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## Biology of Childhood Acute Lymphoblastic Leukemia

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### Introduction

Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy, accounting for 25% of all childhood cancers. In the United States, approximately 3000 children aged 1–19 years are diagnosed with ALL annually.<sup>1</sup> Giant strides have been made in the management of childhood ALL over the past 50 years, which has resulted in improvement in cure rates from approximately 10% to approximately 90%.<sup>2, 3</sup> The rational use of multi-agent systemic chemotherapy over a prolonged duration (2–3 years) and adequate central-nervous-system (CNS)-directed therapy as well as improved antibiotic and blood product support in the 1960s and 1970s were responsible for the early improvements in outcome. However, insights into the heterogenic biology of ALL and monitoring of minimal residual disease (MRD) have helped to refine therapy based on risk of relapse to maximize cure and minimize toxicities. For example, identification of the Philadelphia chromosome in a subset of patients with ALL has made it possible to incorporate ABL tyrosine kinase inhibitors into chemotherapy regimens. This targeted therapy approach has improved the cure rate of patients with Philadelphia chromosome-positive ALL from 35% to around 70% over the last 10 years, even without stem cell transplant.<sup>4</sup>

Leukemic cells have, and are being thoroughly investigated by methods ranging from karyotyping which identifies large chromosomal alterations, to whole genome sequencing,

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which identifies cryptic changes in the entire genome. ALL is particularly amenable to biologic studies because of the relative ease of obtaining sample, which in most cases is an enriched population of blasts. Moreover, because the majority of children with ALL are treated uniformly on large clinical trials, well-annotated clinical information is available to correlate with biologic findings. Extensive collaborative efforts among various study groups internationally have played a vital role in the remarkable progress made in not only improving therapeutic outcomes but also deciphering the complex biology of childhood ALL.<sup>5</sup>

In this review, we summarize various insights gained from biologic studies of childhood ALL, with a focus on recent studies. We also discuss genomic lesions and epigenetic regulatory mechanisms associated with leukemic transformation. Finally we highlight the importance of studying the biology of the host to understand additional heterogeneity in treatment response and toxicities.

## B-ALL

Eighty-five percent of the cases of childhood ALL are of the B-lineage. To keep pace with the growing impact of biologic findings on treatment outcomes, in 2008 the World Health Organization revised the nomenclature from solitary precursor B-ALL to a classification based on 7 specific, recurring genetic lesions (e.g. B-ALL with *ETV6-RUNX1*, B-ALL with hyperdiploidy).<sup>6</sup> Of note, the term B-ALL is not used for Burkitt leukemia/lymphoma which is a mature B-cell malignancy. As newer subtypes are identified, biology-centered classifications need to be continually reviewed and updated (Figure 1).

### Conventional chromosomal alterations

ALL commonly arises from a series of genetic alterations, and in the majority of ALL subtypes, the interplay of these alterations. For the last 3 decades, several conventional cytogenetic studies of genetic aberrations that include chromosomal translocations and alterations in chromosome number have provided information on the pathogenesis of ALL. Common translocations in children with B-ALL include t(12;21) [*ETV6-RUNX1*](25%), t(1;19) [*TCF3-PBX1*](5%), t(9;11) [*BCR-ABL1*](3%) and translocations involving the *MLL* gene with various partner fusion genes (5%). Gains in whole chromosomes, or high-hyperdiploidy (>50 chromosomes) accounts for 25% of childhood ALL, whereas hypodiploidy (< 44 chromosomes) accounts for approximately 1% of cases. Several of these genetic changes have prognostic and therapeutic implications and are important in risk stratification schemes.<sup>7</sup> The overall survival (OS) of patients with *ETV6-RUNX1* or high-hyperdiploid ALL is more than 93%;<sup>8,9</sup> therefore these patients are treated on less intensive regimens, provided that they have an adequate early response to remission induction therapy (as currently assessed by the measurement of MRD). Contemporary therapy has abolished the previously unfavorable prognostic impact of *TCF3-PBX1*(t[1;19]). However, because bone marrow and CNS relapse could be competitive events, patients with *TCF3-PBX1* may need more intensive intrathecal therapy to reduce the risk of CNS relapse with improved systemic therapy.<sup>10</sup> Similarly, the addition of tyrosine kinase inhibitors has dramatically improved the bone marrow control of patients with *BCR-ABL1*-positive ALL<sup>4</sup> and attention should be paid to optimal intrathecal therapy in this genetic subtype of ALL. Hypodiploidy

