

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.

J Clin Oncol 29:551-565. © 2011 by American Society of Clinical Oncology

From St Jude Children's Research Hospital and the University of Tennessee Health Science Center, Memphis, TN; the New York University Cancer Institute, Stephen D. Hassenfeld Children's Center, New York, NY; Fred Hutchinson Comprehensive Cancer Center, Seattle, WA; and the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD.

Submitted May 27, 2010; accepted October 12, 2010; published online ahead of print at www.jco.org on January 10, 2011.

Supported by the National Cancer Institute (Grant No. CA21765), CureSearch, and American Lebanese Syrian Associated Charities. R.J.A. is supported in part through an endowed King Fahd Professorship in Pediatric Oncology.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Corresponding author: Ching-Hon Pui, MD, St Jude Children's Research Hospital, 262 Danny Thomas Pl, Memphis, TN 38105; e-mail: ching-hon.pui@stjude.org.

© 2011 by American Society of Clinical Oncology

0732-183X/11/2905-551/\$20.00

DOI: 10.1200/JCO.2010.30.7405

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)(*CBFβ-*

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

Table 1. Characterizations of the Patients and Treatment Results From Selected Clinical Trials for Childhood ALL

Study	Years of Study	No. of Patients	Age Range (years)	T Cell (%)	WBC Count $\geq 100 \times 10^9/L$ (%)	DNA Index ≥ 1.16 (%)	<i>ETV6</i> - <i>RUNX1</i> (%)	Ph+ (%)	Event-Free Survival at 5 Years		Survival at 5 Years		Cumulative CNS Relapse at 5 Years		Cumulative Secondary Neoplasm at 10 Years		Data Source (first author)
									%	SE	%	SE	%	SE	%	SE	
AIEOP-95	1995-2000	1,743	0-17	11	10.2	19.2	22.4	2.3	75.9	1.0	85.5	0.8	1.2	0.3	0.4	0.2	Conter ¹
BFM-95	1995-2000	2,169	0-18	13.3	11.1	20.3	21.5	2.2	79.6	0.9	86.3	0.6	1.8	0.3	1.7	0.3	Möricke ²
CCG-1900	1996-2002	4,464	0-21	15.9	14.1	21.5*	NA	2.9	76.0	0.7	86.3	0.6	4.6	0.3	1.0	0.2	Gaynon ³
COALL-97	1997-2003	667	0-18	14.1	12.0	NA	23.9	1.9	76.7	1.7	85.4	1.4	2.1	0.6	1.1	0.4	Escherich ⁴
CPH-95	1996-2002	380	0-18	14.8	12.6	NA	22.3	2.3	72.1	2.3	83	1.9	1.2	0.6	0.6	0.4	Stary ⁵
DCOG-9	1997-2004	859	1-18	11.4	12.4	21.5	15.0	1.6	80.6	1.4	86.4	1.2	2.6	0.6	0.1	0.1	Kamps ¹⁴
DFCI 95-01	1996-2000	491	0-18	10.6	11.0	18.0	25.8	NA	81.6	1.8	89.6	1.4	0.7	0.4	0.6		Silverman ⁶
INS 98	1998-2003	315	0-18	19.4	12.1	19.9*	13.7	3.3	78.7	2.3	83.8	2.1	1.9	0.8	1.0	0.5	Stark ⁷
NOPHO-2000	2002-2007	1,023	1-15	11.3	11.5	NA	23.2	1.1	79.4	1.5	89.1	1.1	2.7	0.6	NA		Schmiegelow ⁸
SJCRH-13B	1994-1998	247	0-18	17.4	15.4	18.6	15.8	4.0	80.1	2.6	85.7	2.2	1.7	0.8	3.3	1.2	Pui ⁹
SJCRH-15	2000-2007	498	1-18	15.3	12.7	24.3	19.3	2.0	85.6	2.9	93.5	1.9	2.7	0.8	0.3	0.3	Pui ¹⁰
TCCSG-95-14	1995-1999	597	1-15	9.7	11.8	22.3	NA	4.0	76.8	1.8	84.9	1.5	1.7	0.6	0.7		Tsuchida ¹¹
TPOG-2002	2002-2007	788	0-18	9.7	14.0	NA	13.1	2.1	77.4	1.7	83.5	1.6	3.8	0.8	NA		Liang ¹²
UKALL-97/99	1999-2002	938	1-18	10.0	13.1	NA	NA	2.3	80	1.2	88	1.1	3.0	0.5	NA		Mitchell ¹³

Abbreviations: ALL, acute lymphoblastic leukemia; Ph+, Philadelphia chromosome positive; AIEOP, Associazione Italiana di Ematologia ed Oncologia Pediatrica; BFM, Berlin-Frankfurt-Münster ALL Study Group; CCG, Children's Cancer Group; COALL, Cooperative ALL Study Group; CPH, Pediatric Hematology in the Czech Republic; DCOG, Dutch Childhood Oncology Group; DFCI, Dana-Farber Cancer Institute ALL Consortium; INS, Israeli National Studies of Childhood ALL; NOPHO, Nordic Society of Pediatric Hematology and Oncology; SJCRH, St Jude Children's Research Hospital; TCCSG, Tokyo Children's Cancer Study Group; TPOG, Taiwan Pediatric Oncology Group; UKALL, UK Medical Research Council Working Party on Childhood Leukaemia; NA, not available.

