% of T-ALL	Possibly favorable	
	PICALM-MLLT10	5-10% of T-ALL	Unfavorable (some studies), intermediate (other studies)	Associated with *EZH2* alterations
Other				
t(8;14)(q24;q11)	*TRA-MYC*, *TRC-MYC*	1% of T-ALL	Likely unfavorable	Associated with MYC activation and aggressive phenotype
ETP		10-15% of T-ALL	Unfavorable (some studies), intermediate (other studies)	Associated with Ras pathway mutations; characteristic immunophenotype (CD1a-, CD8-, CD5- or CD5-dim with co-expression of myeloid or stem cell markers)
NOTCH1 mutations		50-60% of T-ALL	Favorable	Associated with *CDKN2A* and *FBXW7* deletions
FBXW7 mutation		15% of T-ALL		Associated with NOTCH1 activation via impairment of

Genetic subtype	Common alterations	Frequency in ALL	Prognosis	Comment
				proteasomal degradation of NOTCH1
t(9;14)(q34;q32)	*NUP214-ABL1*	5-15% of T-ALL	Unfavorable (some studies), intermediate (other studies)	Associated with *HOX11* and *HOX11L2* overexpression
Other T-ALL		20% of T-ALL		
Relapsed ALL				Associated with chemotherapy resistance
	CREBBP mutation	20% of relapsed ALL		Likely confers resistance to glucocorticoids
	NT5C2 mutation	20% of relapsed ALL		Likely confers resistance to anti-metabolite drugs
	MSH6 deletion			
	NR3C1 deletion			
	SETD2 mutation	12% of relapsed ALL		
	KDM6 mutation			
	MLL2 mutation			
	Ras pathway mutations	30-50% of relapsed ALL		

* Frequency of alterations in T-ALL exceeds sum of 100% due to co-occurrence of lesions.

TKI = tyrosine kinase inhibitor