Biology, Risk Stratification, and Therapy of Pediatric Acute Leukemias: An Update
Ching-Hon Pui, William L. Carroll, Soheil Meshinchi, and Robert J. Arceci

ABSTRACT

Purpose
We review recent advances in the biologic understanding and treatment of childhood acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML), identify therapeutically challenging subgroups, and suggest future directions of research.

Methods
A review of English literature on childhood acute leukemias from the past 5 years was performed.

Results
Contemporary treatments have resulted in 5-year event-free survival rates of approximately 80% for childhood ALL and almost 60% for pediatric AML. The advent of high-resolution genome-wide analyses has provided new insights into leukemogenesis and identified many novel subtypes of leukemia. Virtually all ALL and the vast majority of AML cases can be classified according to specific genetic abnormalities. Cooperative mutations involved in cell differentiation, cell cycle regulation, tumor suppression, drug responsiveness, and apoptosis have also been identified in many cases. The development of new formulations of existing drugs, molecularly targeted therapy, and immunotherapies promises to further advance the cure rates and improve quality of life of patients.

Conclusion
The application of new high-throughput sequencing techniques to define the complete DNA sequence of leukemia and host normal cells and the development of new agents targeted to leukemogenic pathways promise to further improve outcome in the coming decade.


INTRODUCTION

The treatment outcome for children with acute myeloid leukemia (AML) and especially acute lymphoblastic leukemia (ALL) has improved substantially with the use of risk-directed treatment and improved supportive care. The 5-year event-free survival rates for ALL now range between 76% and 86% in children receiving protocol-based therapy in the developed countries (Table 1),1-14 and those for AML range between 49% and 63% in some of the more successful clinical trials (Table 2).15-30 The improved treatment has diminished or eliminated the impact of many conventional prognostic factors in ALL, such as male sex and black race.6,10 Thus current ALL trials have focused on improving not only the outcome of a few subtypes that remain refractory to treatment (eg, infant ALL with MLL rearrangement, hypodiploid ALL, and poor early responders), but also the quality of life of the patients. By contrast, with the exception of core-binding factor (CBF) leukemias [(t(8;21)(AML1-ETO)] and inv(16)(CBFB-MYH11)] and AML in Down syndrome, most of the AML cases continue to pose a therapeutic challenge.

Childhood acute leukemias have long been the best characterized malignancies from a genetic viewpoint. Despite remarkable progress in cataloging the molecular lesions, our understanding of how such changes cooperate to produce overt leukemia or to induce drug resistance is still rudimentary. Recent genome-wide studies have begun to enlighten our understanding of leukemogenesis and prognosis and, in some instances, to stimulate the development of target therapy. Because of the space constraints, we review here only some of the most recent advances in the biology and treatment of childhood leukemias.

MOLECULAR GENETICS OF ALL

Standard genetic analyses can detect primary genetic abnormalities in more than 75% of the ALL cases, but they cannot identify the full repertoire of the genetic alterations.31 The advent of high-resolution
The prevalence of this subtype of leukemia, suggesting that additional postnatal mutations are necessary for malignant transformation.31

### Genomic Analysis of ALL

Primary somatic genetic abnormalities have important prognostic and therapeutic implications and play a critical role in leukemogenesis (Table 3).10,31,34-58 However, experimental models have established that cooperative mutations are necessary to induce leukemia and contribute to the development of drug resistance. Although high-throughput analyses of global gene expression have identified distinct subgroups and may in the future be used to stratify patients, these profiles do not distinguish pathways that are “drivers” of leukemogenesis from “passengers.” Using single-nucleotide polymorphism (SNP) array, Mullighan et al34 identified an average of six lesions per case.34 These findings are compatible with the known behavior of these leukemias. MLL-rearranged leukemias often present during infancy and have a concordance rate close to 100% in identical twins, indicating in utero development and transplacental metastasis from one fetus to the other.31 By contrast, ETV6–RUNX1 leukemias present after infancy and have a concordance rate of only 10% in identical twins, and this gene fusion can be found in as many as 1% of normal newborn babies, a frequency 100 times higher than the prevalence of this subtype of leukemia, suggesting that additional postnatal mutations are necessary for malignant transformation.31

### Genetic Determinants of High-Risk B-Cell Precursor ALL

In Philadelphia chromosome-positive ALL with constitutively active BCR-ABL1 tyrosine kinase, IKZF1 (encoding the lymphoid transcription factor IKAROS) is deleted in approximately 80% of the cases.31 Interestingly, there is a high-risk subgroup of BCR-ABL1-negative ALL that is characterized by IKZF1 deletion and has a genetic profile similar to that of cases with BCR-ABL1 fusion.46,47 In search of activated tyrosine kinase signaling in this leukemic subtype, Mullighan et al48 identified activating mutations in the Janus kinases (JAK1, JAK2, and JAK3) in approximately 10% of high-risk BCR-ABL1-negative cases. The presence of JAK mutations was associated with alteration of IKZF1 and deletion of CDKN2A/B. Recent studies showed CRLF2 over-expression (predominantly resulting from P2RY8–CRLF2 fusion or IG/H–CRLF2 rearrangement) in 6% to 7% of B-cell precursor ALL cases and strikingly in 50% to 60% of patients with Down syndrome–associated ALL, all of whom lacked recurring translocations commonly associated with non–Down syndrome ALL.40-42,52 CRLF2 alteration was associated with activating mutations of JAK1 or JAK2; these two genetic lesions together resulted in constitutive Jak-Stat activation and the growth of cytokine-dependent mouse B-progenitor cell lines in the absence of exogenous cytokine, indicating that they are cooperative mutations in leukemogenesis.40 Importantly, CRLF2 alteration was associated with Hispanic/Latino ethnicity and a poor treatment outcome.46,41

### Genomic Analysis of T-Cell ALL

On the basis of gene expression profiling, T-cell ALL cases can be classified into several distinct genetic subgroups that correspond to specific T-cell development stages: HOX11L2, LYL1 plus LMO2, TAL1...
Historically, ALL has been categorized based on the age of the patient, with T-cell ALL generally conferring a poor outcome, whereas B-cell ALL is more treatable. However, the B-cell ALL subtype is further divided into hyperdiploid (≥50 chromosomes) and non-hyperdiploid ALL (≤49 chromosomes). Hyperdiploid ALL is associated with a favorable prognosis, and the presence of specific translocations, such as MLL-AF4, is a consistent feature. Fusion of MLL to AF4 has been associated with responsiveness to tyrosine kinase inhibition.43,44,49

Association with responsiveness to tyrosine kinase inhibition.43,44,49

Inherited Susceptibility to ALL

Although candidate gene approaches have implicated inherited polymorphisms of several genes in leukemogenesis, the findings have not been consistent. Two recent genome-wide studies on patients of European ancestry failed to confirm these previously reported gene associations but independently identified germline polymorphisms of the IKZF1 gene and ARID5B gene to be associated with an increased risk of childhood ALL.57,58 The risk alleles of ARID5B, a gene that belongs to a family of transcription factors important in embryonic development, cell type–specific gene expression, and cell growth regulation, were specifically enriched in patients with hyperdiploid ALL and were also associated with greater methotrexate polyglutamate accumulation.57,58 Thus the same genetic variation of ARID5B that predisposes to the development of hyperdiploid ALL may also underlie the superior response of this subtype of ALL to chemotherapy. A subsequent study was performed in patients of African ancestry, showing that ARID5B germline polymorphisms were also associated with the risk of developing hyperdiploid ALL in black patients.59 The lower frequency of this risk allele in the control populations of African ancestry compared with that of European ancestry might also partly

