Childhood Acute Lymphoblastic Leukemia: Progress Through Collaboration


ABSTRACT

Purpose
To review the impact of collaborative studies on advances in the biology and treatment of acute lymphoblastic leukemia (ALL) in children and adolescents.

Methods
A review of English literature on childhood ALL focusing on collaborative studies was performed. The resulting article was reviewed and revised by the committee chairs of the major ALL study groups.

Results
With long-term survival rates for ALL approaching 90% and the advent of high-resolution genome-wide analyses, several international study groups or consortia were established to conduct collaborative research to further improve outcome. As a result, treatment strategies have been improved for several subtypes of ALL, such as infant, MLL-rearranged, Philadelphia chromosome–positive, and Philadelphia chromosome–like ALL. Many recurrent genetic abnormalities that respond to tyrosine kinase inhibitors and multiple genetic determinants of drug resistance and toxicities have been identified to help develop targeted therapy. Several genetic polymorphisms have been recognized that show susceptibility to developing ALL and that help explain the racial/ethnic differences in the incidence of ALL.

Conclusion
The information gained from collaborative studies has helped decipher the heterogeneity of ALL to help improve personalized treatment, which will further advance the current high cure rate and the quality of life for children and adolescents with ALL.

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INTRODUCTION

Remarkable progress has been made in the treatment of childhood acute lymphoblastic leukemia (ALL) in the past two decades, with the proportion of patients surviving for 5 years approaching and even exceeding 90% in many developed countries (Table 1).1-14 Improved outcome can be attributed to the optimal risk-directed therapy that incorporates delayed intensification with vincristine, asparaginase, and dexamethasone; high dose or escalating dose of methotrexate; and early use of intrathecal therapy. These treatment components were primarily established by the randomized clinical trials performed by major cooperative study groups. With the improved cure rates, current research efforts have focused increasingly on small subsets of patients with drug-resistant leukemia, and they require concerted efforts by multiple study groups to identify effective treatment strategies. Parallel to the therapeutic gain have been the major advances in our knowledge of leukemic cell biology, especially with the recent advent of genome-wide analysis.15

Genome-wide studies to identify inherited and somatically acquired genomic variations that influence disease incidence, treatment response, or toxicities also require a large number of patients for discovery and validation studies and to adjust for the ancestral composition of study cohorts. Indeed, many recent advances in genomics have also been the results of national and, in many instances, international collaborations. We highlight here some of the major accomplishments resulting from these collaborations and point to ongoing efforts for further advances.
The Ponte di Legno group, named after the site of the first major meeting, was formed in 1995, and has since been joined by 15 major study groups or institutions, and has held 14 working group meetings. The participants agreed to use the same or similar criteria to present the treatment outcome so that results could be compared among various clinical trials to identify effective treatment strategies for specific subsets of ALL. The first series of 12 articles on long-term results of clinical trials was published in Leukemia in 2000, and the second series of 15 articles was published in Leukemia in 2010. The group also studied challenging subsets of ALL, the key findings of which are described in this review.

Under the leadership of Haig Riehm and Giuseppe Masera, the Berlin-Frankfurt-Münster (BFM) and the Associazione Italiana di Ematologia Pediatrica (AIEOP) study groups set an early example of fruitful international collaboration. Among their many collaborative studies, the two groups recently confirmed that minimal residual disease (MRD) assessment is the most prognostic indicator in both B- and T-cell ALL. They were also instrumental in developing the Intercontinental BFM (IC-BFM) Study Group comprising study groups from more than 30 countries worldwide to address therapeutic questions tailored to their resources and technology. The IC-BFM 2002 (A Randomized Trial of the I-BFM-SG for Management of Childhood Non-B Acute Lymphoblastic Leukemia) study, which enrolled 5,060 patients between 2002 and 2007 showed no significant improvement in outcome with intensive or prolonged delayed-intensification therapy, and it achieved a 5-year event-free survival of 74% and 5-year overall survival of 82%. Although the overall results were inferior to those of contemporaneous BFM studies because of the high rate of treatment-related mortality, the national results have generally improved and, importantly, this network demonstrated their ability to perform randomized clinical trials across continents.

Because of the rarity and distinct characteristics of infant ALL, 17 study groups collaborated on a clinical trial, Interfant-99 (Observational Study and Multicentre, Randomised Trial in Infants Younger Than 1 Year With Acute Lymphoblastic Leukaemia), which enrolled 482 infants age 0 to 12 months between 1999 and 2005 to investigate the efficacy of a hybrid treatment regimen with elements for treating both ALL and acute myeloid leukemia. The study achieved a 4-year event-free survival of 47.0% and overall survival of 55.3%, outcomes better than those achieved with most previous protocols but showing no benefit from delayed-intensification therapy with high-dose cytarabine and methotrexate. The current study, Interfant-06 (NCT00550992: International Collaborative Treatment Protocol for Infants Under One Year With Acute Lymphoblastic or Biphenotypic Leukemia), assesses early intensification with two blocks of acute myeloid leukemia induction therapy to improve outcome and examines the role of hematopoietic stem-cell transplantation in infants at high risk of relapse (age < 6 months, MLL rearrangement, and initial leukocyte count ≥ 300 × 10^9/L).