*Ploidy > 50.

genome-wide analyses of gene expression, DNA copy number alterations (CNA) and loss of heterozygosity, epigenetic changes, and whole-genome sequencing have led to the detection of many novel genetic abnormalities; to date, virtually all patients with ALL can be classified according to specific genetic abnormality (Fig 1A). These studies also provided new insights into the complex interactions of multiple genetic alterations in leukemogenesis and response to therapy.³⁴

Genomic Analysis of ALL

Primary somatic genetic abnormalities have important prognostic and therapeutic implications and play a critical role in leukemogenesis (Table 3).^{10,31,34-50} 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 CNAs per case of childhood ALL. In general, deletions outnumbered amplifications by a ratio of 2:1. The lesions target genes regulating lymphoid differentiation, tumor suppression, cell cycle, apoptosis, signaling, microRNAs, and drug responsiveness. There were substantial differences in the frequency of CNAs among various subtypes. *MLL*-rearranged cases had less than one CNA per case, suggesting that *MLL* is a potent oncogene that requires very few cooperating lesions to induce leukemia transformation, whereas *ETV6-RUNX1* (formerly known as *TEL-AML1*) and *BCR-ABL1* leukemias demonstrated more than 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.⁵¹ 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 *IGH@-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.^{40,41}

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*, *TALI*

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

Study*	Years of Study	No. of Patients†‡	Early Deaths (%)	CR Rate (%)	Time of CR Evaluation	Anthracyclines (mg/m ²)§	Cytarabine (g/m ²)	Etoposide (g/m ²)	5-Year EFS		5-Year OS		Data Source (first author)
									%	SE	%	SE	
POG 8821	1988-1993	511	3.9	77	2 courses	360	55.7	2.25	31	2	40.4	2	Ravindranath ²⁵
CCG 2891	1989-1995	750	4	77	2 courses	350	28.3	1.9	34	3	45	3	Smith ²¹
MRC-AML 10	1988-1995	303	4	93	4 courses	550	10.6	0.5-1.5	49		58		Gibson ²⁷
PINDA-92	1992-1998	151	21	74	Not specified	350	7.64	0.45	36		37		Quintana ²⁹
LAME- 91	1991-1998	247	6.4	91	2 courses	460	9.8-13.4	0.4	48	4	62.3	4	Perei ²⁴
TCCSG M91-13/M96-14	1991-1998	192	3.6	88	Not specified	495	69.4-99.4	3.75-5.75	56		62		Tomizawa ¹⁷
BFM-93	1993-1998	427	7.4	82	4 courses	300-400	23-41	0.95	50	2	57	2	Creutzig ²⁰
CCG 2961	1996-1999	901	6	83	2 courses	360	15.2-19.6	1.6	42	3	52	4	Lange ¹⁵
EORTC-CLG 58,921	1993-2000	166	1	84	2 courses	380	23-29	1.35	49	4	62	4	Entz-Werle ²³
GATLA-AML90	1993-2000	179	20	70	Not specified	300	41.1	1.45	31	4	41	4	Armendariz ¹⁸
AIEOP LAM-92	1992-2001	160	6	89	2 courses	Not provided	Not provided	Not provided	54	4	60	4	Pession ²²
NOPHO-AML 93	1993-2001	223	2	92	3 courses	300-375	49.6-61.3	1.6	50	3	66	3	Lie et al ¹⁹
MRC-AML 12	1994-2002	455	4	92	4 courses	300-610	4.6-34.6	1.5	56		66		Burnett ¹⁹ /Gibson ²⁷
AML99	2000-2002	260	1.7	94	2 courses	300-375	59.4-78.4	3.15-3.2	61	3	75	3	Tsukimoto ²⁸
BFM-98	1998-2003	473	3	88	4 courses	420	41-47	0.95	49	3	62	3	Creutzig ²⁰
SJCRH AML02¶	2002-2008	230	1	94	2 courses	300-550	34-48	1-1.5	63	4	71	4	Rubnitz ²⁶
COG AAML03P1¶#	2003-2005	350	2.6	83	2 courses	300-480	21.6-45.6	1-1.75	53	6	66	5	Cooper ³⁰

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-AML, United Kingdom's Medical Research Council Acute Myelogenous Leukemia study; PINDA, National Program for Antineoplastic Drugs for Children; LAME, Leucemie Aigue 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 Meiloide; 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 for 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.

†No. of patients excludes patients with Down syndrome.

‡Ages include patients from 0 up to 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 5×, mitoxantrone 5×, doxorubicin 1×. Another conversion factor for idarubicin and mitoxantrone that has been used is 3×.

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

¶SJCRH AML02 and the COG AAML03P1 have EFS and OS at 3-year follow-up.