### Table 2. Characteristics and Treatment Results From Selected Clinical Trials for Childhood AML

<table>
<thead>
<tr>
<th>Study</th>
<th>Years of Study</th>
<th>No. of Patients</th>
<th>Early Deaths (%)</th>
<th>CR Rate (%)</th>
<th>Time of CR Evaluation</th>
<th>Anthracyclines</th>
<th>Cytarabine (g/m²)</th>
<th>Etoposide (g/m²)</th>
<th>5-Year EFS (%)</th>
<th>5-Year OS (%)</th>
<th>Data Source (first author)</th>
</tr>
</thead>
<tbody>
<tr>
<td>POG 8821</td>
<td>1988-1993</td>
<td>511</td>
<td>3.9</td>
<td>77</td>
<td>2 courses</td>
<td>360</td>
<td>56.7</td>
<td>2.25</td>
<td>31</td>
<td>32</td>
<td>40.4</td>
</tr>
<tr>
<td>CCG 2991</td>
<td>1985-1995</td>
<td>750</td>
<td>4</td>
<td>77</td>
<td>2 courses</td>
<td>350</td>
<td>28.3</td>
<td>1.9</td>
<td>34</td>
<td>43</td>
<td>45</td>
</tr>
<tr>
<td>MRC-AAML 10</td>
<td>1986-1995</td>
<td>303</td>
<td>4</td>
<td>93</td>
<td>4 courses</td>
<td>550</td>
<td>10.6</td>
<td>0.5-1.5</td>
<td>49</td>
<td>58</td>
<td>68</td>
</tr>
<tr>
<td>FIPNA-92</td>
<td>1992-1998</td>
<td>151</td>
<td>21</td>
<td>74</td>
<td>Not specified</td>
<td>350</td>
<td>7.6</td>
<td>0.45</td>
<td>36</td>
<td>45</td>
<td>37</td>
</tr>
<tr>
<td>LAME-91</td>
<td>1991-1998</td>
<td>247</td>
<td>6.4</td>
<td>91</td>
<td>2 courses</td>
<td>460</td>
<td>9.8-13.4</td>
<td>0.4</td>
<td>48</td>
<td>43</td>
<td>62</td>
</tr>
<tr>
<td>TCCSG M91-13/M98-14</td>
<td>1991-1998</td>
<td>192</td>
<td>3.6</td>
<td>88</td>
<td>Not specified</td>
<td>495</td>
<td>69.4-99.4</td>
<td>3.75-6.75</td>
<td>56</td>
<td>62</td>
<td>Tomizawa77</td>
</tr>
<tr>
<td>BFM-93</td>
<td>1993-1997</td>
<td>427</td>
<td>7.4</td>
<td>82</td>
<td>4 courses</td>
<td>300-400</td>
<td>23.41</td>
<td>0.95</td>
<td>50</td>
<td>52</td>
<td>57</td>
</tr>
<tr>
<td>CCG 2961</td>
<td>1996-1999</td>
<td>901</td>
<td>6</td>
<td>83</td>
<td>2 courses</td>
<td>360</td>
<td>15.2-19.6</td>
<td>1.6</td>
<td>42</td>
<td>3</td>
<td>52</td>
</tr>
<tr>
<td>EORTC-CLG 88912</td>
<td>1993-2000</td>
<td>166</td>
<td>1</td>
<td>84</td>
<td>2 courses</td>
<td>380</td>
<td>23.28</td>
<td>1.35</td>
<td>49</td>
<td>4</td>
<td>62</td>
</tr>
<tr>
<td>GATLA-AKL-90</td>
<td>1993-2000</td>
<td>179</td>
<td>20</td>
<td>70</td>
<td>Not specified</td>
<td>300</td>
<td>41.1</td>
<td>4.15</td>
<td>31</td>
<td>41</td>
<td>44</td>
</tr>
<tr>
<td>AIEOP LAM-92</td>
<td>1992-2001</td>
<td>161</td>
<td>6</td>
<td>89</td>
<td>2 courses</td>
<td>Not provided</td>
<td>Not provided</td>
<td>Not provided</td>
<td>54</td>
<td>60</td>
<td>64</td>
</tr>
<tr>
<td>NOPHO-AKL 93</td>
<td>1993-2001</td>
<td>223</td>
<td>2</td>
<td>92</td>
<td>3 courses</td>
<td>300-375</td>
<td>49.6-61.3</td>
<td>1.6</td>
<td>50</td>
<td>3</td>
<td>66</td>
</tr>
<tr>
<td>MRC-AAML 12</td>
<td>1994-2002</td>
<td>455</td>
<td>4</td>
<td>92</td>
<td>4 courses</td>
<td>300-610</td>
<td>4.6-34.6</td>
<td>1.5</td>
<td>56</td>
<td>66</td>
<td>Burmeister50/Gibson57</td>
</tr>
<tr>
<td>AML99</td>
<td>2000-2002</td>
<td>268</td>
<td>1.7</td>
<td>94</td>
<td>4 courses</td>
<td>300-375</td>
<td>50.7-64.6</td>
<td>3.15-3.2</td>
<td>61</td>
<td>3</td>
<td>75</td>
</tr>
<tr>
<td>BFM-98</td>
<td>1998-2003</td>
<td>473</td>
<td>3</td>
<td>88</td>
<td>4 courses</td>
<td>420</td>
<td>41.7-47.5</td>
<td>0.95</td>
<td>49</td>
<td>3</td>
<td>62</td>
</tr>
<tr>
<td>SJCRH AML-92</td>
<td>1992-2008</td>
<td>230</td>
<td>1</td>
<td>94</td>
<td>2 courses</td>
<td>300-550</td>
<td>34-48</td>
<td>1.15</td>
<td>63</td>
<td>4</td>
<td>71</td>
</tr>
<tr>
<td>COG AAML03P1§¶</td>
<td>2003-2005</td>
<td>350</td>
<td>2.6</td>
<td>83</td>
<td>2 courses</td>
<td>300-480</td>
<td>21.6-45.6</td>
<td>1.15</td>
<td>53</td>
<td>6</td>
<td>66</td>
</tr>
</tbody>
</table>

Abbreviations: AML, acute myeloid leukemia; CR, complete response; EFS, event-free survival; OS, overall survival; POG, Pediatric Oncology Group; CCG, Children’s Cancer Group; MRC-AAML, United Kingdom’s Medical Research Council Acute Myelogenous Leukemia study; PINDA, National Program for Antineoplastic Drugs for Children; LAME, Leucemie Acute Myeloblastique Enfant; TCCSG, Tokyo Children’s Cancer Study Group; BFM, Berlin-Frankfurt-Münster; EORTC-CLG, European Organisation for Research and Treatment of Cancer Children’s Leukemia Group; GATLA-AML, Argentine Group for the Treatment of Acute Leukemia; AIEOP LAM, Associazione Italiana di Ematologia ed Oncologia Pediatrica Leucemia Acuta Mieloide; NOPHO-AML, Nordic Society of Pediatric Hematology and Oncology—Acute Myeloid Leukemia; SJCRH, St Jude Children’s Research Hospital; COG, Children’s Oncology Group.

§Results are reported for only those trials that had ≥ 150 patients and information provided for each of the column headings. The AIEOP LAM-92 did not allow definitive dose calculations of drugs because consolidation therapy was given based on the treating physician’s judgement, but usually included anthracyclines, etoposide, and high-dose cytarabine.

ΠNo. of patients excludes patients with Down syndrome.

†Ages include patients from 0 to 1 and including age 15 years: BFM-98 included patients from 0 to less than 17 years of age; CCG-2961 included patients from 0 to < 21 years of age.

‡Anthracycline conversions were according to daunorubicin equivalents, including idarubicin 5x, mitoxantrone 5x, doxorubicin 1x. Another conversion factor for daunorubicin and mitoxantrone that has been used is 3x.

||LAME-91 also used 450 mg/m² of amsacrine; MRC-10 and MRC-12 both also included 500 mg/m² of amsacrine.

#COG AAML03P1 enrolled patients ≥ 1 month and ≤ 21 years of age.