To identify treatment components responsible for improved treatment outcome, the Childhood ALL Collaborative Group was formed in 1994 to systematically review and analyze results from relevant randomized trials. Analysis of data from four clinical trials that enrolled patients between 1972 and 1984 showed that anthracyclines reduced hematologic relapse but failed to improve event-free survival, partly because of the increased induction failures and deaths in remission. Meta-analysis of trials started between 1965 and 1998 showed that the addition of vincristine plus prednisone or prednisolone pulses during postremission therapy improved event-free survival; the lack of improvement with vincristine and dexamethasone pulses in the most recent trials was attributed to the greater intensity of the early therapy. Meta-analysis of three trials that enrolled patients between 1992 and 2002 suggested that thioguanine improved event-free survival compared with mercaptopurine for males younger than age 10 years, but its lack of effect on survival and association with a high risk of veno-occlusive disease of the liver made mercaptopurine the standard thiopurine of choice. Meta-analysis of 47 trials of CNS-directed therapy conducted between 1970 and 1999 showed that CNS radiotherapy can generally be replaced by intrathecal therapy, including vincristine and methotrexate, which was also supported by the Berlin-Frankfurt-Münster study group. The IC-BFM 2012 study (NCT01575621: A Phase II Randomized Trial of the I-BFM-SG for Children With Early-Stage Acute Lymphoblastic Leukemia) was conducted to further improve the efficacy of first-line therapy for early-stage ALL.

Table 1. Patient Characteristics and Treatment Results From Selected Clinical Trials

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Years of Study</th>
<th>No. of Patients</th>
<th>Age Range (years)</th>
<th>T-Cell ALL (%)</th>
<th>5-Year Cumulative Rate of Isolated CNS Relapse (% ± SE)</th>
<th>5-Year EFS (% ± SE)</th>
<th>5-Year Overall Survival (% ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIEOP-95</td>
<td>1995-2000</td>
<td>1,743</td>
<td>0-18</td>
<td>11</td>
<td>1.2 ± 0.3</td>
<td>75.9 ± 1.0</td>
<td>85.5 ± 0.8</td>
</tr>
<tr>
<td>BFM-95</td>
<td>1995-1999</td>
<td>2,169</td>
<td>0-18</td>
<td>13</td>
<td>1.8 ± 0.3</td>
<td>79.6 ± 0.9</td>
<td>87.0 ± 0.7</td>
</tr>
<tr>
<td>CoALL-97</td>
<td>1997-2003</td>
<td>667</td>
<td>1-18</td>
<td>14</td>
<td>4.0 ± 0.8</td>
<td>76.7 ± 1.7</td>
<td>85.4 ± 1.4</td>
</tr>
<tr>
<td>COG</td>
<td>2000-2005</td>
<td>7,153</td>
<td>0-21</td>
<td>7</td>
<td>NA</td>
<td>NA</td>
<td>90.4 ± 0.5</td>
</tr>
<tr>
<td>DCOG-9</td>
<td>1997-2004</td>
<td>859</td>
<td>1-18</td>
<td>11</td>
<td>2.6 ± 0.6</td>
<td>80.6 ± 1.4</td>
<td>86.4 ± 1.2</td>
</tr>
<tr>
<td>DFCI 00-01</td>
<td>2000-2004</td>
<td>492</td>
<td>1-18</td>
<td>11</td>
<td>NA</td>
<td>80.0 ± 2</td>
<td>91 ± 1</td>
</tr>
<tr>
<td>EORTC-CLG</td>
<td>1998-2008</td>
<td>1,047</td>
<td>15-18</td>
<td>15</td>
<td>1.7 ± 0.3</td>
<td>82.7 ± 0.9</td>
<td>89.7 ± 0.7</td>
</tr>
<tr>
<td>IC-BFM 2002</td>
<td>2002-2007</td>
<td>5,060</td>
<td>1-13</td>
<td>13</td>
<td>1.9 ± 0.1</td>
<td>74 ± 1</td>
<td>62 ± 1</td>
</tr>
<tr>
<td>JCLLSG ALL 2000</td>
<td>2000-2004</td>
<td>305</td>
<td>1-15</td>
<td>9.8</td>
<td>0.9 ± 0.1</td>
<td>79.7 ± 2.4</td>
<td>89.2 ± 1.8</td>
</tr>
<tr>
<td>Ma-Spore ALL 2003</td>
<td>2002-2011</td>
<td>556</td>
<td>0-18</td>
<td>8.8</td>
<td>1.4</td>
<td>80.6 ± 3.5</td>
<td>89.2 ± 2.7</td>
</tr>
<tr>
<td>MRC UKALL 2003</td>
<td>2003-2011</td>
<td>3,136</td>
<td>1-25</td>
<td>12</td>
<td>1.9 ± 0.6</td>
<td>87.3 ± 1.4</td>
<td>91.6 ± 1.2</td>
</tr>
<tr>
<td>NOPHO-2000</td>
<td>2002-2007</td>
<td>1,023</td>
<td>1-15</td>
<td>11</td>
<td>2.7 ± 0.6</td>
<td>79.4 ± 1.5</td>
<td>89.1 ± 1.1</td>
</tr>
<tr>
<td>SJCRH XV</td>
<td>2000-2007</td>
<td>498</td>
<td>1-18</td>
<td>15</td>
<td>2.7 ± 0.8</td>
<td>87.3 ± 2.9</td>
<td>93.5 ± 1.9</td>
</tr>
<tr>
<td>TPOG</td>
<td>1999-2010</td>
<td>152</td>
<td>0-18</td>
<td>7.2</td>
<td>1.4 ± 1.0</td>
<td>84.2 ± 3.0</td>
<td>90.2 ± 2.4</td>
</tr>
</tbody>
</table>