continues to be a high-risk feature,<sup>11</sup> necessitating further understanding of oncogenic mechanisms and the rational use of targeted therapy (e.g. for RAS pathway inhibition).<sup>12</sup> The frequency and prognostic impact of *MLL* rearrangements differ by age. Approximately 80% of infants younger than 1 year harbor *MLL* rearrangements and their overall outcome is poor (5-year survival 50%) despite receiving very intensive therapy.<sup>13</sup>

Fusion gene products that result from chromosomal translocations provide the lymphoid progenitor or stem cell with leukemogenic potential, such as constitutional activation of tyrosine kinases (e.g. *ABL1*) or disruption of genes that regulate normal lymphoid development (e.g. *ETV6*, *PAX5*). Additional genetic hits are often required for the ultimate development of leukemia and include loss of the tumor suppressor gene *CDKN2A* and deletion of the non-translocated *ETV6* allele in *ETV6-RUNX1*-positive ALL.<sup>14</sup> High-resolution genomic studies are revealing the entire range of co-operating genetic changes and pathogenic mechanisms.<sup>12, 15–20</sup>

### Submicroscopic alterations

The advent of genome-wide profiling of RNA and DNA and next-generation sequencing (NGS) technologies has greatly increased our ability to identify and catalog submicroscopic genetic alterations and sequence mutations in ALL, which in turn help define new molecular subtypes. Some novel genomic lesions have prognostic and therapeutic significance and may be used to refine risk stratification schemes in the near future; for example, *IKZF1* deletion predicts poor prognosis in children with B-ALL.<sup>18</sup> In addition, the recognition of specific molecular lesions and critical oncogenic pathways paves the way for developing novel targeted approaches to therapy (e.g. inhibition of the ABL tyrosine kinase or JAK-STAT pathways). Single nucleotide polymorphism (SNP) array analyses revealed that gross genomic instability is not present in most children with ALL.<sup>17</sup> A mean of 6.4 genomic lesions were present per case, with wide variability within the genetic subtypes of ALL. Structural alterations in genes encoding transcriptional regulators of B-lymphoid development and differentiation occur in more than 40% of patients with B-ALL. *PAX5* is the most common target; others targets include *IKZF1* and *EBF1*. A number of additional lesions have also been identified in lymphoid signaling, transcription factors and tumor suppressors. Several of these alterations cooperate in leukemogenesis. For example, the deletion of *IKZF1* accelerates the onset of ALL in murine models of *BCR-ABL1*-ALL.<sup>20</sup> The assortment and accumulation of “driver” and “passenger” mutations and the sequence of events in leukemia development and progression continue to be investigated.

## High-risk subtypes of B-ALL

### *BCR-ABL*-like ALL

*IKZF1* deletions are a hallmark of *BCR-ABL1*-positive ALL, but these deletions also occur in a subset of patients with poor-response, high-risk ALL without any known chromosomal rearrangement.<sup>18</sup> Using genome-wide analyses, two groups of investigators independently identified a subgroup of B-ALL which has a gene expression profile similar to that of *BCR-ABL1*-positive ALL including a high frequency of *IKZF1* alterations but lacks the *BCR-ABL1* fusion protein; they termed this genetic subtype as *BCR-ABL1*-like or Philadelphia