### Genetic Determinants of Relapse in ALL

To explore the genetic basis of relapse, genome-wide studies were conducted using matched diagnosis and relapse samples from the same patients.43,55 Although 90% of the cases exhibited differential CNAs (gaining or losing genetic lesions) from diagnosis to relapse, most relapse samples are donor-related to diagnosis samples, and backtracking studies showed that the relapse clones were often present as minor populations at diagnosis, suggesting that they were selected during treatment. Notably, many of the genetic alterations that emerge in the predominant clone at relapse involve genes that have been implicated in treatment resistance (eg, CDKN2A/B, IKZF1).43,55

Interestingly, some cases had focal deletion of MSH6, reduced expression of which was associated with resistance to mercaptopurine and prednisone, providing another plausible mechanism of drug resistance at relapse.43 Parallel gene expression studies, which have identified a proliferative gene signature that emerges at relapse and consistent upregulation of genes such as survivin, provide attractive targets for novel therapeutic intervention.56

### Inherited Susceptibility to ALL

Although candidate gene approaches have implicated inherited polymorphisms of several genes in leukemogenesis, the findings have not been consistent. Two recent genome-wide studies on patients of European ancestry failed to confirm these previously reported gene associations but independently identified germline polymorphisms of the IKZF1 gene and ARID5B gene to be associated with an increased risk of childhood ALL.57,58 The risk alleles of ARID5B, a gene that belongs to a family of transcription factors important in embryonic development, cell type–specific gene expression, and cell growth regulation, were specifically enriched in patients with hyperdiploid ALL and were also associated with greater methotrexate polyglutamate accumulation.57,58 Thus the same genetic variation of ARID5B that predisposes to the development of hyperdiploid ALL may also underlie the superior response of this subtype of ALL to chemotherapy. A subsequent study was performed in patients of African ancestry, showing that ARID5B germline polymorphisms were also associated with the risk of developing hyperdiploid ALL in black patients.59 The lower frequency of this risk allele in the control populations of African ancestry compared with that of European ancestry might also partly
Fig 1. Estimated frequency of specific genotypes in childhood leukemias. (A) Genetic abnormalities in acute lymphoblastic leukemia (ALL). Data were modified from Pui et al. by including recently identified genotypes. The genetic lesions that are exclusively seen in cases of T-cell ALL are indicated in purple. (B) Genetic abnormalities in acute myeloid leukemia (AML). Panel to the left demonstrates the most common karyotypic alterations. Eighty percent of all children have disease-associated genomic structural alterations. Mutation profile in those without cytogenetic abnormalities (normal karyotype) is shown in the right panel. Seventy-six percent of those in the normal karyotype population have one of the known mutations; thus, more than 95% of all children with AML have at least one known genomic abnormality. (With permission from Reaman and Smith).
explain the lower incidence of hyperdiploid B-cell precursor ALL in the black population.

**CELLULAR AND MOLECULAR ORIGINS OF AML**

Despite the extremely heterogeneous nature of AML, the various subtypes seem to share some common pathways leading to leukemogenesis, and the hierarchical nature of the disease is generally well established. Several lines of evidence have led to a model that AML arises from the cooperation between two classes of genetic alterations that regulate self-renewal and differentiation. For example, mutations or epigenetic alterations involving *CBF* or *MLL* genes, termed type II alterations, play key roles in modifying the ability of precursor cells to differentiate, but are usually insufficient by themselves to generate AML. In support of this concept is the finding that the *AML1-ETO* fusion transcript resulting from the t(8;21) translocation can be detected in neonatal blood from teenagers with AML characterized by this translocation. Such fusion transcripts have also been identified to persist in the bone marrow of some patients in long-term morphologic remission. When a cooperative mutation or type I mutation (such as *FLT/ITD* or *c-KIT* point mutations) occurs, a proliferative signal is provided, leading to overt AML. That type II mutations such as *AML-ETO* can be detected far more frequently in neonatal samples than the incidence of leukemia further supports the concept of cooperating mutations in leukemogenesis. Genomewide studies have identified additional genetic or epigenetic changes that lead to type I and II mutations. Currently, more than 90% of pediatric AML cases are identified to have at least one known genomic alteration (Fig 1B).

**Altered Transcription and Chromatin-Modifying Factors**

Similar to ALL, many of the chromosomal abnormalities in AML result in alteration of the function of transcription factors critical for normal hematopoiesis and have diagnostic and
therapeutic implications (as discussed in Risk-Adapted Treatment of AML). The t(8;21)(q22;q22) and the inv(16)(p13q22) involving CBFA2 and β subunits respectively account for about a quarter of all cases. These translocations impair CBF transcriptional activation potential. Less common translocations include the t(15;17), 11q23/MLL rearrangements and the t(1;22). The t(15;17) generated PML-RARA fusion protein is resistant to physiologic concentrations of retinoic acid but sensitive to pharmacologic levels of all-trans-retinoic acid (ATRA), which destabilize the repressor complex, allowing for the expression of genes permissive for differentiation and making this subtype the first successfully treated acute leukemia by molecular approaches. The initial whole-genome sequencing of an adult with M1 AML (COG) trials are using a mutant to wild-type allelic ratio of ≥ 0.4 as a criterion for allogeneic hematopoietic stem-cell transplantation (HSCT) in first remission. Point mutations in the activating loop of the receptor (FLT3/ALM) also result in constitutively activated FLT3 but do not confer a poor prognosis.73 This finding may be in part due to differences in signaling pathway activation by the different forms of FLT3. FLT3/ALM occurs in only 6% to 8% of children with AML and is mutually exclusive of FLT3-ITD.73

Activating mutations of the c-KIT receptor also result in cytokine-independent proliferation, survival, and drug resistance. Such mutations preferentially activate STAT3 signaling and appear to be clustered with CBF abnormalities, occurring in up to 25% of CBF AML cases but in only ≤ 5% of overall childhood AML.74,77 In contrast to initial reports,74,75 recent studies failed to demonstrate c-KIT mutations to have adverse prognostic impact.78 Mutations involving EFS and PDGFR, class III type of tyrosine kinases, have been reported in adults but not in children with hematologic malignancies.

N-RAS has been reported to be mutated in approximately 10% of children and up to 30% of adults with AML.29 These activating mutations most frequently involve single-base changes of codons 12, 13, and 61, resulting in inhibition of RAS-GTPase. No clinical significance has been associated with mutations in RAS in pediatric AML, and RAS-directed therapies have not been successful thus far.80 However, the recent application of synthetic lethal screening has started to reveal some alternative pathways that could be therapeutically targeted.81 NPM1 mutations are associated with a favorable prognosis. They appear more frequently in AML with normal karyotypes and up to 20% of childhood and 50% of adult cases.82,83 NPM1 mutations seem to be present in leukemia-initiating cell populations and are usually maintained at relapse, suggesting they may represent a primary oncogenic event.84,85

Gene Mutations

Biallelic mutations in CEBPA, a key leucine zipper containing transcription factor regulating differentiation of several cell types, including myeloid precursors, have been identified in approximately 4% of the cases.70 The end result of such biallelic mutations is often that of a null phenotype or loss of function.71 WT1, originally described as a key oncogene in Wilms tumor, functions in non-nephrogenic tissues, including hematopoietic stem cells. Increased expression of WT1 has been used as a surrogate marker for minimal residual disease (MRD) in AML. Inactivating WT1 mutations were reported in 8% to 12% of patients with AML, and their prognostic significance remains uncertain and is likely treatment-dependent.

GATA1 is critical in regulating the differentiation of hematopoiesis, particularly for the erythroid and megakaryocyte lineages. Mutations leading to truncated forms of GATA1 are primarily observed in the AMKL or the megakaryoblastic transient myeloproliferative disease that occur in children with Down syndrome.72 Although not sufficient by themselves in causing leukemia, these GATA1 mutations have been established as a first genetic hit in these disorders and have even been detected prenatally.