Abbreviations: AIEOP, Associazione Italiana di Ematologia Pediatrica; ALL, acute lymphoblastic leukemia; BFM, Berlin-Frankfurt-Münster; CoALL, Cooperative ALL (study group); COG, Children’s Oncology Group; DCOG, Dutch Children’s Oncology Group; DFCI, Dana-Farber Cancer Institute (consortium); EFS, event-free survival; EORTC-CLG, European Organisation for Research and Treatment of Cancer-Children’s Leukemia Group; IC-BFM, Intercontinental BFM; JCLLSG, Japanese Children’s Cancer and Leukemia Study Group; Ma-Spore, Malaysia-Singapore; MRC UKALL, Medical Research Council United Kingdom Acute Lymphoblastic Leukaemia; NA, not available; NOPHO, Nordic Society of Paediatric Haematology and Oncology; SJCRH, St Jude Children’s Research Hospital; TPOG, Taiwan Pediatric Oncology Group.
and triple intrathecal therapy should be used with effective systemic therapy such as intravenous high-dose methotrexate to realize its full benefit of CNS control without the hazard of increased systemic relapse.\textsuperscript{26}

Several working groups investigated the optimal use of asparaginase. In one review, intensive use of asparaginase during the intensification phase of therapy was credited for improved outcome.\textsuperscript{27} Two studies showed improved outcome with therapeutic drug monitoring during Escherichia coli asparaginase treatment; detection of silent inactivation of the drug as a result of asparaginase antibody allowed for early replacement with Erwinia asparaginase.\textsuperscript{2,28}

**CLINICAL ADVANCES IN SPECIFIC SUBTYPES OF ALL**

Table 2 summarizes the findings of a few selected collaborative studies that have had a major impact on clinical management.

### ALL With 11q23 Rearrangement

There is apparent clinical heterogeneity within each of the subtypes of rearrangement of MLL at 11q23. In a study of 497 patients with 11q23 rearrangements diagnosed between 1983 and 1995, infants younger than 1 year fared significantly worse than older patients, and any category of 11q23 abnormality conferred a dismal prognosis among infants,\textsuperscript{29} a finding confirmed by the Interfant-99 study.\textsuperscript{21} In the largest subgroup of infants and children with t(4;11)(q21;q23), any type of transplantation, even with a matched-related donor, failed to improve outcome compared with chemotherapy alone.\textsuperscript{29} A separate analysis of the same cohort showed that among patients with t(4;11)(q21;q23), infants fared significantly worse than older patients.\textsuperscript{30} The Interfant-99 study showed that young age and MLL rearrangement independently predicted poor outcome in infant ALL.\textsuperscript{21} Importantly, hematopoietic stem-cell transplantation failed to improve the outcome of infants with MLL rearrangement in the Interfant-99 study, with the exception of a small subset of infants younger than age 6 months with either poor response to steroids or presenting leukocyte count \(\geq 300 \times 10^9/L\) who seemed to benefit from transplantation.\textsuperscript{22}

### Hypodiploid ALL

The clinical and biologic characteristics of hypodiploid ALL have been poorly defined. In a study of 139 hypodiploid patients diagnosed between 1986 and 1996, even after excluding nine patients with Philadelphia chromosome (Ph), the remaining 130 patients still had a poor outcome with an event-free survival of 38.3\% at 8 years; interestingly, there were no induction failures, but information on the level