chromosome-like ALL.<sup>18, 21</sup> This subtype comprises 10% of the cases of B-ALL in children and 25% of the cases of ALL in adolescents and young adults.<sup>22</sup> NGS techniques and downstream functional experiments show that *BCR-ABL1*-like ALL is characterized by genetic changes which result in constitutive activation of cytokine receptor and/or tyrosine kinase signaling.<sup>19, 22</sup> The spectrum of genetic alterations is extremely diverse; however, several rearrangements involve tyrosine kinases such as *ABL* and *PDGFR* which respond to imatinib and dasatinib in vitro and in vivo.<sup>22, 23</sup> Even though risk-directed therapy including intensive chemotherapy with or without transplant based on MRD level during remission induction therapy can abolish the poor prognosis of this group of patients, it is important to look for genetic lesions responsive to *ABL* tyrosine kinase inhibitor so that some patients can be spared from transplantation.<sup>24</sup> Several other rearrangements target *JAK* and *EPOR*, which are sensitive to *JAK* inhibitors in preclinical models.<sup>25</sup> In addition, re-arrangements involving the cytokine receptor gene *CRLF2* have been identified in 50% of patients with *BCR-ABL1*-like ALL, with frequent co-existing *JAK* mutations, also potentially sensitive to *JAK* inhibition.<sup>19, 25</sup> In view of the therapeutic implications for this high-risk subset of patients, array and sequencing based methodologies are being developed for rapid classification of patients with *BCR-ABL1*-like ALL and identification of targetable lesions; the incorporation of tyrosine kinase inhibitors in frontline therapy is also planned.<sup>26</sup>

#### **ALL with intrachromosomal amplification of chromosome 21 (iAMP 21)**

iAMP21 ALL was originally discovered by the observation of multiple copies of the *RUNX1* gene during routine screening for *ETV6-RUNX1* by fluorescent in situ hybridization. This particular ALL subtype is characterized by the instability of chromosome 21.<sup>27</sup> The incidence of iAMP21 is approximately 2%, and the median age of patients is 9–11 years. Intensification of chemotherapy has abolished the poor prognosis once associated with this ALL subtype.<sup>28</sup>

#### **Down syndrome ALL**

Patients with Down syndrome are at an approximately 20-fold increased risk of developing ALL, though the precise role of the extra chromosome 21 in leukemogenesis is unknown.<sup>29</sup> These patients have low frequencies of T-ALL and common ALL translocations such as *ETV6-RUNX1*. Patients with Down syndrome ALL have inferior outcome due to increased risk of relapse and high rate of treatment-related mortality.<sup>30</sup> High-resolution SNP profiling has identified a submicroscopic deletion of the pseudoautosomal regions of chromosomes X and Y which leads to the *P2RY8-CRLF2* fusion in approximately 50% of patients with Down syndrome-ALL.<sup>31</sup> These fusions and other *CRLF2* alterations were associated with *JAK* mutations. Together, these lesions activate the JAK-STAT pathway and promote cytokine-independent growth. Therefore, the inhibition of *JAK* tyrosine kinase is a potentially useful therapeutic strategy in patients with Down syndrome-ALL.

#### **T-ALL**

T-ALL accounts for 10%–15% of the cases of childhood ALL. The outcome of children with T-ALL, which has been historically poor, has improved gradually with the use of intensified therapy, including dexamethasone, asparaginase, and high-dose methotrexate.<sup>3, 8</sup>

However, children who relapse have a dismal outcome even with hematopoietic stem cell transplantation.<sup>32</sup> Therefore, it is critical to identify aberrant molecular pathways and targets for therapeutic intervention for T-ALL. Genetic lesions in T-ALL are diverse and complex and a multitude of alterations contribute in the pathogenesis of various subtypes of T-ALL.<sup>33, 34</sup> Chromosomal translocations are present in approximately 50% of patients with T-ALL cases, but unlike B-ALL, their prognostic impact is not well defined and they are not used for risk stratification. Some translocations result in the juxtaposition of oncogenes to T-cell receptor (TCR) genes, leading to overexpression of the oncogene in T-cell progenitor cells (e.g. *TLX1-TCRδ*), whereas others result in the fusion of 2 transcription factor oncogenes (e.g. *STIL-TALI*). In addition, rearrangements of the *MLL* gene occur in 5%–10% of patients with T-ALL. Gene expression profiling studies have identified 4 major subtypes of T-ALL on the basis of the predominant oncogenic pathway activation (*TLX1*, *LYL1*, *TAL/LMO2* and *TLX3*).<sup>35</sup>