Activating mutations of several cytokine receptors have been shown to result in altered signal transduction and increased leukemia cell proliferation, survival, and chemotherapeutic resistance. FLT3-ITD, which involves a duplication of the internal juxtamembrane domain leading to constitutive receptor activation, is rare in infants but is present in 5% to 10% of 5- to 10-year-old patients, 20% of young adult patients, and more than 35% of patients older than 55 years of age with AML.73 An increased FLT3-ITD mutant to wild-type allelic ratio portends a poor prognosis.74,75 Children's Oncology Group

Recent genome-wide microarray studies and direct-sequencing approaches have revealed important sub-karyotypic abnormalities. One important finding has been the high percentage of acquired uniparental disomy, resulting in homozygosity of chromosomal regions that occur after the acquisition of a mutation in one allele.56,67 Examples of this have included FLT3-ITD and CEBPA mutations.68,69

Gene Mutations

Biallelic mutations in CEBPA, a key leucine zipper containing transcription factor regulating differentiation of several cell types, including myeloid precursors, have been identified in approximately 4% of the cases.70 The end result of such biallelic mutations is often that of a null phenotype or loss of function.71 WT1, originally described as a key oncogene in Wilms tumor, functions in non-nephrogenic tissues, including hematopoietic stem cells. Increased expression of WT1 has been used as a surrogate marker for minimal residual disease (MRD) in AML. Inactivating WT1 mutations were reported in 8% to 12% of patients with AML, and their prognostic significance remains uncertain and is likely treatment-dependent.

GATA1 is critical in regulating the differentiation of hematopoiesis, particularly for the erythroid and megakaryocyte lineages. Mutations leading to truncated forms of GATA1 are primarily observed in the AMKL or the megakaryoblastic transient myeloproliferative disease that occur in children with Down syndrome.72 Although not sufficient by themselves in causing leukemia, these GATA1 mutations have been established as a first genetic hit in these disorders and have even been detected prenatally.

Activating mutations of several cytokine receptors have been shown to result in altered signal transduction and increased leukemia cell proliferation, survival, and chemotherapeutic resistance. FLT3-ITD, which involves a duplication of the internal juxtamembrane domain leading to constitutive receptor activation, is rare in infants but is present in 5% to 10% of 5- to 10-year-old patients, 20% of young adult patients, and more than 35% of patients older than 55 years of age with AML.73 An increased FLT3-ITD mutant to wild-type allelic ratio portends a poor prognosis.74,75 Children's Oncology Group

Gene Mutations

Biallelic mutations in CEBPA, a key leucine zipper containing transcription factor regulating differentiation of several cell types, including myeloid precursors, have been identified in approximately 4% of the cases.70 The end result of such biallelic mutations is often that of a null phenotype or loss of function.71 WT1, originally described as a key oncogene in Wilms tumor, functions in non-nephrogenic tissues, including hematopoietic stem cells. Increased expression of WT1 has been used as a surrogate marker for minimal residual disease (MRD) in AML. Inactivating WT1 mutations were reported in 8% to 12% of patients with AML, and their prognostic significance remains uncertain and is likely treatment-dependent.

GATA1 is critical in regulating the differentiation of hematopoiesis, particularly for the erythroid and megakaryocyte lineages. Mutations leading to truncated forms of GATA1 are primarily observed in the AMKL or the megakaryoblastic transient myeloproliferative disease that occur in children with Down syndrome.72 Although not sufficient by themselves in causing leukemia, these GATA1 mutations have been established as a first genetic hit in these disorders and have even been detected prenatally.

Activating mutations of several cytokine receptors have been shown to result in altered signal transduction and increased leukemia cell proliferation, survival, and chemotherapeutic resistance. FLT3-ITD, which involves a duplication of the internal juxtamembrane domain leading to constitutive receptor activation, is rare in infants but is present in 5% to 10% of 5- to 10-year-old patients, 20% of young adult patients, and more than 35% of patients older than 55 years of age with AML.73 An increased FLT3-ITD mutant to wild-type allelic ratio portends a poor prognosis.74,75 Children's Oncology Group

Gene Mutations

Biallelic mutations in CEBPA, a key leucine zipper containing transcription factor regulating differentiation of several cell types, including myeloid precursors, have been identified in approximately 4% of the cases.70 The end result of such biallelic mutations is often that of a null phenotype or loss of function.71 WT1, originally described as a key oncogene in Wilms tumor, functions in non-nephrogenic tissues, including hematopoietic stem cells. Increased expression of WT1 has been used as a surrogate marker for minimal residual disease (MRD) in AML. Inactivating WT1 mutations were reported in 8% to 12% of patients with AML, and their prognostic significance remains uncertain and is likely treatment-dependent.

GATA1 is critical in regulating the differentiation of hematopoiesis, particularly for the erythroid and megakaryocyte lineages. Mutations leading to truncated forms of GATA1 are primarily observed in the AMKL or the megakaryoblastic transient myeloproliferative disease that occur in children with Down syndrome.72 Although not sufficient by themselves in causing leukemia, these GATA1 mutations have been established as a first genetic hit in these disorders and have even been detected prenatally.

Activating mutations of several cytokine receptors have been shown to result in altered signal transduction and increased leukemia cell proliferation, survival, and chemotherapeutic resistance. FLT3-ITD, which involves a duplication of the internal juxtamembrane domain leading to constitutive receptor activation, is rare in infants but is present in 5% to 10% of 5- to 10-year-old patients, 20% of young adult patients, and more than 35% of patients older than 55 years of age with AML.73 An increased FLT3-ITD mutant to wild-type allelic ratio portends a poor prognosis.74,75 Children's Oncology Group
the CEBPA promoter and another characterized by CEBPA mutations illustrates the critical role that epigenetic changes play in AML.94 Of further interest, the distinctive myeloid/T-lymphoid characteristics of this subtype have been linked to the finding that decreased CEBPA expression leads to increased expression of T-lineage genes in hematopoietic precursors.94,95 On a genome-wide scale, altered CpG methylation profiles have been associated with distinct subsets of adult AML.96 A 15-gene methylation classifier has been reported to have prognostic significance.97

Inherited Susceptibility of AML

Several inherited syndromes associated with an increased incidence of AML have been instrumental in demonstrating the importance of key molecular pathways in the development of somatically acquired AML.98,99 Transient myeloproliferative disease and AMKL in patients with Down syndrome are characterized by specific mutations in the GATA1 gene, which have been documented even during prenatal development.72 Fanconia anemia (mutations in DNA repair genes), dyskeratosis congenita (X-linked mutations in dyskerin or autosomal-recessive forms with mutations in genes involved in telomere maintenance and RNA processing), Schwachman-Diamond’s syndrome (SBDS gene involved in ribosome processing), and Kostmann’s syndrome (defects in neutrophil elastase ELA2) are all associated with an increased incidence of AML. Mutations in the neurofibromin gene, a RAS-inactivating GTPase, are the cause of neurofibromatosis type I, along with its increased incidence of both juvenile myelomonocytic leukemia and AML. Similarly, mutations of the PTPN11 gene, which encodes the SHP-2 tyrosine phosphatase, cause Noonan’s syndrome, which is also associated with the development of juvenile myelomonocytic leukemia and, less commonly, AML. This group of mutations are all linked to the activation of the RAS pathway. Familial platelet disorder with a propensity to develop AML, as well as congenital amegakaryocytic thrombocytopenia, are associated with an increased incidence of AML and caused by mutations in CFFA2 and the thrombopoietin receptor, c-mpl, respectively. Inherited mutations in the CEBPA have also been linked to familial AML.

**RISK-ADAPTED TREATMENT OF ALL**

The principal and strategies of contemporary risk-adapted treatment of ALL have been the subject of recent reviews.31,100 Therefore, only a few selected high-risk subgroups will be discussed here.