<table>
<thead>
<tr>
<th>Subgroup of ALL</th>
<th>Years of Study</th>
<th>No. of Study Groups</th>
<th>No. of Patients</th>
<th>Major Findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>11q23 rearranged</td>
<td>1983-1995</td>
<td>11</td>
<td>497</td>
<td>Prognosis was particularly dismal in infants; allogeneic transplantation did not improve outcome in patients with t(4;11) ALL. Poor early response to prednisone or age younger than 3 months conferred a particularly dismal prognosis among infants with t(4;11).</td>
<td>Pui et al\textsuperscript{29,30}</td>
</tr>
<tr>
<td>Hypodiploid</td>
<td>1986-1996</td>
<td>11</td>
<td>139</td>
<td>Prognosis was very poor, especially among patients with fewer than 44 chromosomes.</td>
<td>Nachman et al\textsuperscript{31}</td>
</tr>
<tr>
<td>Hypodiploid</td>
<td>2008-2013</td>
<td>2</td>
<td>126</td>
<td>Genome profiling and sequencing identifies distinct genetic alterations in subtypes of hypodiploid ALL. Near-haploid patients have Ras pathway mutations and IKZF3 mutations; low-hypodiploid patients have IKZF2 alterations and TPRS mutations, many of which are inherited.</td>
<td>Holmfeldt et al\textsuperscript{41}</td>
</tr>
<tr>
<td>Ph positive</td>
<td>1986-1996</td>
<td>10</td>
<td>326</td>
<td>Presenting age, leukocyte counts, and response to initial treatment with glucocorticoids and intrathecal methotrexate affected treatment outcome; matched-related transplantation improved outcome.</td>
<td>Aricò et al\textsuperscript{42}</td>
</tr>
<tr>
<td>Ph positive</td>
<td>1995-2005</td>
<td>10</td>
<td>610</td>
<td>Both matched-related and matched-unrelated transplantation improved treatment outcome.</td>
<td>Aricò et al\textsuperscript{43}</td>
</tr>
<tr>
<td>Ph-like</td>
<td>2004-2009</td>
<td>10</td>
<td>178</td>
<td>Imatinib combined with intensive chemotherapy was well tolerated and might improve outcome.</td>
<td>Biondi et al\textsuperscript{44}</td>
</tr>
<tr>
<td>Ph-like</td>
<td>2008-2009</td>
<td>2</td>
<td>221</td>
<td>Genetic alteration of IKZF1 was associated with high levels of minimal residual disease and a very poor outcome.</td>
<td>Mullighan et al\textsuperscript{35}</td>
</tr>
<tr>
<td>Ph-like</td>
<td>2008-2009</td>
<td>2</td>
<td>297</td>
<td>These cases were associated with a high frequency of deletions in genes involved in B-cell development, resistance to asparaginase and daunorubicin, and unfavorable outcome.</td>
<td>Den Boer et al\textsuperscript{36}</td>
</tr>
<tr>
<td>Ph-like</td>
<td>2014</td>
<td>5</td>
<td>264</td>
<td>More than 90% of the patients had kinase-activating alterations, some of which were amenable to inhibition with tyrosine kinase inhibitors such as imatinib or dasatinib.</td>
<td>Roberts et al\textsuperscript{37}</td>
</tr>
<tr>
<td>Early T-cell precursor</td>
<td>1992-2006</td>
<td>2</td>
<td>30</td>
<td>These patients had distinctive immunophenotypic (CD1a\textsuperscript{-}, CD5\textsuperscript{-}, CD34\textsuperscript{+/low} with stem-cell or myeloid markers) and high risk of remission induction failure or hematologic relapse.</td>
<td>Coustan-Smith et al\textsuperscript{38}</td>
</tr>
<tr>
<td>Down syndrome</td>
<td>1995-2004</td>
<td>16</td>
<td>653</td>
<td>Compared with other patients, these patients have lower event-free survival as a result of increased relapse hazard and treatment-related mortality. Younger age and leukocyte count (&lt; 10 \times 10^9/L) at diagnosis and the presence of high hyperdiploidy and ETV6-RUNX1 are favorable prognostic factors.</td>
<td>Butenkamp et al\textsuperscript{39}</td>
</tr>
<tr>
<td>Induction failure</td>
<td>1985-2000</td>
<td>14</td>
<td>1,041</td>
<td>Patients with induction failure are highly heterogeneous. Although allogeneic transplantation improved outcome for T-cell ALL, chemotherapy should be the treatment of choice for B-ALL without other adverse features.</td>
<td>Schrappe et al\textsuperscript{40}</td>
</tr>
</tbody>
</table>

**Abbreviations:** ALL, acute lymphoblastic leukemia; Ph, Philadelphia chromosome.
of MRD was not available. There were no significant differences in outcome among patients with near haploidy (24 to 29 chromosomes), low hypodiploidy (33 to 39 chromosomes), or high hypodiploidy (40 to 43 chromosomes). Patients with fewer than 44 chromosomes fared significantly worse than patients with 44 chromosomes, among whom those with monosomy 7, a dicentric chromosome, or both had a significantly worse event-free survival. There was no difference in outcome between patients with or without doubling of their hypodiploid clone. The efficacy of hematopoietic stem-cell transplantation could not be adequately evaluated because only nine patients underwent this procedure.

Ph-Positive ALL

Before the development of ABL tyrosine kinase inhibitors, there was no consensus on the optimal treatment of Ph-positive ALL. A combined study of 326 children and young adults diagnosed between 1985 and 1996 disclosed a 5-year event-free survival (± SE) of only 28% ± 3% and that older age, high initial leukocyte count, and poor response to initial treatment with glucocorticoids and intrathecal methotrexate adversely affected treatment outcome. Compared with chemotherapy alone, matched-related transplantation improved outcome. A follow-up study of 610 patients diagnosed between 1995 and 2005 and treated without tyrosine kinase inhibitor showed that the event-free survival had improved to 32% ± 2% at 7 years, and both matched-related and matched-unrelated transplantation improved outcome compared with chemotherapy alone. The results of this study served as a historical reference to the subsequent Children's Oncology Group (COG) and European intergroup studies, showing that postinduction imatinib mesylate plus intensive chemotherapy improved disease-free survival to 70% ± 12% at 5 years and could spare patients with good initial response to remission induction from transplantation. Whether dasatinib, a potent ABL tyrosine kinase inhibitor, given in conjunction with a BFM high-risk chemotherapy backbone can result in noninferior or improved outcome while reducing the need for transplantation is being evaluated in a multicenter study (NCT01460160: A Phase 2 Multi-Center, Historically Controlled Study of Dasatinib Added to Standard Chemotherapy in Pediatric Patients With Newly Diagnosed Philadelphia Chromosome Positive Acute Lymphoblastic Leukemia).

Ph-Like (BCR-ABL1-like) ALL

By using single nucleotide polymorphism microarrays, transcriptional profiling, and gene resequencing, two group studies independently identified a novel high-risk subtype of B-ALL that exhibits a gene expression profile similar to that of Ph-positive ALL with a high frequency of alterations of the IKZF1 gene but lacks the BCR-ABL1 fusion protein. These patients were characterized by resistance to asparaginase and daunorubicin, a high level of MRD at the end of induction, and overall poor outcome. The BCR-ABL1–like expression profile and IKZF1 deletions had independent poor prognostic impact in a European collaborative study. Although risk-directed therapy, including intensive chemotherapy and allogeneic transplantation, could abolish the adverse prognostic significance of this genotype, a recent large collaborative study showed that a substantial proportion of patients have genomic alterations that are amenable to inhibition with ABL tyrosine kinase inhibitors and potentially JAK inhibitors, which may spare some patients from transplantation.