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

plus *LMO1* or *LMO2*, *HOX11*, and *MLL-ENL* (Table 3).⁴³ Although *HOX11L2* generally confers a poor outcome, *HOX11* and *MLL-ENL* are associated with a favorable outcome.^{43,53} Using SNP and other genome-wide platforms, many novel genomic alterations have recently been identified, including focal deletions leading to dysregulated expression of *TAL1* and *LMO2*, deletion and mutation of *PTEN*, mutations of *NOTCH1* and *FBXW7*, deletions of *RBI1*, duplications of *MYB*, deletions of *RBI1*, and fusion of *SET* or *ABL1* to *NUP214*.³⁴ Hence T-cell ALL is also a heterogeneous disease. Thus far, mutations of *NOTCH1* and *FBXW7* (observed in 50% of T-ALL) have generally been associated with a favorable prognosis, and *NUP214-ABL1* fusion has been associated with responsiveness to tyrosine kinase inhibition.^{43,44,49}

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.^{54,55} Although 90% of the cases exhibited differential CNAs (gaining or losing genetic lesions) from diagnosis to relapse, most relapse samples are clonally 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*).^{54,55} 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.⁵⁴ 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

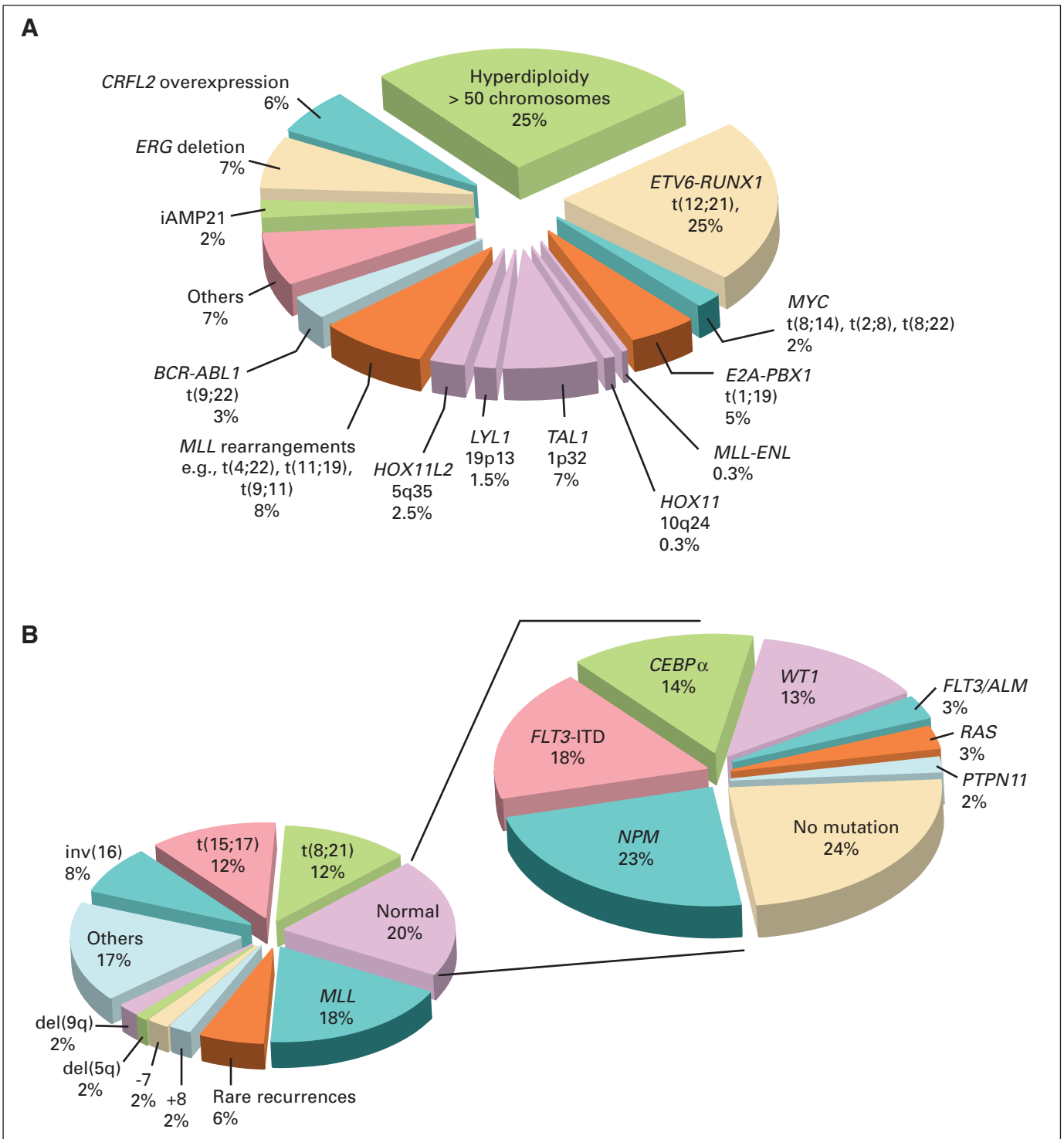


Fig 1. Estimated frequency of specific genotypes in childhood leukemias. (A) Genetic abnormalities in acute lymphoblastic leukemia (ALL). Data were modified from Pui et al³² by including recently identified genotypes. The genetic lesions that are exclusively seen in cases of T-cell ALL are indicated 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 Smith³³).