### NOTCH activation in T-ALL

Constitutive activation of *NOTCH* signaling, primarily via somatic mutations, is seen in more than 50% of patients with T-ALL; a finding that is not restricted to specific subtypes of T-ALL.<sup>36</sup> In general, the presence of *NOTCH1* mutations indicates a favorable prognosis. *NOTCH1* is a transmembrane receptor crucial for T-cell development, lineage commitment, cell growth, and survival. Activation of *NOTCH1* and the presence of co-operating lesions, such as deletion of the tumor suppressor *CDKN2A* (found in 70% of patients with T-ALL) can lead to leukemic transformation. In addition, mutations in *FBXW7*, which encodes an ubiquitin protein ligase (found in 8%–10% of patients with T-ALL), attenuate the degradation of activated *NOTCH1*, further enhancing its downstream signaling.<sup>37</sup> Thus, the inhibition of *NOTCH1*, either by small molecule inhibitors of gamma secretase (that impede the release of activated *NOTCH1*) or by anti-*NOTCH1* antibodies is being actively pursued as a therapeutic strategy for T-ALL.<sup>38,39</sup>

### Early T-cell precursor ALL

Early T-cell precursors (ETPs) are a subset of immature thymocytes that retain stem-cell-like features and can differentiate into multiple lineages, including lymphoid and myeloid lineage. Complementary studies of flow cytometry, gene expression and DNA copy number, showed that the genetic profile of approximately 12% of patients with T-ALL is similar to that of these immature thymocytes.<sup>40</sup> A whole-genome study showed that ETP-ALL has frequent mutations of genes involved in hematopoietic development, cytokine receptor and RAS signaling, and chromatin modification.<sup>41</sup> The incidence of activating *NOTCH1* mutations is low in ETP-ALL which also lacks a unifying chromosomal abnormality. In general, the outcome of patients with ETP-ALL is poor, but myeloid-directed and epigenetic therapies may be beneficial for these patients.<sup>41</sup> A recent, small study suggested that patients with ETP-ALL have an intermediate outcome when treated with intensive chemotherapy that includes pegylated asparaginase and dexamethasone (5-year event-free survival of 76.7%);<sup>42</sup> a finding that requires confirmation.

## Epigenetics in ALL

In recent years, the importance of epigenetic regulatory mechanisms in normal and malignant hematopoiesis has become increasingly evident. Alterations in the methylation of DNA promoters and the modification of histone can significantly perturb transcriptional regulation and modify gene expression. Different subtypes of ALL are characterized by distinct DNA methylation signatures, which in turn correlate with gene expression profiles.<sup>15</sup> Several genes related to lymphoid development that are targets of somatic mutations in ALL are also inactivated by aberrant methylation, suggesting that multiple mechanism of silencing of critical genes may contribute to leukemic transformation. Mutations in histone writers, erasers and readers are more frequent in T-ALL than in most other pediatric cancers.<sup>43</sup> Also, several studies show that various *MLL* fusion proteins characteristically modulate chromatin structure through histone modifications; thus *MLL*-rearranged leukemia is considered an epigenetic malignancy.<sup>13, 44</sup> Epigenetic can also influence chemoresistance in ALL as manifested by increased global promoter methylation at relapse.<sup>16</sup> Therapy with demethylating agents led to re-expression of hypermethylated genes and restored chemosensitivity in an experimental model.<sup>45</sup> The interplay among the altered epigenetic landscape and structural changes in the genome, and the development of epigenetic therapies such as histone deacetylase inhibitors and demethylating agents provides exciting opportunities for therapeutic interventions.