Early T-Cell Precursor ALL

On the basis of gene expression profiling and flow cytometry, a collaborative study identified a distinct group of patients who represent approximately 12% of T-cell ALL with leukemia originating from a subset of thymocytes that retain a stem-cell–like feature. Although the initial report showed that these patients had a high risk of remission induction failure or hematologic relapse, a recent study showed that they have intermediate-risk outcome (5-year event-free survival of 76.7%) when treated with intensive chemotherapy, including daunomycin and pegylated asparaginase.

Down Syndrome ALL

Patients with Down syndrome who have ALL have inferior survival because of increased risk of relapse and treatment-related mortality. The specific treatment phase responsible for increased death related to toxicity and the prognostic factors among these patients were not well characterized in previous small studies. In a large collaborative study, these patients were found to have increased risk of relapse, which tended to occur late, and to have increased treatment-related mortality throughout treatment, which resulted in lower event-free survival and overall survival but lower incidence of secondary malignancies compared with other children with ALL. Independent favorable prognostic factors included age younger than 6 years, leukocyte count less than $10^5/L$, high hyperdiploidy, and ETV6-RUNX1 fusion. The lower rates of high hyperdiploidy (9% ± 33%) and ETV6-RUNX1 fusion (8% ± 26%) than that found in other children may contribute partly to the increased risk of relapse in patients with Down syndrome. The low rates of relapse among patients with Down syndrome with ETV6-RUNX1 fusion (3%) and especially those with high hyperdiploidy and trisomies 4 and 10 (0%) suggested that treatment reduction may be warranted for these subgroups of patients. However, one study suggested that insufficient treatment intensity during maintenance therapy (based on median WBC count) could contribute to poor prognosis of patients with Down syndrome. A Dutch Children's Oncology Group (DCOG) study showed that the IKZF1 deletion, which occurred in one third of patients with Down syndrome, was a strong adverse prognostic factor. Because up to 60% of patients with Down syndrome are Ph-like and are characterized by CRLF2 overexpression and JAK-STAT activation, they may potentially benefit from future therapy targeting kinase pathways.

ALL With Induction Failure

Because induction failure occurs in only 2% to 3% of all patients, little was known about the heterogeneity among this group of patients who were generally offered allogeneic transplantation as the treatment of choice. In a large collaborative study, these patients, as expected, were associated with older age, high leukocyte count, T-cell phenotype, Ph-positive ALL, and 11q23 chromosomal rearrangement. Interestingly, although patients with T-cell ALL had improved outcome with matched-related transplantation, children younger than 6 years with B-ALL and no other adverse genetic features had an excellent 10-year survival of 72% ± 5% when treated with chemotherapy alone. The relatively favorable outcome of these patients with B-ALL may be the result of increased sensitivity of the blast cells to methotrexate and mercaptopurine, which are generally not used during remission induction. Thus, this rare subset of patients with B-ALL who readily achieve remission with subsequent consolidation therapy
with high-dose methotrexate and oral mercaptopurine could be spared from transplantation.

**Next-Generation Sequencing Studies**

Genome-wide studies have revolutionized our understanding of the genetic basis of ALL. These studies, using microarray profiling of DNA copy number alterations and gene expression, candidate gene sequencing and, more recently, second-generation sequencing (exome, transcriptome, and/or whole-genome sequencing), have resulted in three major advances: (1) revision of the genetic subclassification of ALL by identifying new subtypes, notably Ph-like, CRLF2-rearranged, ERG-deregulated, intrachromosomal amplification of chromosome 21 (iAMP21), and early T-cell precursor ALL (Fig 1); (2) definition of the constellations of gross and submicroscopic structural genetic alterations and sequence mutations that characterize each ALL subtype; and (3) identification of genomic aberrations or profiles with diagnostic or prognostic implication (eg, IKZF1 alterations) or as targets for therapy (eg, kinase-activating alterations).

Importantly, these studies have enabled comprehensive genomic characterization of the three high-risk subtypes as described in this section.

**IKZF1 alterations in high-risk B-ALL and Ph-like ALL.** In 2009, two groups of investigators using a different gene expression profiling approach identified a subtype of high-risk B ALL that lacked a known chromosomal rearrangement but exhibited a gene expression profile similar to that of Ph-positive ALL with deletions and, less commonly, sequence mutations of IKZF1. A recent study of this entity of Ph-like or BCR-ABL1–like ALL showed that the prevalence increases from 10% of standard-risk childhood ALL to more than one quarter of young adult (age 21 to 39 years) ALL. The genetic basis of Ph-like ALL is complex, including more than 30 rearrangements involving 13 kinase, cytokine, or cytokine-receptor genes, but several key subgroups with therapeutic implications have been identified: (1) ABL-class rearrangements targeting ABL1, ABL2, CSF1R, and PDGFRB, which are sensitive to imatinib and dasatinib; (2) EPOR and JAK2 rearrangements sensitive to JAK inhibitors; (3) CRLF2 rearrangements, frequently with concomitant activating JAK point mutations that may also be sensitive to JAK inhibition; (4) other JAK-STAT activating mutations and deletions, including those involving IL7R, FLT3, SH2B3, and others; and (5) infrequent targets of rearrangement including NTRK3 and others. Patients with Ph-like ALL commonly exhibit high-risk features and have a suboptimal response to therapy, and anecdotal reports are emerging of profound and lasting response to the addition of tyrosine kinase inhibitors to patients with Ph-like ALL with ABL1-class rearrangements. These findings have led to prospective studies to identify Ph-like ALL (by low-density gene expression arrays and/or sequencing) and the underlying rearrangements and to direct patients to logical kinase inhibitor therapy.