Table 3. Characteristics and Clinical Outcomes of Selected Subtypes of Childhood ALL

Subtype	Frequency (%)	Clinical Implication	Estimated 5-Year Event-Free Survival (%)	Data Source (first author)
B-cell precursor				
Hyperdiploidy > 50	20-30	Excellent prognosis with antimetabolite-based therapy	85-95	Pui ^{10,31}
t(12;21)(p13;q22) <i>ETV6-RUNX1</i>	15-25	Expression of myeloid-associated antigens CD13 and CD33; excellent prognosis with intensive asparaginase therapy	80-95	Pui ^{10,31}
Trisomies 4 and 10	20-25	Excellent prognosis with antimetabolite therapy	85-90	Salzer ³⁵
t(1;19)(q23;p13) <i>TCF3-PBX1</i>	2-6	Increased incidence in blacks; excellent prognosis with high-dose methotrexate treatment; increased risk of CNS relapse in some studies	80-85	Pui ^{10,31}
Intrachromosomal amplification of chromosome 21	2-3	More common in older children and adolescents; poor prognosis; benefit from intensive induction and early re-intensification therapy	30-40	Attarbaschi ³⁶
t(4;11)(q21;q23) <i>MLL-AF4</i>	1-2	Poor prognosis and predominance in infancy, especially those < 6 months of age; overexpression of <i>FLT3</i>	30-40	Pui ^{10,31}
t(9;22)(q34;q11.2) <i>BCR-ABL1</i>	2-4	Imatinib plus intensive chemotherapy improve early treatment outcome	80-90 at 3 years	Schultz ³⁷
t(8;14)(q23;q32.3)	2	Favorable prognosis with short-term intensive therapy with high-dose methotrexate, cytarabine, and cyclophosphamide	75-85	Pui ³⁸
Hypodiploidy < 44 chromosomes	1-2	Poor prognosis	35-40	Nachman ³⁹
<i>CRLF2</i> overexpression	6-7	Poor prognosis; common in patients with Down syndrome (55%)	?	Mullighan, ⁴⁰ Cario, ⁴¹ Harvey ⁴²
T-cell				
<i>TAL/LMO</i> rearrangement	15-30	Good prognosis in some studies; potentially responsive to histone deacetylase inhibitor	?	Meijerink ⁴³
<i>HOX11</i> rearrangement	7-8	Good prognosis	?	Meijerink ⁴³
<i>HOX11L2 (TLX3)</i> rearrangement	20-24	Poor prognosis in some studies	?	Meijerink ⁴³
<i>HOXA</i> rearrangement	4-5	Poor prognosis; potentially sensitive to histone H3K79 methyltransferases inhibitor	?	Meijerink ⁴³
<i>NUP214-ABL1</i>	6	Sensitive to tyrosine kinase inhibitor	~50 (survival)	Graux ⁴⁴
<i>MLL-ENL</i>	2-3	Favorable prognosis	80-90	Pui ³¹
Early T-cell precursor	12	Poor prognosis; expressed myeloid or stem-cell markers	30-35	Coustan-Smith ⁴⁵
Cooperation mutations				
B-cell precursor				
<i>1KZF1</i> deletions/mutations	15-30	Poor prognosis; resistant to asparaginase and daunorubicin	50-55	Mullighan ⁴⁶ Den Boer ⁴⁷
<i>JAK</i> mutations	?2-5	Predominance in high-risk patients; <i>JAK2</i> mutations in 20% of Down syndrome cases; potentially responsive to <i>JAK2</i> inhibitors	~60	Mullighan ⁴⁸
T-cell				
<i>NOTCH/FBXW7</i> mutations	50	Favorable prognosis; potentially responsive to <i>NOTCH</i> inhibitor	90	Breit ⁴⁹
PTEN-P13K-AKT pathway	~50	? Poor prognosis	?	Gutierrez ⁵⁰
<i>CDKN2A/2B</i> deletions	~70	? Potentially responsive to DNA methyltransferases inhibitor	?	Meijerink ⁴³

Abbreviation: ALL, acute lymphoblastic leukemia.

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.⁶⁰ 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.⁶¹ 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 charac-

terized by this translocation.⁶² Such fusion transcripts have also been identified to persist in the bone marrow of some patients in long-term morphologic remission.⁶³ 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.⁶⁴ Genome-wide 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 CBF α and β subunits respectively account for about a quarter of all cases. These translocations impair CBF transcriptional activation potential. These 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 targeting. The incidences of chromosomal rearrangements involving the *MLL* gene are age-dependent, being highest in children younger than 2 years and subsequently decreasing to less than 5% in adults.⁶⁵ The t(1;22) translocation results in the fusion of the *OTT* gene on chromosome 1 to the Megakaryocytic Acute Leukemia gene on chromosome 22. The translocation is associated closely if not exclusively with acute megakaryoblastic leukemia (AMKL) and has been detected in up to one third of such childhood cases. Monosomy 7, monosomy 5, or 5q deletions are present in only approximately 2% to 4% of cases compared with more than 10% in adults.