Philadelphia Chromosome–Positive ALL

The Philadelphia chromosome–positive ALL had historically been associated with a dismal outcome, even with allogeneic HSCT, especially in older patients who presented with a high leukocyte count or had a slow early response to initial therapy. In a recent COG study, the addition of continuous exposure of imatinib into an intensive chemotherapy regimen has yielded a 3-year event-free survival of 80%, more than twice that of the historical controls.37 Patients who were treated with intensive chemotherapy plus imatinib fared as least as well as those historical controls who underwent matched-related or matched-unrelated transplantation. Continuous exposure of imatinib seemed to abrogate the prognostic impact of age, leukocyte count, high level of MRD at the end of induction, and even induction failure. Additional follow-up is needed to determine whether the treatment improved the cure rate rather than merely prolonging the disease-free survival. Meanwhile, many pediatric oncologists have reserved transplantation for therapy after relapse in children with Philadelphia chromosome–positive ALL. Future studies will need to address whether the aggressive backbone therapy that was used in the COG study can be safely omitted or reduced in combination with a tyrosine kinase inhibitor and whether the new generation of tyrosine kinase inhibitors (eg, dasatinib, nilotinib) can further improve outcome. Because of its dual targeting of ABL and SRC, more potent suppression of the BCR-ABL1 signaling, and efficacy to most imatinib-resistant BCR-ABL1 mutants (except for T3151), as well as good tolerability in adult clinical trials,101 dasatinib is now being tested in children.

High-Risk T-Cell ALL

With the use of intensive treatment including asparaginase and dexamethasone, T-cell cases fared as well as B-cell precursor cases in some studies.6,10 Although high-dose methotrexate at 5 g/m2 has been suggested to improve outcome in T-cell ALL, methotrexate was given parenterally at only 30 mg/m2 in the DFCI 95-01 study, which featured intensive asparaginase and doxorubicin for consolidation therapy and has perhaps the best treatment result for this subtype of ALL, with a 5-year event-free survival rate of 85%.102 In the current COG study for T-cell ALL, the relative efficacy and safety of high-dose methotrexate at 5 g/m2 with leucovorin rescue are being compared with escalating medium doses of methotrexate without leucovorin rescue plus PEG-asparaginase. In the same study, patients are also randomly assigned to receive or not receive nelarabine (a prodrug of 9-Î²-D-arabinofuranosylguanine), which has considerable antileukemic effects for T-cell ALL in phase II studies.103

Early T-cell precursor ALL, a subset of T-cell ALL characterized by a gene expression profile similar to that of normal early thymic precursor cells with multilineage differentiation potential and a distinct immunophenotype (CD1a-negative, CD8-negative, CD5-weak, and the expression of stem-cell or myeloid markers), was recently identified.45 These cases have extremely poor outcome, despite the use of transplantation. Because of the significantly improved outcome of patients with T-cell ALL who were treated with high-dose dexamethasone (10 mg/m2 per day) during remission induction in the recently completed Associazione Italiana Ematologia ed Oncologia Pediatrica Berlin-Frankfurt-Muenster AIEOP-BFM-2000 study, albeit with increased toxicity,104 this dose of dexamethasone is being tested during remission induction for this subset of patients in the current St Jude Total Therapy Study XVI.

**Infant ALL**

Even with intensive therapy including high doses of cytarabine and methotrexate, treatment outcome for infants with ALL remains poor, and for those with MLL gene rearrangement, the event-free survival rates range between 30% and 40% only.100,105,106 Although several small studies have suggested that allogeneic HSCT improves outcome, results from large group studies failed to show any benefit of this treatment modality.105,106 The Interfant-06 Study Group is conducting a randomized trial to test whether the addition of two early intensification courses typically used for AML might improve outcomes for infants with medium-risk or high-risk ALL, including those with MLL rearrangement. The role of allogeneic HSCT in first
remission is being investigated in very high-risk cases, as defined by MLL rearrangement, age less than 6 months, and WBCs more than 300 × 10^9/L. The current COG trial randomly assigns MLL-rearranged infant cases to receive the FLT3 inhibitor lestaurtinib, which has been shown to have schedule-dependent synergistic effects with chemotherapy for MLL-rearranged leukemia in preclinical studies.107 Recent findings suggested that aberrant DNA methylation occurs in the majority of infant ALL case with MLL rearrangement,108,109 raising the possibility of using DNA methyltransferase inhibitors in these patients.

Adolescent ALL

Older adolescents 15 to 21 years of age have had an inferior outcome as compared with that of children and younger adolescents with ALL, partly due to an increased incidence of Philadelphia chromosome–positive and T-cell ALL, lower incidence of ETV6-RUNX1 fusion and hyperdiploidy, and poorer compliance.31,110-112 The relatively poor outcome of older adolescents 16 to 21 years of age with ALL has prompted comparisons of outcome of patients in this age group treated in pediatric versus adult clinical trials in North America and Western Europe. Consistently, pediatric trials have yielded significantly better outcome than adult trials.112-114 This finding, in all likelihood, reflects differences in the more intensive use of nonmyelosuppressive agents such as dexamethasone, vincristine, and asparaginase; the incorporation of high-dose methotrexate; and early and frequent administration of intrathecal therapy in pediatric regimens.

The importance of these treatment components is supported by several other pediatric trials. In the Children's Cancer Group 1961 trial, which included 262 adolescents 16 to 21 years of age treated from 1996 to 2002,112 the 5-year event-free survival rate was 71.5%. This randomized trial demonstrated that augmented rather than standard postinduction intensification therapy with additional doses of vincristine and PEG-asparaginase in lieu of mercaptopurine during interim maintenance therapy and increased doses of intravenous methotrexate without leucovorin rescue improved outcome for patients with a rapid early response. The Dana-Farber Cancer Institute (DFCI) protocols (1991 to 2000) and St Jude Total Study XV (2000 to 2007),115 which yielded excellent results for adolescents, likewise featured early intensification therapy with vincristine and asparaginase. Although some contemporary adult ALL trials have recommended allogeneic transplantation as the best treatment option for good-risk adult ALL patients in first remission,116 the excellent results obtained in these pediatric trials do not support the routine use of transplantation in older adolescents with ALL.

There are three other important observations from the Children's Cancer Group 1961 study.112 First, there was no statistical difference in treatment outcome between adolescents with a rapid response who were randomly assigned to receive either one or two courses of postinduction intensification therapy. Second, there was an increased risk of toxic deaths among older adolescents, with 18% of the first events being nonrelapse deaths that occurred during induction or in first remission. Third, there was an increased risk of osteonecrosis. Thus it will be of interest to test whether prednisone pulses, which are commonly given during maintenance therapy, can be omitted in adolescents and young adults with ALL and rapid early response.

The excellent results recently achieved in older adolescents with ALL have prompted many centers and cooperative groups to start treating young adults with pediatric-like regimens. A recent report showed 6-year event-free survival rates of 60% for the 35 adolescents 15 to 18 years old and 63% for the 46 young adults 19 to 30 years old.117 The DFCI Consortium attained a 2-year rate of 72.5% among 75 adults 18 to 50 years of age.118 Investigators at Princess Margaret Hospital in Toronto have also adapted the DFCI treatment regimen and achieved a 3-year relapse-free survival rate of 77% among 64 adults with BCR-ABL–negative ALL who did not undergo transplantation.119 To this end, a US adult intergroup study is testing a pediatric-like regimen in adolescents and young adults up to 39 years of age.

Other High-Risk Subgroups

Hypodiploidy (< 44 chromosomes), t(17;19)(q22;p13.3) [TCF3-HLF], remission induction failure, and the presence of MRD more than 1% at the end of remission induction are also associated with dismal outcome and collectively occur in approximately 5% of children with ALL.31 Although allogeneic transplantation is frequently used to treat these patients, there is no strong evidence to document the efficacy of this approach. Among many novel therapeutics under investigation (Table 4),101 killer-cell immunoglobulin-like receptor–mismatch natural cell therapy120,121 and immunotherapy with a T-cell–engaging CD19/CD3-bispecific antibody construct (blinatumomab) seem to be particularly promising.122

Cases at High Risk of CNS Relapse

Two recent studies showed that with effective chemotherapy, prophylactic cranial irradiation can be safely omitted altogether in the treatment of childhood ALL10,123 showing isolated CNS relapse rates of 2.7% and 2.6%. The complete omission of prophylactic cranial irradiation in these studies allowed the clear identification of risk factors for CNS relapse, which were any CNS involvement at diagnosis, t(1;19)[TCF3-PBX1], and T-cell ALL.10,123 In the current St Jude Total Therapy Study XVI, triple intrathecal therapy is further intensified for these patients with twice weekly administration in the first 2 weeks of remission induction.