## Biology of relapsed ALL

Studies of matched diagnosis-relapse samples have shed light on the clonal evolution leading to relapse, pathways associated with chemoresistance, and potential targets for therapy. In one study, 86% of the patients at relapse had outgrowth of a minor subclone present at diagnosis, which has genetic alterations both similar to and different from the major clone at diagnosis.<sup>46</sup> In some patients, relapse may have genetic alterations either identical to, or entirely different from those seen at diagnosis. The latter scenario likely represents a second malignancy. Preclinical studies and clinical experience show that leukemic blasts are more resistant to various chemotherapeutic agents at relapse than at initial diagnosis.<sup>47, 48</sup> Mechanisms of resistance may include selection of a pre-existing resistant subclone or the acquisition of additional genomic lesions under the selective pressure of chemotherapy. In a recent study, gain-of-function mutations in *NT5C2* were identified in leukemic blasts of approximately 20% of patients at relapse.<sup>49, 50</sup> *NT5C2* encodes a 5' nucleotidase enzyme that catalyzes the inactivation of nucleoside analogs such as mercaptopurine and thioguanine. As mercaptopurine and methotrexate are the mainstay of the maintenance therapy for ALL, acquisition of the *NT5C2* mutation can lead to emergence of drug-resistant clones and early relapse. Other genomic lesions at relapse include mutations in *CREBBP* (which mediates glucocorticoid response and histone acetylation)<sup>51</sup> and focal deletions in the mismatch repair gene *MSH6*<sup>52</sup> and the glucocorticoid receptor *NR3C1*.<sup>53</sup>



## Biology of the host

### ALL susceptibility

Besides constitutional trisomy 21 (Down syndrome) and rare DNA damage repair defects (e.g. ataxia–telangiectasia and Bloom syndrome), little is known about the genetic predisposition to ALL.<sup>54</sup> The frequency of hematologic malignancies is 4% in patients with common cancer predisposition syndromes such as Li–Fraumeni syndrome (caused by inherited mutations in *TP53*), approximately half of which are ALL.<sup>55</sup> However, a study of hypodiploid ALL with whole genome and exome sequencing revealed that 91% of patients with low–hypodiploid ALL (32–39 chromosomes) harbored somatic *TP53* alterations.<sup>12</sup> In 43% of these patients, *TP53* mutations were also present in non–tumor DNA, indicating a previously unrecognized link of Li–Fraumeni syndrome to low-hypodiploid ALL and implications for genetic counseling. Recently, whole exome sequencing identified a new familial leukemia syndrome in kindreds harboring a novel germline variant in *PAX5* on chromosome 9.<sup>56</sup> *PAX5* is a lymphoid transcription factor that plays a crucial role in B–cell development and is a common target for submicroscopic deletion in B–ALL blasts. Leukemic samples from family members with ALL showed 9p deletion with loss of heterozygosity and retention of the mutant *PAX5* allele, leading to significantly reduced transcriptional activity and perhaps leukemic transformation.

In the search for common, inherited ALL susceptibility variants, several large genome–wide association studies (GWAS) have identified polymorphisms in *ARID5B*, *IKZF1*, *CEBPE*, *CDKN2A* and *PIP4K2A–BM11* were over–represented in patients with ALL compared with non– ALL controls.<sup>57–59</sup> There were significant differences in the prevalence of these variants among patients of different ancestries, possibly contributing to racial differences in the incidence of ALL. Each of these variants account for a modest increase in the risk of developing ALL (odds ratio 1.5–2), but they independently and cumulatively contribute to genetic susceptibility to ALL.<sup>58</sup> *CDKN2A* and *IKZF1* are also targeted by somatic alterations in ALL,<sup>17</sup> suggesting that both inherited and somatic genetic variations cooperate in the pathogenesis of ALL. Interestingly, the risk of developing various subtypes of ALL is also influenced by genetic inheritance. An intronic SNP in *TP63* (a member of the *TP53* family of transcription factors) conferred susceptibility to *ETV6–RUNX1*–positive ALL<sup>60</sup> and SNPs in *GATA3* conferred susceptibility to *BCR–ABL1*–like ALL and its underlying somatic lesions (*IKZF1* deletions, *CRLF2* rearrangements and *JAK* mutations).<sup>61</sup>