**Early T-cell precursor ALL.** By St Jude criteria, early T-cell precursor (ETP) ALL is defined as a T-cell (cytoplasmic CD3+ ) leukemia that lacks expression of markers otherwise observed in T-cell ALL (CD1a, CD8, and weak and/or absent CD5) and has aberrant expression of myeloid or stem-cell markers. ETP ALL lacks a unifying chromosomal alteration, has a low frequency of genetic alterations otherwise common in T-ALL (eg, NOTCH1 mutations), and is associated with MEF2C abnormalities in one European study.

One collaborative study of ETP ALL with whole-genome sequencing identified frequent mutations in three pathways: hematopoietic development (eg, GATA3, IKZF1, RUNX1), cytokine receptor and Ras signaling (eg, IL7R, NRAS, KRAS), and chromatin modification (eg, the polycomb repressor complex 2 and SETD2). Moreover, human ETP ALL cells and a mouse model of Il7r-mutant ETP ALL exhibit JAK-STAT activation that is inhibited by the JAK inhibitor ruxolitinib. These findings raise the prospect of targeted or pathway-directed therapies, such as JAK inhibitors or epigenetic modifiers, to improve the outcome of ETP ALL.

**Hypodiploid ALL.** Until recently, the genetic basis for this high-risk hypodiploid ALL subtype has been poorly understood. A collaborative study of 126 patients with hypodiploid ALL identified distinct submicroscopic alterations in each subtype. Patients with the near-haploid subtype had a high frequency of activating Ras pathway mutations (particularly novel deletions in NF1) and IKZF3 (Aiolos) alterations, and patients with low-hypodiploid IKZF2 (Helios) mutations. A remarkable finding was the near universal presence of TP53 mutations in low-hypodiploid ALL, which were in nontumor cells in more than half of patients and were shown to be inherited in a kindred with other non-ALL tumors. Both near-haploid and low-hypodiploid leukemic cells had activation of the PI3K and Ras-Raf-MEK-ERK pathway.
signaling pathways, with sensitivity to PI3K/mTOR inhibitors, but not MEK inhibitors. These findings have important clinical implications: patients with low-hypodiploid ALL should be tested for TP53 status to enable appropriate surveillance and counseling, and preclinical studies are evaluating the potential for PI3K and MEK inhibitors alone and in combination for hypodiploid ALL.

**Inherited Genetic Determinants of Leukemogenesis**

Children with certain constitutional genetic abnormalities (eg, trisomy 21) are at increased risk of developing ALL, and inherited mutations in TP53 and PAX5 have also been described recently in familial (as well as sporadic) ALL. However, the vast majority of mutations in inherited genetic determinants of leukemogenesis are evaluating the potential for PI3K and MEK inhibitors alone and in combination for hypodiploid ALL.

**Table 3. Genome-Wide Association of Studies of Susceptibility of Childhood ALL**

<table>
<thead>
<tr>
<th>Study Group and Location</th>
<th>Study Population</th>
<th>Sample Size</th>
<th>Susceptibility Loci</th>
<th>OR 95% CI</th>
<th>Comment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>COG, SJC, RJH</td>
<td>Europeans</td>
<td>441</td>
<td>ARID5B (rs10821936) IKZF1 (rs11978267)</td>
<td>1.91 1.6 to 2.2</td>
<td>ARID5B SNP discriminated hyperdiploid ALL from other subtypes of ALL and was associated with accumulation of methotrexate polyglutamates.</td>
<td>Treviño et al62</td>
</tr>
<tr>
<td>UKCCS, MRC</td>
<td>Europeans</td>
<td>907</td>
<td>ARID5B (rs7089424) IKZF1 (rs4132601) CEBPE (rs2239633)</td>
<td>1.65 1.5 to 1.7</td>
<td>ARID5B variant was over-represented in hyperdiploid ALL.</td>
<td>Papaemmanuil et al62</td>
</tr>
<tr>
<td>UK, BFM, Spain, Hungary, Canada</td>
<td>Europeans and European Canadians</td>
<td>3,293</td>
<td>ARID5B IKZF1 CEBPE CDKN2A (rs3731217)</td>
<td>0.71 0.6 to 07</td>
<td>CDKN2A encoded both p16 and p14 and was associated with the development of both B- and T-ALL.</td>
<td>Sherborne et al63</td>
</tr>
<tr>
<td>COG, SJC, RJH</td>
<td>Europeans, African Americans, Hispanic Americans</td>
<td>2,450</td>
<td>ARID5B (rs10821936) IKZF1 (rs11978267) CEBPE (rs4962731) CDKN2A (rs17756311) PIP4K2A (rs7088318) BMI1 (rs474793)</td>
<td>1.86 1.7 to 2.0</td>
<td>ARID5B, BMI1-PIP4K2A, and IKZF1 associations were consistent across ethnicity, and the frequencies of the ARID5B and BMI1-PIP4K2A variants differed in parallel with the ethnic differences in ALL incidence. These variants independently and cumulatively contribute to ALL risk.</td>
<td>Xu et al64</td>
</tr>
<tr>
<td>AIEOP, BFM, CoALL</td>
<td>Europeans</td>
<td>1,370</td>
<td>TP63 (rs17505102) PTPRJ (rs3942852)</td>
<td>0.63 0.5 to 0.7</td>
<td>In addition to IKZF1, DCC, ARID5B, and CEBPE, variants in TP63 (a member of the TP53 gene family) and PTPRJ (a receptor type protein tyrosine phosphatase) were associated with ETV6-RUNX1 ALL.</td>
<td>Ellinghaus et al65</td>
</tr>
<tr>
<td>COG, SJC, RJH</td>
<td>Americans of different ancestry</td>
<td>682</td>
<td>GATA3 (rs3824662)</td>
<td>3.85 2.7 to 5.4</td>
<td>GATA3 was associated with Ph-like ALL and risk of ALL relapse.</td>
<td>Perez-Andreu et al67</td>
</tr>
<tr>
<td>UKCCS, MRC, UKALL, BFM</td>
<td>Europeans</td>
<td>3,107</td>
<td>ARID5B IKZF1 CEBPE CDKN2A PIP4K2A (rs10828317) GATA3 (rs3824662)</td>
<td>1.23 1.1 to 1.3</td>
<td>GATA3 influenced ALL risk and was associated with worse prognosis.</td>
<td>Migliorini et al68</td>
</tr>
</tbody>
</table>