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 occurs after the acquisition of a mutation in one allele.^{66,67} 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

(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 $\leq 5\%$ 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 *FMS* 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.⁷⁹ 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}

Genome-Wide Analysis

The initial whole-genome sequencing of an adult with M1 AML and normal karyotype disclosed 10 somatic mutations and two leukemia-specific SNPs.⁸⁶ Two of the mutations involved *FLT3* and *NPM1*, whereas the other eight genes involved nonsense and missense mutations that had not previously been described in AML.⁸⁶ These novel mutations were not identified in 200 additional AML samples, raising the question of whether they were merely passenger mutations. Analysis of the second case of cytogenetically normal AML revealed 12 mutations in annotated protein-coding genes or regulatory RNAs and 52 mutations in noncoding regions of the genome.⁸⁷ Four genes, *NPM1*, *NRAS*, *IDH1*, and a conserved region on chromosome 10, were found in at least one additional sample of 180 adult AMLs. Although the *IDH1* mutation was observed in 16% of 80 cytogenetically normal adult AML samples,⁸⁷ it has not been found in childhood AML.⁸⁸ Independent analyses of *IDH1* and *IDH2* mutations showed that they were more frequently associated with AML having a normal karyotype, but had no prognostic or therapeutic impact.⁸⁹⁻⁹²

Although RNA and microRNA expression signatures can accurately discriminate cases with specific chromosomal translocations and identify novel subsets of AML,⁹³ two independent pediatric studies failed to identify an expression pattern with consistent prognostic significance.^{94,95} Some of the differences observed in gene expression studies are also beginning to be understood in terms of epigenetic regulation of chromatin function. The finding of a similar expression profile between an AML subset with the methylation and silencing of

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 Fanconi 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 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 at 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- β -D-arabino-furanosyl-guanine), 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 $300 \times 10^9/L$. 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 pre-clinical 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)⁶ 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 therapy^{120,121} 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 ALL,^{10,123} 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

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

Category and Agent	Properties
New formulations	
Pegylated asparaginase	Long half-life, reduced immunogenicity (ALL)
Sphingosomal vincristine	Decreased neuropathy, higher tissue concentration, non-vesicant (ALL)
Liposomal annamycin	Decreased cardiotoxicity (ALL/AML)
Liposomal doxorubicin	Decreased cardiotoxicity (ALL/AML)
Liposomal cytarabine	Long half-life, potential neurotoxicity when administered intrathecally (ALL/AML)
Nucleoside analogues	
Clofarabine	Effective for ALL and AML
Nelarabine	Selective for T-cell ALL, potential neurotoxicity
Forodesine (BCX-1777)	Oral phosphorilase inhibitor (ALL)
Monoclonal antibodies	
Rituximab (anti-CD20)	Potential chemotherapy for CD20 ⁺ B-lineage ALL
Alemtuzumab (anti-CD52)	Potential chemotherapy for CD52 ⁺ ALL
Epratuzumab (anti-CD22)	May have synergistic effect with anti-CD20 antibody
CAT-8015; HA22; inotuzumab ozogamicin (anti-CD22)	Cytotoxic for CD22 ⁺ ALL
Gemtuzumab ozogamicin (anti-CD33)	Cytotoxic for CD33 ⁺ leukemia (AML)
Blinatumomab	Bispecific antibodies that direct CD3 ⁺ T-cell against CD19 ⁺ ALL
Molecularly targeted agents	
Tyrosine kinase inhibitors (imatinib, nilotinib, dasatinib, MK-0457, bosutinib, AP24534, DCC2036, sorafenib); Aurora kinase inhibitor (danusertib); Src-family kinase inhibitor; VEGF inhibitors (bosutinib); bevacizumab, SU5416, AZD2171	Potential chemotherapy for Ph ⁺ ALL; targeting c-KIT, VEGF in AML
Fms-like tyrosine kinase -3 (Lestaurtinib, Midostaurin, Tandutinib)	Phase I studies on <i>MLL</i> -rearranged leukemia/ <i>FLT3-ITD</i> positive AML
NOTCH1 inhibitors (γ -secretase inhibitor, antagonists targeting NOTCH transactivation complex, NOTCH1 receptor inhibitors)	Preclinical studies for T-cell ALL
mTOR inhibitors (rapamycin, temsirolimus, everolimus)	Testing in post-transplantation use to decrease graft-versus-host disease and to suppress leukemia (ALL); in combination with chemotherapy in AML
Demethylating agents (decitabine, azacytidine)	Potential chemotherapy by epigenetic modulation of leukemia cells with <i>MLL</i> rearrangement and in AML/MDS
Histone deacetylase inhibitor (vorinostat, valproic acid, depsipeptide)	Potential chemotherapy by epigenetic moderation of leukemia cells in ALL and AML
Heat shock protein inhibitor (17-allylaminogeldanamycin)	Potential chemotherapy of histone deacetylase inhibitor
Proteasome inhibitor (bortezomib, carfilzomib, ONX 0912)	Potential mTOR inhibitors and anthracyclines; clinical trial in AML
Farnesylation (tipifarnib)	Directed at RAS inhibition in AML
JAK2 inhibitor (lestaurtinib)	Phase I trial for ALL and AML with <i>JAK</i> mutations
Microenvironment	
Integrin and cell adhesion inhibition (AMD3100, plerixafor)	CXCR4 inhibition; phase I trials in AML planned
Immunomodulation	
IL-2, IL-6, GM-CSF, WT1, RHAMM-R3 and PR1 peptides, GVAX, dendritic cells	Immunostimulatory approaches to increase T-lymphocyte mediated antileukemic responses in AML

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.