### Toxicity

An individual’s genetic make–up can influence drug transport and metabolism and subsequently efficacy and toxicity. Thiopurine S–methyl transferase (TPMT) enzymatic activity is deficient in approximately 10% of individuals with polymorphisms in the *TPMT* gene. The reduced activity of TPMT leads to excessive cellular accumulation of active thiopurine metabolites and thereby excessive hematopoietic toxicity. In one study, the cumulative incidences of mercaptopurine–related myelosuppression were 100%, 35%, and 7% for patients with homozygous, heterozygous, and wild–type genotypes respectively.<sup>62</sup> Tailoring the dose of mercaptopurine on the basis of TPMT genotype results in equivalent systemic exposure, tolerance, and efficacy, and is an excellent example of widely used

pharmacogenetic-guided therapy in clinical care. Germline variants associated with steroid-induced osteonecrosis,<sup>63</sup> vincristine-induced peripheral neuropathy,<sup>64</sup> anthracycline-induced cardiotoxicity<sup>65</sup> and asparaginase allergy<sup>66</sup> have also been identified by candidate gene and genome-wide studies. Validation of these associations is warranted to determine their clinical relevance.

## Response

Inherited genetic variation can contribute to inter-patient variability in ALL treatment response, by influencing host disposition of anti-leukemic agents, interactions between tumor-microenvironment and ALL, and tumor biology itself. At a genome-wide level, a study of more than 400,000 host germline polymorphisms in 487 children with ALL, identified 102 SNPs (representing 71 unique genomic loci) that were significantly associated with MRD at the end of remission induction therapy<sup>67</sup>. Twenty percent of these SNP genotypes associated with high MRD were related to decreased exposure to methotrexate and etoposide, either because of increased clearance of these agents or decreased intracellular accumulation of active methotrexate polyglutamates. These findings underscore the effect of host genetic makeup on the response to multiple agents. A subsequent GWAS of 2,535 children with ALL identified 134 SNPs consistently associated with outcome, most of which remained prognostic even after adjusting for known risk factors (ex. MRD, molecular subtype). In particular, risk variants in *PDE4B* predisposed patients to relapse, plausibly via affecting methotrexate pharmacodynamics and tumor sensitivity to steroids.<sup>68</sup>

The outcome of Hispanic children with ALL is historically inferior to that of patients of European descent. This racial disparity may in part be due to increased frequency of germline variants associated with Native American ancestry in Hispanic patients which negatively influence MRD and relapse.<sup>69</sup> Patients with Hispanic ethnicity are also predisposed to developing high-risk subtypes of ALL (e.g. *GATA3* germline polymorphism in *BCR-ABL1*-like ALL).<sup>61</sup> Importantly, the addition of an extra block of delayed intensification therapy for Hispanic children seemed to mitigate the ancestry-related difference in outcome.<sup>69</sup>

## Summary

Modern-day management of childhood ALL exemplifies the successful integration of biology into therapeutic decision making. In addition to the prognostic impact of conventional chromosomal translocations and aneuploidy, functional studies of key genetic alterations have contributed to our understanding of ALL pathogenesis. With the advent of high-throughput genomics and NGS technologies, knowledge of specific molecular lesions and critical pathways of leukemogenesis has exponentially increased. The incorporation of targeted therapy is expected to improve outcome for high-risk patients, particularly patients with *BCR-ABL1*-positive ALL and those with the novel *BCR-ABL1*-like ALL subtype. In addition to genomic lesions, alterations in the epigenome modulate gene expression and contribute significantly to leukemic transformation and resistance to therapy. Therefore, epigenetic therapy is another strategy being actively pursued in the clinic. A deeper understanding of the effect of inherited genetic variations can provide the opportunity to modify therapy to decrease toxicity without compromising efficacy. Furthermore, strong



associations have been identified between inherited genetic variants and ALL susceptibility. Many of these polymorphisms are in genes that are targets of somatic lesions in ALL, highlighting plausible interactions between the biology of the disease and the host (Figure 2).