Abbreviations: AIEOP, Associazione Italiana di Ematologia Pediatrica; ALL, acute lymphoblastic leukemia; BFM, Berlin-Frankfurt-Münster; CoALL, Cooperative ALL (study group); COG, Children’s Oncology Group; MRC, Medical Research Council; OR, odds ratio; Ph, Philadelphia chromosome; SJC, SJC, St Jude Children’s Research Hospital; SNP, single nucleotide polymorphism; UK, United Kingdom; UKALL, United Kingdom Acute Lymphoblastic Leukemia (study group); UKCCS, United Kingdom Childhood Cancer Study.
and the COG discovered novel ALL risk variants at the $\text{PIP4K2A-BMI1}$ locus and, for the first time, focused on multiethnic populations.\textsuperscript{64} The inclusion of non-European patients in this study not only improved the statistical power of detecting association signals at the $\text{PIP4K2A}$ variants but also revealed striking differences in the prevalence of these genetic risk variants among groups with different ancestries and their contribution to racial and ethnic differences in the incidence of ALL. Notably, several germline susceptibility loci are targeted by somatic genomic alterations in ALL blasts ($\text{IKZF1}, \text{CDKN2A/CDKN2B}$),\textsuperscript{68} suggesting that inherited and acquired genetic variations should be regarded as a continuum in the development of childhood ALL.

GWASs of susceptibility to specific ALL subtypes have received increasing attention. In 2012, a collaborative study reported that variants in the $\text{TP63}$ and $\text{PTPRJ}$ genes were associated with the acquisition of the $\text{ETV6-RUNX1}$ fusion.\textsuperscript{69} More recently, inherited variants in the $\text{GATA3}$ gene were associated with the development of Ph-like ALL and also the increased risk of relapse in two separate studies,\textsuperscript{65,67} illustrating interactions between genetic variations in the host and those in the cancer cells and their unique contributions to pathogenesis and treatment outcomes of ALL.

GWASs have been successful in unequivocally establishing an inherited genetic basis for ALL susceptibility with seven risk loci discovered thus far (Table 3). Future studies will require even more extensive collaboration to characterize the role of germline genetic variations in uncommon ALL phenotypes and to characterize the combined effects of multiple variants acting within the same pathway.\textsuperscript{69}

### Genomic Determinants of Drug Toxicity

Pharmacokinetic, pharmacodynamic, and pharmacogenomic studies have shown important determinants of interpatient differences in treatment response that can affect clinical outcome. Recent technologies for interrogating inherited and acquired genome variations have accelerated the discovery of genomic determinants of de novo drug resistance. Initially, by using genome-wide gene expression arrays, the expression (mRNA) of a relatively small number of genes in tumor cells was linked to de novo sensitivity of glucocorticoids,\textsuperscript{70} multidrug cross-resistance,\textsuperscript{71} in vivo response to initial treatment with high-dose methotrexate,\textsuperscript{72} and inherited genome variants related to response to remission induction therapy.\textsuperscript{73} Indeed, both germline and somatic genome variation can influence the effects of ALL chemotherapy,\textsuperscript{74-78} which emphasizes the importance of assessing both inherited and somatic genome variation in cancer pharmacogenomics. Furthermore, genome-wide mRNA expression analyses have identified genome variation that influences the accumulation of the active polyglutamyalted metabolites of methotrexate in ALL cells after in vivo methotrexate treatment.\textsuperscript{79,80} These studies and others have provided mechanistic insights into the need for different dosages of methotrexate in certain ALL subtypes (eg, higher dosage in T-ALL) and have revealed drug-specific differences in genomic determinants of de novo drug resistance in childhood ALL. These findings also hold promise for developing strategies to mitigate drug resistance by targeting proteins whose overexpression or enhanced function leads to resistance to conventional antileukemic agents. Ongoing genome-wide interrogation of germline and somatic DNA variation (genetic and epigenetic) should lead to greater insights into the mechanism of drug resistance and yield new treatment strategies to further enhance the effectiveness of ALL treatment.