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 sensitive to ATRA and arsenic trioxide. Children with APL also benefit from maintenance therapy with ATRA and antimetabolites.^{129,130} Allogeneic transplantation is not recommended for these children in first remission. Survival after relapse is approximately 70% after reinduction, usually with arsenic trioxide, and then autologous or allogeneic transplantation.^{131,132}

MLL-rearranged AML represents a diverse group with quite variable outcomes.⁶⁵ In an international study, survival was 100%, 63%, 27%, and 22% for patients with the t(1;11), the t(9;11), the t(4;11), and the t(6;11), respectively.⁶⁵ 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}

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 5. Characteristics and Clinical Outcomes of Selected Subtypes of Childhood AML

Subtype	Affected Genes	Gene Functions	Frequency (%)	Clinical Characteristics	5-Year EFS (%)	5-Year OS (%)
Rearrangements						
t(8;21)(q22;q22)	<i>ETO-AML1</i>	Transcription factors	12	Associated with chloromas	55-71	75-85
inv(16)(p13;q22)	<i>MYH11-CBF</i>	Muscle protein/transcription factor	8	Eosinophilia with dysplastic basophilic granules	72-88	75-85
t(8;16)	<i>MOZ-CBP</i>	Transcription factors	1	High WBCs, chloromas, etoposide-related secondary AML	ID*†	ID
t(15;17)(q22;q12)	<i>PML-RAR</i>	Transcription factors/retinoid receptor	12	Associated with FAB M3, Auer rods common; FAB M3; ATRA-sensitive	71†	90†
t(11;17)(q23;q12)	<i>PLZF-RARA</i>	Transcription factors/retinoid receptor	Rare	Associated with FAB M3, Auer rods common; FAB M3; ATRA-resistant	ID	ID
t(1;22)	<i>RBM15-MKL1</i>	RNA binding protein, DNA binding protein	2-3	Associated with FAB M7 in Down syndrome and non-Down syndrome	ID	ID
t(6;9)(p23;q34)	<i>DEK-NUP214(CAN)</i>	Transcription factor/nuclear transport	Rare	Basophilia and multilineage dysplasia; associated with <i>FLT3-ITD</i> and TdT+	ID	ID
MLL						
t(1;11)(q21;q23)	<i>MLL</i> (partner genes) AF1q (MLLT11)	Histone methyltransferase Function unknown, causes short half-life	18 3	76% < age 2 years; 20% and 48% FAB M4 and M5, respectively	92	100
t(4;11)(q21;q23)	AF4 (MLLT2)	Associated with EAP ¹⁰	2	61% < age 2 years; 17% and 42% FAB M4 and M5, respectively	29	27
t(6;11)(q27;q23)	AF6 (MLLT4)	Functions as dimerization domain	5	9% < age 2 years; 57% ≥ 10 years; 35% and 41% FAB M4 and M5, respectively	11	22
t(9;11)(p22;q23)	AF9 (MLLT3)	ENL homolog, associated with EAP	43	42% < age 2 years; 81% is FAB M5	50	63
t(10;11)(p11.2;q23)	AF10 (MLLT10)	Interacts with DOT1L	2	75% < age 2 years; 27% and 55% FAB M4 and M5, respectively	17	27
t(10;11)(p12;q23)	AF10 (MLLT10)	Interacts with DOT1L	13	62% < age 2 years; 72% is FAB M5	31	45
t(11;19)(q23;p13)	ELL or ENL (MLLT1)	Binds histone H3, Assembles EAP	4	58% < age 2 years; 42% and 45% FAB M4 and M5, respectively	49	49
t(11;19)(q23;p13.1)	ELL or ENL (MLLT1)	Binds histone H3, Assembles EAP	4	41% < age 2 years; 30% and 33% FAB M4 and M5 respectively	46	61
t(11;19)(q23;p13.3)	ELL or ENL (MLLT1)	Binds histone H3, Assembles EAP	3	36% < age 2 years; 44% ≥ age 10 years; 20% and 40% FAB M4 and M5	46	47
t(11;17)(q23;q21)	AF17 (MLLT6), LASP1	F-actin rick cytoskeletal activity	2	33% < age 2 years; 42% ≥ age 10 years; 33% and 50% FAB M4 and M5	11	22
Other			19	50% < age 2 years; 29% and 50% FAB M4 and M5, respectively	39	54
Normal karyotype			20			
Gene mutations						
<i>NPM</i>	Nucleophosmin	Nuclear transporter RNA processing	23	8%-10% of childhood AML	65-80	75-85
<i>CEBPα</i>	CCAAT/enhancer binding protein α	Transcription factor	14	4%-6% of all childhood AML; more common in older patients, FAB M1 or M2	70	83
<i>FLT3/ALM</i>	Fms-like tyrosine kinase 3 activation loop domain	Receptor for FLT3	3	6%-7% of all childhood AML	50-60	60-70
<i>FLT3-ITD</i>	Fms-like tyrosine kinase 3 internal tandem duplication	Receptor for FLT3	18	10%-15% of all childhood AML	< 35	< 35
<i>WT1</i>	Wilms tumor 1	Transcription factor	13	8%-10% of all childhood AML	22-35	35-56
<i>RAS</i>	Rat sarcoma gene	Signal transduction	3	5% of all childhood AML	ID	ID
<i>PTPN11</i>	Protein tyrosine phosphatase, non-receptor type 11	Tyrosine phosphatase	2	Most commonly associated with JMML	ID	ID
No known mutations			24		40	50
Poor-risk cytogenetics			< 15		< 40	< 40
Del 5q/E5			1			
-7			2			