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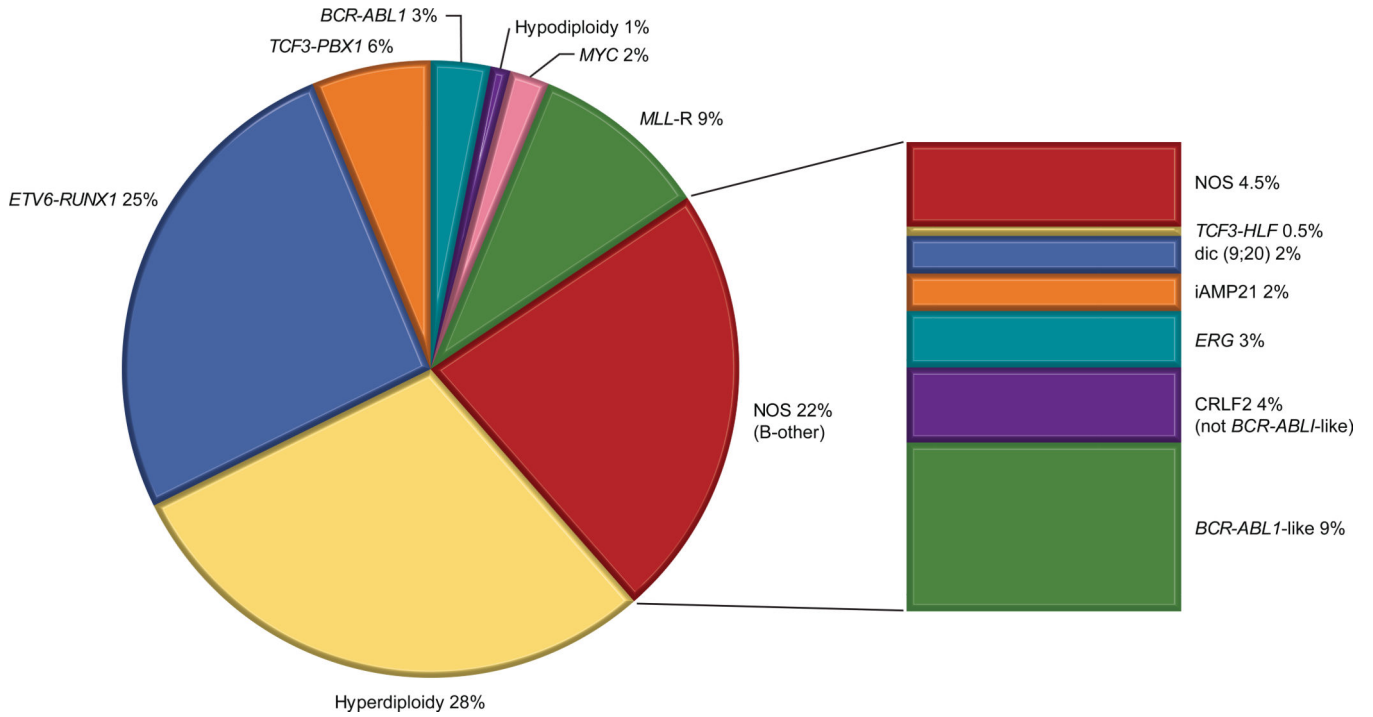
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**Key points**