RISK-ADAPTED TREATMENT OF AML

Similar to ALL, risk-adapted therapy has also become an important approach in childhood AML. Many traditional prognostic factors have been replaced by cytogenetic and molecular features, as well as flow cytometric assessment of MRD (Table 5).

AML Characterized by Chimeric Transcription Factors: “Mostly Low-Risk” AML

With the introduction of intensively dosed and/or timed chemotherapy regimens, AML characterized by alterations of core-binding and transcription factors have emerged as a favorable prognostic group. Both t(8;21) and inv(16) AML have an approximately 80% overall survival rate when treated with three to four courses of intensive chemotherapy.15-28 Although patients with t(8;21) AML may have a higher relapse rate than those with inv(16), they are still able to be cured with allogeneic transplantation after attaining a second remission.127
from maintenance therapy with ATRA and antimetabolites.129,130 Al-sitive to ATRA and arsenic trioxide. Children with APL also benefit neic transplantation.131,132

production, usually with arsenic trioxide, and then autologous or alloge-first remission. Survival after relapse is approximately 70% after rein-logeneic transplantation is not recommended for these children in

In part because of the variability in outcomes, small numbers of patients, and lack of definitive data demon-

strating the superiority of HSCT, most cooperative trials do not include patients with MLL-rearranged AML in the high-risk group requiring allogeneic HSCT in first remission.133 Once associated with a poor prognosis,134 AMKL with the t(1;22) has improved outcome with contemporary treatment, and HSCT may not be indicated in these patients.26,135

Similar to adults, children with acute promyelocytic leukemia (APL) have an excellent overall survival in the 75% to 85% range, with some reports as high as 90%.124,128 This leukemia is particularly sen-sitive to ATRA and arsenic trioxide. Children with APL also benefit from maintenance therapy with ATRA and antimetabolites.129,130 Al-logeneic transplantation is not recommended for these children in first remission. Survival after relapse is approximately 70% after rein-
duction, usually with arsenic trioxide, and then autologous or alloge-
neic transplantation.131,132

AML Characterized by Mutation Altered Genes: The “Good” and the “Bad”

FLT3-ITD mutations, particularly when associated with a high mutant to wild-type allelic ratio, are associated with an overall survival rate of usually less than 30%.136 Although controversial, there are some indications that transplantation might improve outcome of children with this type of AML.137 Some clinical trials

### Table 4. Novel Therapeutics Under Investigation in Childhood ALL and AML

<table>
<thead>
<tr>
<th>Category and Agent</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>New formulations</strong></td>
<td>Long half-life, reduced immunogenicity (ALL)</td>
</tr>
<tr>
<td>Pegylated asparaginase</td>
<td>Decreased neuropathy, higher tissue concentration, non-vesicant (ALL)</td>
</tr>
<tr>
<td>Sphingosomal vincristine</td>
<td>Decreased cardiotoxicity (ALL/AML)</td>
</tr>
<tr>
<td>Liposomal annamycin</td>
<td>Decreased cardiotoxicity (ALL/AML)</td>
</tr>
<tr>
<td>Liposomal doxorubicin</td>
<td>Long half-life, potential neurotoxicity when administered intrathecally (ALL/AML)</td>
</tr>
<tr>
<td>Liposomal cytarabine</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Nucleoside analogues</strong></th>
<th>Effective for ALL and AML</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clofarabine</td>
<td>Selective for T-cell ALL, potential neurotoxicity</td>
</tr>
<tr>
<td>Nelarabine</td>
<td>Oral phosphorylase inhibitor (ALL)</td>
</tr>
<tr>
<td>Forodesine (BCX-1777)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Monoclonal antibodies</strong></th>
<th>Potentiate chemotherapy for CD20+ B-lineage ALL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rituximab (anti-CD20)</td>
<td>Potentiate chemotherapy for CD52+ ALL</td>
</tr>
<tr>
<td>Alemtuzumab (anti-CD52)</td>
<td>May have synergistic effect with anti-CD20 antibody</td>
</tr>
<tr>
<td>Epratuzumab (anti-CD22)</td>
<td>Cytotoxic for CD22+ ALL</td>
</tr>
<tr>
<td>CAT-8015, HA22; inotuzumab ozogamicin (anti-CD22)</td>
<td>Cytotoxic for CD33+ leukemia (AML)</td>
</tr>
<tr>
<td>Gemtuzumab ozogamicin (anti-CD33)</td>
<td>Bispecific antibodies that direct CD3+ T-cell against CD19+ ALL</td>
</tr>
<tr>
<td>Blinatumomab</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Molecularly targeted agents</strong></th>
<th>Potentiate chemotherapy for Ph+ ALL; targeting c-KIT, VEGF in AML</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyrosine kinase inhibitors (imatinib, nilotinib, dasatinib, MK-O457, bosutinib, AP24534, DCC2036, sorafenib); Aurora kinase inhibitor (danusertib); Src-family kinase inhibitor; VEGF inhibitors (bosutinib); bevaccimab, SU8416, AZD2171 Fms-like tyrosine kinase – 3 (Lestaurntinib, Midostaurin, Tandutinib)</td>
<td>Phase I studies on MLL-rearranged leukemia/FLT3-ITD positive AML</td>
</tr>
<tr>
<td>NOTCH1 inhibitors (γ-secretase inhibitor, antagonists targeting NOTCH transcription complex, NOTCH1 receptor inhibitors)</td>
<td>Preclinical studies for T-cell ALL</td>
</tr>
<tr>
<td>mTOR inhibitors (rapamycin, temsirolimus, everolimus)</td>
<td>Testing in post-transplantation use to decrease graft-versus-host disease and to suppress leukemia (ALL); in combination with chemotherapy in AML</td>
</tr>
<tr>
<td>Demethylating agents (decitabine, azacytidine)</td>
<td>Potentiate chemotherapy by epigenetic modulation of leukemia cells with MLL rearrangement and in AML/MDS</td>
</tr>
<tr>
<td>Histone deacetylase inhibitor (vorinostat, valproic acid, depsipeptide)</td>
<td>Potentiate chemotherapy by epigenetic modulation of leukemia cells in ALL and AML</td>
</tr>
<tr>
<td>Heat shock protein inhibitor (17-allylamino-geldanamycin)</td>
<td>Potentiate the cytotoxicity of histone deacetylase inhibitor</td>
</tr>
<tr>
<td>Proteasome inhibitor (bortezomib, carfilzomib, ONX 0912)</td>
<td>Directe at RAS inhibition in AML</td>
</tr>
<tr>
<td>Farnesylation (tipifarnib)</td>
<td>Phase I trial for ALL and AML with JAK mutations</td>
</tr>
<tr>
<td>JAK2 inhibitor (lestaurtinib)</td>
<td></td>
</tr>
<tr>
<td>Microenvironment</td>
<td></td>
</tr>
<tr>
<td>Integrin and cell adhesion inhibition (AMD3100, plerixafor)</td>
<td>CXC4R inhibition; phase I trials in AML planned</td>
</tr>
<tr>
<td>Immunomodulation</td>
<td>Immunosuppressive approaches to increase T-lymphocyte mediated antileukemic responses in AML</td>
</tr>
</tbody>
</table>