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 Lange et al,¹⁵ Creutzig et al,²⁰ Smith et al,²¹ Ravindranath et al,²⁵ Rubnitz et al,²⁶ Gibson et al,²⁷ and Cooper et al.³⁰

†Data extracted from Ho et al,⁷⁰ Meshinchi et al,⁷³ Brown et al,⁸² de Botton et al,¹²⁴ Hollink et al,¹²⁵ and Ho et al.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* (eg, *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)-, -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 (ie, 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 $10 \times 10^9/L$, 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 \leq or more than $10 \times 10^9/L$, 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 anthracycline 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 *MLL*¹⁴⁹ and *bcl-2*¹⁵⁰ 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}

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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 "U" are those for which no compensation was received; those relationships 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

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

AUTHOR CONTRIBUTIONS

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

REFERENCES

- Conter V, Aricò M, Basso G, et al: Long-term results of the Italian Association of Pediatric Hematology and Oncology (AIEOP) Studies 82, 87, 88, 91 and 95 for childhood acute lymphoblastic leukemia. *Leukemia* 24:255-264, 2010
- Mörücke A, Zimmermann M, Reiter A, et al: Long-term results of five consecutive trials in childhood acute lymphoblastic leukemia performed by the ALL-BFM study group from 1981 to 2000. *Leukemia* 24:265-284, 2010
- Gaynon PS, Angiolillo AL, Carroll WL, et al: Long-term results of the Children's Cancer Group studies for childhood acute lymphoblastic leukemia 1983-2002: A Children's Oncology Group Report. *Leukemia* 24:285-297, 2010
- Escherich G, Horstmann MA, Zimmermann M, et al: Cooperative study group for childhood acute lymphoblastic leukaemia (COALL): Long-term results of trials 82,85,89,92 and 97. *Leukemia* 24:298-308, 2010
- Stary J, Jabali Y, Trka J, et al: Long-term results of treatment of childhood acute lymphoblastic leukemia in the Czech Republic. *Leukemia* 24:425-428, 2010
- Silverman LB, Stevenson KE, O'Brien JE, et al: Long-term results of Dana-Farber Cancer Institute ALL Consortium protocols for children with newly diagnosed acute lymphoblastic leukemia (1985-2000). *Leukemia* 24:320-334, 2010
- Stark B, Nirel R, Avrahami G, et al: Long-term results of the Israeli National Studies in childhood acute lymphoblastic leukemia: INS 84, 89 and 98. *Leukemia* 24:419-424, 2010
- Schmiegelow K, Forestier E, Hellebostad M, et al: Long-term results of NOPHO ALL-92 and ALL-2000 studies of childhood acute lymphoblastic leukemia. *Leukemia* 24:345-354, 2010
- Pui CH, Pei D, Sandlund JT, et al: Long-term results of St Jude Total Therapy Studies 11, 12, 13A, 13B, and 14 for childhood acute lymphoblastic leukemia. *Leukemia* 24:371-382, 2010
- Pui CH, Campana D, Pei D, et al: Treating childhood acute lymphoblastic leukemia without cranial irradiation. *N Engl J Med* 360:2730-2741, 2009
- Tsuchida M, Ohara A, Manabe A, et al: Long-term results of Tokyo Children's Cancer Study Group trials for childhood acute lymphoblastic leukemia, 1984-1999. *Leukemia* 24:383-396, 2010
- Liang DC, Yang CP, Lin DT, et al: Long-term results of Taiwan Pediatric Oncology Group studies 1997 and 2002 for childhood acute lymphoblastic leukemia. *Leukemia* 24:397-405, 2010
- Mitchell C, Richards S, Harrison CJ, et al: Long-term follow-up of the United Kingdom medical research council protocols for childhood acute lymphoblastic leukaemia, 1980-2001. *Leukemia* 24:406-418, 2010
- Kamps WA, van der Pal-de Bruin KM, Veerman AJ, et al: Long-term results of Dutch Childhood Oncology Group studies for children with acute lymphoblastic leukemia from 1984 to 2004. *Leukemia* 24:309-319, 2010
- Lange BJ, Smith FO, Feusner J, et al: Outcomes in CCG-2961, a Children's Oncology Group phase 3 trial for untreated pediatric acute myeloid leukemia: A report from the Children's Oncology Group. *Blood* 111:1044-1053, 2008
- Burnett AK, Hills RK, Milligan DW, et al: Attempts to optimize induction and consolidation treatment in acute myeloid leukemia: Results of the MRC AML12 trial. *J Clin Oncol* 28:586-595, 2010
- Tomizawa D, Tabuchi K, Kinoshita A, et al: Repetitive cycles of high-dose cytarabine are effective for childhood acute myeloid leukemia: Long-term outcome of the children with AML treated on two consecutive trials of Tokyo Children's Cancer Study Group. *Pediatr Blood Cancer* 49:127-132, 2007
- Armendariz H, Barbieri MA, Freigeiro D, et al: Treatment strategy and long-term results in pediatric patients treated in two consecutive AML-GATLA trials. *Leukemia* 19:2139-2142, 2005
- Lie SO, Abrahamsson J, Clausen N, et al: Long-term results in children with AML: NOPHO-AML Study Group—Report of three consecutive trials. *Leukemia* 19:2090-2100, 2005
- Creutzig U, Zimmermann M, Ritter J, et al: Treatment strategies and long-term results in paediatric patients treated in four consecutive AML-BFM trials. *Leukemia* 19:2030-2042, 2005
- Smith FO, Alonzo TA, Gerbing RB, et al: Long-term results of children with acute myeloid leukemia: A report of three consecutive phase III trials by the Children's Cancer Group: CCG 251, CCG 213 and CCG 2891. *Leukemia* 19:2054-2062, 2005
- Pession A, Barbieri E: Treatment and prevention of tumor lysis syndrome in children. *Contrib Nephrol* 147:80-92, 2005
- Entz-Werle N, Suciu S, van der Werff ten Bosch J, et al: Results of 58872 and 58921 trials in acute myeloblastic leukemia and relative value of chemotherapy vs allogeneic bone marrow transplantation in first complete remission: The EORTC Children Leukemia Group report. *Leukemia* 19:2072-2081, 2005
- Perel Y, Auvrignon A, Leblanc T, et al: Treatment of childhood acute myeloblastic leukemia: Dose intensification improves outcome and maintenance therapy is of no benefit—Multicenter studies of the French LAME (Leucemie Aigue Myeloblastique Enfant) Cooperative Group. *Leukemia* 19:2082-2089, 2005
- Ravindranath Y, Chang M, Steuber CP, et al: Pediatric Oncology Group (POG) studies of acute myeloid leukemia (AML): A review of four consecutive childhood AML trials conducted between 1981 and 2000. *Leukemia* 19:2101-2116, 2005
- Rubnitz JE, Inaba H, Dahl G, et al: Minimal residual disease-directed therapy for childhood acute myeloid leukaemia: Results of the AML02 multicentre trial. *Lancet Oncol* 11:543-552, 2010
- Gibson BE, Wheatley K, Hann IM, et al: Treatment strategy and long-term results in paediatric patients treated in consecutive UK AML trials. *Leukemia* 19:2130-2138, 2005
- Tsukimoto I, Tawa A, Horibe K, et al: Risk-stratified therapy and the intensive use of cytarabine improves the outcome in childhood acute myeloid leukemia: The AML99 trial from the Japanese Childhood AML Cooperative Study Group. *J Clin Oncol* 27:4007-4013, 2009
- Quintana J, Advis P, Becker A, et al: Acute myelogenous leukemia in Chile PINDA protocols 87 and 92 results. *Leukemia* 19:2143-2146, 2005
- Franklin J, Alonzo T, Hurwitz CA, et al: COG AAML03P1: Efficacy and safety in a pilot study of intensive chemotherapy including gemtuzumab in children newly diagnosed with acute myeloid leukemia (AML). *ASH Annual Meeting Abstracts* 112:56, 2008 (abstr 13)
- Pui CH, Robison LL, Look AT: Acute lymphoblastic leukaemia. *Lancet* 371:1030-1043, 2008
- Pui CH, Relling MV, Downing JR: Acute lymphoblastic leukemia. *N Engl J Med* 350:1535-1548, 2004
- Reaman GH, Smith FO: *Childhood Leukemias*. Heidelberg, Germany, Springer Verlag, 2010 (in press)
- Mullighan CG, Goorha S, Radtke I, et al: Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. *Nature* 446:758-764, 2007
- Salzer WL, Devidas M, Carroll WL, et al: Long-term results of the pediatric oncology group studies for childhood acute lymphoblastic leukemia 1984-2001: A report from the Children's Oncology Group. *Leukemia* 24:355-370, 2010