### Genomic Determinants of Drug Toxicities

#### Table 4. Selected Examples of Genomic Determinants of Drug Toxicities Involving Multiple Collaborative Study Groups

<table>
<thead>
<tr>
<th>Drug</th>
<th>Gene Name</th>
<th>Comments</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asparaginase</td>
<td>$\text{HLA-DRB1}$*$\text{07:01}$</td>
<td>$\text{HLA-DRB1}*$07:01-encoded protein is associated with the development of anti-asparaginase antibody and a high frequency of asparaginase hypersensitivity, probably through its high binding affinity for asparaginase epitopes.</td>
<td>Fernandez et al\textsuperscript{81}</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>$\text{SLCO1B1}$</td>
<td>Methotrexate clearance is associated with polymorphisms of $\text{SLCO1B1}$ which encode a hepatic solute carrier organic anion transporter that mediates disposition of many medications, including methotrexate.</td>
<td>Trevino et al,\textsuperscript{75} Ramsey et al,\textsuperscript{77} Ramsey et al\textsuperscript{82}</td>
</tr>
<tr>
<td>Mercaptopurine,</td>
<td>$\text{TPMT}$</td>
<td>Genetic polymorphisms in $\text{TPMT}$ (thiopurine S-methyltransferase) are known to have a marked effect on mercaptopurine metabolism and myelosuppressive toxicity.</td>
<td>Ramsey et al,\textsuperscript{82} Lennard et al,\textsuperscript{83} Stannulla et al,\textsuperscript{84} Schmiegelow et al,\textsuperscript{85} Relling et al,\textsuperscript{86} Evans et al\textsuperscript{87}</td>
</tr>
<tr>
<td></td>
<td>$\text{NUDT15}$</td>
<td>A polymorphism of $\text{NUDT15}$ is associated with mercaptopurine intolerance and is most common in East Asians and Hispanics.</td>
<td>Yang et al\textsuperscript{88}</td>
</tr>
<tr>
<td>Vincristine</td>
<td>$\text{CEP72}$</td>
<td>A polymorphism of $\text{CEP72}$ reduces expression of its encoded centrosomal protein 72 kD, which functions as the major microtubule-organizing center, regulates proper bipolar spindle formation, and is associated with an increased risk of vincristine-induced neuropathy.</td>
<td>Diouf et al\textsuperscript{89}</td>
</tr>
</tbody>
</table>

### Genomic Determinants of Drug Toxicities

Many serious toxicities of ALL therapy are relatively uncommon, and they vary substantially in prevalence by treatment regimen. Although genome-wide approaches have shown tremendous potential for uncovering novel mechanisms and new risk factors of drug toxicities, large sample sizes from collaborations are necessary to have adequate power to uncover underlying genomic determinants with hazard ratios on the order of 1.5-fold to three-fold. Indeed, collaborations have already identified inherited genomic variation associated with asparaginase allergy,\textsuperscript{81} and with high-dose methotrexate clearance and toxicity (Table 4).\textsuperscript{75,82} Other examples of inherited genomic variation that contribute to ALL toxicity phenotypes include the association of $\text{TPMT}$ variant,\textsuperscript{83-87} and an $\text{NUDT15}$ variant\textsuperscript{88} with thiopurine-induced myelosuppression; $\text{CEP72}$ polymorphism with vincristine-related peripheral neuropathy;\textsuperscript{89} $\text{CRHR1}$ polymorphisms and steroid-induced low bone density, which was replicated in a pediatric asthma population;\textsuperscript{90} several genes associated with osteonecrosis;\textsuperscript{91,92} and methotrexate-induced leukoencephalopathy.\textsuperscript{93}
The underlying genomic risk factors for some adverse effects are likely to differ by treatment regimen. For example, asparaginase may potentiate the osteonecrosis risk of glucocorticoids, partly via effects on lipid homeostasis, and partly by affecting glucocorticoid disposition. Dosage may also play a critical role; for example, anthracycline-induced cardiomyopathy is related to inherited variants in HLA, but only in patients who received higher cumulative doses of anthracycline. By contrast, genetic risk factors for vincristine neuropathy are apparent only at lower doses of the drug, because at higher doses, almost all patients are at risk for the adverse effect.

CONCLUSIONS AND FUTURE DIRECTIONS

Collaborative group studies have refined the molecular classification of ALL and have substantially improved strategies for personalized treatment by identifying new targets for therapeutic intervention. These collaborative studies should continue to address many unresolved issues and questions. Next-generation sequencing studies to analyze the genome, transcriptome, and epigenome of patients will comprehensively identify all genetic variations contributing to leukemogenesis and treatment outcome; interpreting the complex protein-protein and pathway interactions will be the challenge. Detailed analysis of germline genetic variations will unravel the role of inherited polymorphisms in leukemia susceptibility and the acquisition of somatic genetic abnormalities. Experimental models will be developed to faithfully recapitulate the human leukemias, and novel, rationally targeted therapeutic approaches to reverse or mitigate the driver mutations with small molecules will become available for most patients. Effective immunotherapy and cellular therapy will be developed, especially for patients with no known genetic lesions that are responsive to available molecular-targeted therapy. Collaborative clinical trials will be initiated for patients with challenging or drug-resistant leukemia. These efforts will be extended beyond the current study groups and consortia that investigate patients residing mainly in the United States (eg, Therapeutic Advances in Childhood Leukemia & Lymphoma Consortium) and Europe (eg, International Study for Treatment of Childhood Relapsed ALL Consortium). The VIVA-Asia Acute Leukemia group has recently been formed to collaborate on and address the topics relevant to patients in Asia, which not only has the largest patient population but also has different leukemia subtypes, environmental characteristics, population genetics, and resources.

Historically, older adolescents and young adults had poorer treatment outcomes than young children and received little research attention. Because pediatric-based regimens improved outcome for this age group, pediatric and adult oncologists have started to form consortia to focus on the unique clinical, biologic, psychosocial, and survivorship of this age group. There is a great hope and expectation that their treatment outcomes will dramatically improve in the coming years.

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