IL-2, IL-6, GM-CSF, WT1, RHAMM-R3 and PR1 peptides, GVAX, dendritic cells

**Abbreviations:** ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; VEGF, vascular endothelial growth factor; Ph+, Philadelphia chromosome positive; mTOR, mammalian target of rapamycin; MDS, myelodysplastic syndrome; IL, interleukin; GM-CSF, granulocyte-macrophage colony-stimulating factor.
<table>
<thead>
<tr>
<th>Subtype</th>
<th>Affected Genes</th>
<th>Gene Functions</th>
<th>Frequency (%)</th>
<th>Clinical Characteristics</th>
<th>5-Year EFS (%)</th>
<th>5-Year OS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rearrangements</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t(8;21)(q22;q22)</td>
<td>ETO-AML1</td>
<td>Transcription factors</td>
<td>12</td>
<td>Associated with chloromas</td>
<td>55-71</td>
<td>75-85</td>
</tr>
<tr>
<td>inv(16)(p13;q22)</td>
<td>MYYH11-CBF</td>
<td>Muscle protein/transcription factor</td>
<td>8</td>
<td>Eosinophilia with dysplastic basophilic granules</td>
<td>72-88</td>
<td>75-85</td>
</tr>
<tr>
<td>t(1;16)</td>
<td>MOZ-CBP</td>
<td>Transcription factors</td>
<td>1</td>
<td>High WBCs, chloromas, etoposide-related secondary AML</td>
<td>ID†</td>
<td>ID</td>
</tr>
<tr>
<td>t(15;17)(q22;q12)</td>
<td>PML-RAR</td>
<td>Transcription factors/retinoid receptor</td>
<td>12</td>
<td>Associated with FAB M3, Auer rods common; FAB M3; ATRA-sensitive</td>
<td>71†</td>
<td>90†</td>
</tr>
<tr>
<td>t(11;17)(q23,q12)</td>
<td>PLZF-RARA</td>
<td>Transcription factors/retinoid receptor</td>
<td>Rare</td>
<td>Associated with FAB M3, Auer rods common; FAB M3; ATRA-resistant</td>
<td>ID</td>
<td>ID</td>
</tr>
<tr>
<td>t(1;22)</td>
<td>RBM15-MKL1</td>
<td>RNA binding protein, DNA binding protein</td>
<td>2-3</td>
<td>Associated with FAB M7 in Down syndrome and non-Down syndrome</td>
<td>ID</td>
<td>ID</td>
</tr>
<tr>
<td>t(6;9)(p23;q34)</td>
<td>DEK-NUP214(CAN)</td>
<td>Transcription factor/nuclear transport</td>
<td>Rare</td>
<td>Basophilia and multilineage dysplasia; associated with FLT3-ITD and TdT+</td>
<td>ID</td>
<td>ID</td>
</tr>
<tr>
<td>MLL</td>
<td>MLL (partner genes)</td>
<td>Histone methyltransferase</td>
<td>18</td>
<td></td>
<td>92</td>
<td>100</td>
</tr>
<tr>
<td>t(1;11)(q21;q23)</td>
<td>AF1q (MLLT11)</td>
<td>Function unknown, causes short half-life</td>
<td>3</td>
<td>76% &lt; age 2 years; 20% and 48% FAB M4 and M5, respectively</td>
<td>92</td>
<td>100</td>
</tr>
<tr>
<td>t(4;11)(q21;q23)</td>
<td>AF4 (MLLT2)</td>
<td>Associated with EAP</td>
<td>2</td>
<td>61% &lt; age 2 years; 17% and 42% FAB M4 and M5, respectively</td>
<td>29</td>
<td>27</td>
</tr>
<tr>
<td>t(6;11)(q27;q23)</td>
<td>AF6 (MLLT4)</td>
<td>Functions as dimerization domain</td>
<td>5</td>
<td>9% &lt; age 2 years; 57% ≥ 10 years; 35% and 41% FAB M4 and M5, respectively</td>
<td>11</td>
<td>22</td>
</tr>
<tr>
<td>t(9;11)(p22;q23)</td>
<td>AF9 (MLLT3)</td>
<td>ENL homolog, associated with EAP</td>
<td>43</td>
<td>42% &lt; age 2 years; 81% is FAB M5</td>
<td>50</td>
<td>63</td>
</tr>
<tr>
<td>t(10;11)(p11.2;q23)</td>
<td>AF10 (MLLT10)</td>
<td>Interacts with DOT1L</td>
<td>2</td>
<td>75% &lt; age 2 years; 27% and 55% FAB M4 and M5, respectively</td>
<td>17</td>
<td>27</td>
</tr>
<tr>
<td>t(10;11)(p12;q23)</td>
<td>AF10 (MLLT10)</td>
<td>Interacts with DOT1L</td>
<td>13</td>
<td>62% &lt; age 2 years; 72% is FAB M5</td>
<td>31</td>
<td>45</td>
</tr>
<tr>
<td>t(11;19)(q23;p13)</td>
<td>ELL or ENL (MLLT1)</td>
<td>Binds histone H3, Assemblies EAP</td>
<td>4</td>
<td>58% &lt; age 2 years; 42% and 45% FAB M4 and M5, respectively</td>
<td>49</td>
<td>49</td>
</tr>
<tr>
<td>t(11;19)(q23;p13.1)</td>
<td>ELL or ENL (MLLT1)</td>
<td>Binds histone H3, Assemblies EAP</td>
<td>4</td>
<td>41% &lt; age 2 years; 30% and 33% FAB M4 and M5 respectively</td>
<td>46</td>
<td>61</td>
</tr>
<tr>
<td>t(11;19)(q23;p13.3)</td>
<td>ELL or ENL (MLLT1)</td>
<td>Binds histone H3, Assemblies EAP</td>
<td>3</td>
<td>36% &lt; age 2 years; 44% ≥ age 10 years; 20% and 40% FAB M4 and M5</td>
<td>46</td>
<td>47</td>
</tr>
<tr>
<td>t(11;17)(q23;q21)</td>
<td>AF17 (MLLT6), LASP1</td>
<td>F-actin rick cytoskeletal activity</td>
<td>2</td>
<td>33% &lt; age 2 years; 42% ≥ age 10 years; 33% and 50% FAB M4 and M5</td>
<td>11</td>
<td>22</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Normal karyotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Gene mutations</strong></td>
<td>NPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CEBPa</td>
<td>Nucleophosmin</td>
<td>Nuclear transporter RNA processing</td>
<td>23</td>
<td>8%-10% of childhood AML</td>
<td>65-80</td>
<td>75-85</td>
</tr>
<tr>
<td>FL3/ALM</td>
<td>CCAAT/enhancer binding protein α</td>
<td>Transcription factor</td>
<td>14</td>
<td>4%-6% of all childhood AML; more common in older patients, FAB M1 or M2</td>
<td>70</td>
<td>83</td>
</tr>
<tr>
<td>FL3-ITD</td>
<td>Fms-like tyrosine kinase 3 activation loop domain</td>
<td>Receptor for FLT3</td>
<td>3</td>
<td>6%-7% of all childhood AML</td>
<td>50-60</td>
<td>60-70</td>
</tr>
<tr>
<td>WT1</td>
<td>Fms-like tyrosine kinase 3 internal tandem duplication</td>
<td>Receptor for FLT3</td>
<td>18</td>
<td>10%-15% of all childhood AML</td>
<td>&lt; 35</td>
<td>&lt; 35</td>
</tr>
<tr>
<td>RAS</td>
<td>Wilms tumor 1</td>
<td>Transcription factor</td>
<td>13</td>
<td>8%-10% of all childhood AML</td>
<td>22-35</td>
<td>35-56</td>
</tr>
<tr>
<td>PTPN11</td>
<td>Rat sarcoma gene</td>
<td>Signal transduction</td>
<td>3</td>
<td>5% of all childhood AML</td>
<td>ID</td>
<td>ID</td>
</tr>
<tr>
<td></td>
<td>Protein tyrosine phosphatase, non-receptor type 11</td>
<td>Tyrosine phosphatase</td>
<td>2</td>
<td>Most commonly associated with JMML</td>
<td>ID</td>
<td>ID</td>
</tr>
<tr>
<td>No known mutations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Poor-risk cytogenetics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 15</td>
<td>Del 5q/E5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: AML, acute myeloid leukemia; EFS, event-free survival; ID, insufficient data; FAB, French-American-British; ATRA, all-trans-retinoic acid; JMML, juvenile myelomonocytic leukemia.