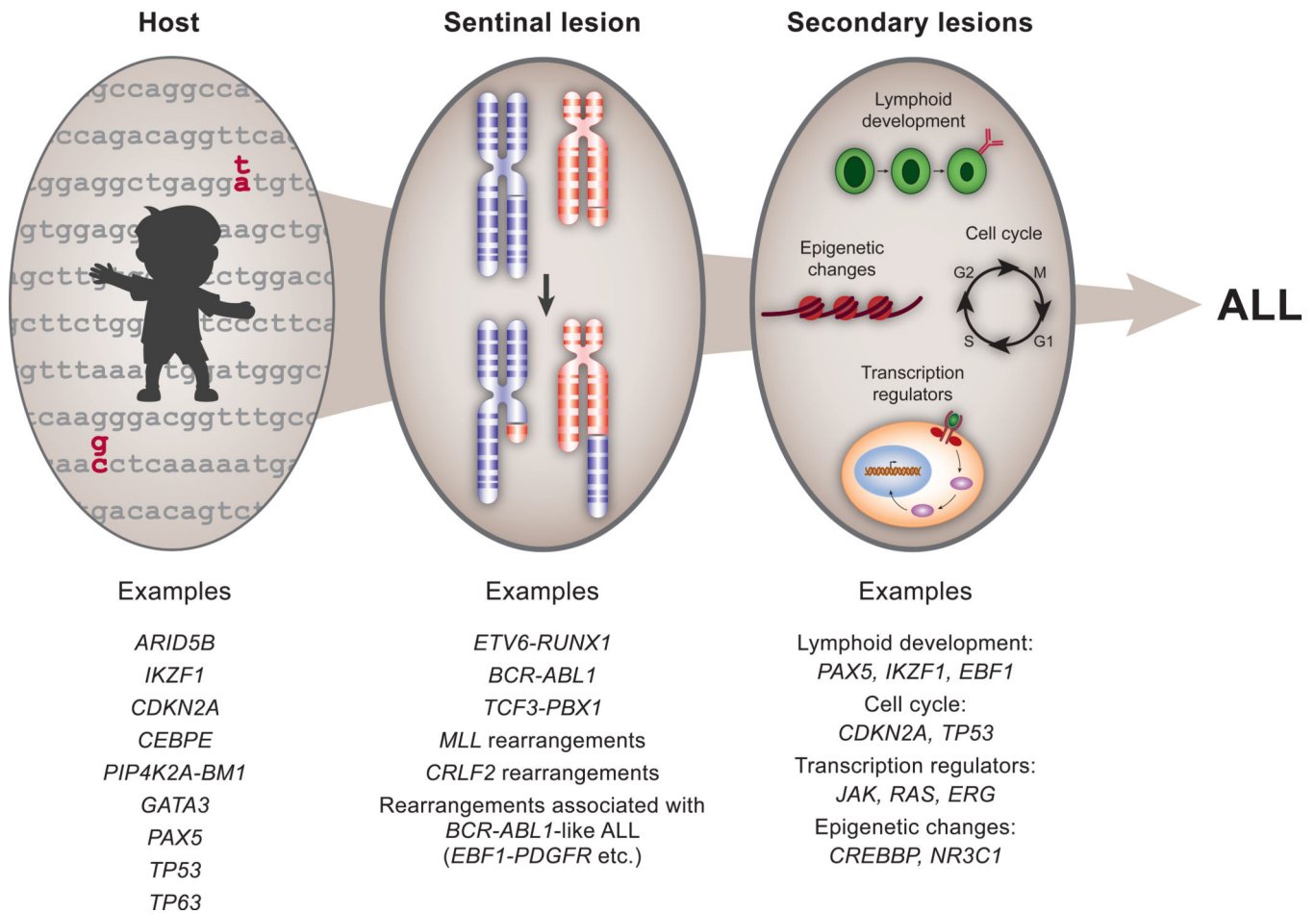
- Childhood ALL is a heterogeneous disease with multiple distinct biologic subtypes.
- High-throughput genomic profiling and next-generation sequencing technologies have identified submicroscopic genomic lesions and sequence mutations that define novel subtypes of ALL.
- The discovery of various oncogenic pathways and candidate genes has led to the development of biologically based targeted therapy.
- Host germline polymorphisms influence susceptibility to ALL, chemotherapy-related toxicities and response to therapy.



**Figure 1. Distribution of molecular subtypes of childhood B-ALL**

The pie chart on the left depicts molecular subtypes that were identified prior to 2004, incorporated in the 2008 WHO classification and are currently used for risk stratification. Subtype was unknown in 22% of patients (termed B-other). Since then, various novel molecular subtypes have been characterized shown in the bar graph on the right. Data has been modified from Pui CH, Relling MV, Downing JR. Acute lymphoblastic leukemia. *N Engl J Med.* 2004;350: 1535–1548; and from Pui CH, Mullighan CG, Evans WE, Relling MV. Pediatric acute lymphoblastic leukemia: Where are we going and how do we get there? *Blood.* 2012;120: 1165–1174.  
 NOS: not otherwise specified





**Figure 2. Pathogenesis of ALL**

Several inherited variants associated with susceptibility to ALL have been identified by genome-wide association studies and studies of familial ALL. Within hematopoietic cells, in addition to a sentinel event such as a chromosomal translocation, a multitude of secondary genetic events contribute to leukemic transformation.