†Data extracted from Ho et al,70 Meshinchi et al,73 Brown et al,25 Rubnitz et al,26 Gibson et al,27 and Cooper et al.20

‡Data extracted from Pui et al,10,125,126
are testing the efficacy of various FLT3 inhibitors with different degrees of specificity plus allogeneic transplantation in these children. In the next COG trial for newly diagnosed AML, patients with a high FLT3-ITD to wild-type allelic ratio will be offered an allogeneic HSCT in first remission. Of note, patients with point mutations of FLT3 (e.g., FLT3/ALM) do not have poor outcome and should be treated with chemotherapy only.

The c-KIT mutations, most commonly observed in CBF AML, has been reported to portend a poor prognosis in some studies, but not in a recent COG study.138 Similarly, RAS mutations have not been definitively associated with a poor prognosis. Thus patients with these mutations are usually treated with chemotherapy only.

NPM mutations convey both increased chemosensitivity and improved outcome.83 However, whether or not the presence of NPM mutations can partially abrogate the adverse affect of coexpressed FLT3-ITD is controversial.82 CEBPA mutations are often associated with normal karyotype AML and improved overall survival.70 Thus HSCT in first remission is not recommended for pediatric patients with isolated NPM or CEBPA mutations. WT1 mutations had an independent, poor prognostic impact in one study,125 but not in another (except in patients with coexpression of FLT3-ITD).126 Most clinical trials do not recommend allogeneic transplantation based on isolated WT1 mutations.

The Importance of MRD

Other than a Berlin-Frankfurt-Munster study, the detection of MRD has been associated with adverse prognostic significance.26,139 COG and St Jude trials are using this factor to stratify patients for risk-directed treatment.

Combined Approaches to Risk Stratification

In COG, the combination of cytogenetic, molecular, and MRD information is being used to stratify patients into two groups for risk-directed therapy. Low-risk AML includes patients with mutations involving CBF, CEBPA, and NPM and those with no MRD at the end of induction therapy. This group represents approximately 73% of patients, with a predicted survival close to 75%. The high-risk group (the remaining 27% of patients with survival <35%) includes patients with adverse cytogenetic abnormalities (monosomy 7, del(5q)γ, −5), high FLT3-ITD to wild-type allelic ratio, or MRD at the end of induction and will be offered HSCT in first remission with the most suitable donor.

Molecularly targeted drugs can be tested in both low- and high-risk groups with the intention of reducing toxic therapy in the former group while improving survival of the latter group (Table 4). For example, the use of bortezomib to augment chemotherapy effects on AML stem cells will be randomized in the next COG phase III trial. In addition, FLT3-ITD inhibitors (i.e., sorafenib) will be tested in the high-risk group with a high FLT3-ITD to wild-type allelic ratio.

Special Subtypes

Down syndrome. In view of superior prognosis of Down syndrome patients with AML,140 current trials are testing strategies to reduce overall drug exposure, especially cardiotoxic drugs, because of special concerns in this population.141

APL. Since the demonstration that ATRA significantly improved the outcome of patients with APL, these patients have been treated separately from other patients with AML, resulting in 5-year overall survival rates as high as 87%.128 A major, adverse prognostic factor is presenting WBC more than 10 × 10⁹/L, associated with an event-free survival of approximately 60%.128 Approximately 3% of patients died during induction from hemorrhagic complications, accounting for half of the induction failures. The microgranular variant (M3v), a bcr3 PML breakpoint, and the presence of FLT3-ITD had also been associated with a poor prognosis,142 partly due to increased presenting WBCs. In the current pediatric studies, patients are considered low risk or high risk on the basis of WBCS ≤ or more than 10 × 10⁹/L, respectively.

Although intensive anthracycline treatment (cumulative doses ranging from 400 to 750 mg/m²) has been attributed to improve outcome,130 one of the key issues for pediatric patients has been to reduce exposure to these cardiotoxic drugs. The efficacy of arsenic in relapsed and newly diagnosed patients has made it an attractive alternative to anthracyclines.143-145 Thus the current COG trial replaces a course of anthracycline-containing chemotherapy with arsenic, reducing anthracyline exposure to 355 mg/m² of daunorubicin equivalents for standard-risk patients with negative MRD and to 455 mg/m² for high-risk patients and standard-risk patients with positive MRD after the third treatment course. This trial also includes high-dose cytarabine based on the survival advantage observed in a European trial.145 Maintenance therapy with ATRA plus antimetabolites will be given to all patients for approximately 2 years. This trial is similar to the European trial, with one primary difference being the introduction of arsenic during consolidation. Future studies may include other targeted agents, such as anti-CD33 monoclonal antibody therapy.146,147

AML in neonates and infants. Because spontaneous remissions have been reported more frequently in the neonate age group, it is commonly recommended to initially observe such patients and, if cytoreduction is necessary, to perform an exchange transfusion. However, AML-directed therapy with dosing adjustments is usually required. Outcome in this age group is worse than that in older children, despite similar therapy, as a result of treatment toxicities and resistant disease.148 Novel approaches to directly target MLL149 and bcl-2150 are being developed (Table 4). In contrast to neonates, infants more than 1 month old seem to do as well as older children when treated with current, intensive regimens.15-30 The role of transplantation in infants is controversial because it has not been shown to definitely improve outcome and has significant adverse sequelae.

In conclusion, cure rates for childhood leukemias have improved largely through more intensive use of conventional cytotoxic agents evaluated in the context of large, randomized clinical trials. Clinical factors, genetic features of the leukemia, and initial response to therapy are now used in concert to personalize treatment for all patients. Advances in high-throughput genomics have led to the discovery of additional recurrent somatic lesions in the leukemic cell that offers not only additional prognostic information, but also opportunities for the application of novel targeted treatment. Finally, the recognition of host factors that are associated with the risk of leukemic transformation and the response to therapy will likely lead to more sophisticated treatment strategies in the near future.151,152
Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "C" are those for which no compensation was received; these relationships are marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO’s conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

**Employment or Leadership Position:** None Consultant or Advisory Role: Ching-Hon Pui, Genzyme (C) Stock Ownership: None Honoraria: Ching-Hon Pui, Enzon Pharmaceuticals, sanofi-aventis

**REFERENCES**


**AUTHORS’ DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST**

**AUTHOR CONTRIBUTIONS**

**Research Funding:** None
**Expert Testimony:** None
**Remuneration:** None

**Conception and design:** Ching-Hon Pui, Robert J. Arceci
**Financial support:** Ching-Hon Pui
**Administrative support:** Ching-Hon Pui
**Provision of study materials or patients:** Ching-Hon Pui, Robert J. Arceci
**Collection and assembly of data:** Ching-Hon Pui, Robert J. Arceci
**Data analysis and interpretation:** Ching-Hon Pui, Robert J. Arceci
**Manuscript writing:** Ching-Hon Pui, William L. Carroll, Soheil Meshinchi, Robert J. Arceci
**Final approval of manuscript:** Ching-Hon Pui, William L. Carroll, Soheil Meshinchi, Robert J. Arceci

© 2011 by American Society of Clinical Oncology

Information downloaded from jco.ascopubs.org and provided by Christian Medical College-Vellore on August 9, 2011 from Copyright © 2011 American Society of Clinical Oncology. All rights reserved.
84. Schnittger S, Kern W, Tschulk C, et al: Minimal residual disease levels assessed by NPM1
mutation-specific RQ-PCR provide important prognostic information in AML. Blood 114:2222-2231, 2009
108. Pui et al


144. Gore SD, Hermes-DeSantis ER: Enhancing survival outcomes in the management of patients with higher-risk myelodysplastic syndromes. Cancer Control 16:2-10, 2009 (suppl)


149. Liedtke M, Cleary ML: Therapeutic targeting of MLL. Blood 113:6061-6068, 2009