36. Attarbaschi A, Mann G, Panzer-Grümayer R, et al: Minimal residual disease values discriminate between low and high relapse risk in children with B-cell precursor acute lymphoblastic leukemia and an intrachromosomal amplification of chromosome 21: The Austrian and German acute lymphoblastic leukemia Berlin-Frankfurt-Munster (ALL-BFM) trials. *J Clin Oncol* 26:3046-3050, 2008
37. Schultz KR, Bowman WP, Aledo A, et al: Improved early event-free survival with imatinib in Philadelphia chromosome-positive acute lymphoblastic leukemia: A Children's Oncology Group study. *J Clin Oncol* 27:5175-5181, 2009
38. Pui CH, Evans WE: Treatment of acute lymphoblastic leukemia. *N Engl J Med* 354:166-178, 2006
39. Nachman JB, Heerema NA, Sather H, et al: Outcome of treatment in children with hypodiploid acute lymphoblastic leukemia. *Blood* 110:1112-1115, 2007
40. Mullighan CG, Collins-Underwood JR, Phillips LA, et al: Rearrangement of CRLF2 in B-progenitor- and Down syndrome-associated acute lymphoblastic leukemia. *Nat Genet* 41:1243-1246, 2009
41. Cario G, Zimmermann M, Romey R, et al: Presence of the P2RY8-CRLF2 rearrangement is associated with a poor prognosis in non-high-risk precursor B-cell acute lymphoblastic leukemia in children treated according to the ALL-BFM 2000 protocol. *Blood* 115:5393-5397, 2010
42. Harvey RC, Mullighan CG, Chen IM, et al: Rearrangement of CRLF2 is associated with mutation of JAK kinases, alteration of IKZF1, Hispanic/Latino ethnicity, and a poor outcome in pediatric B-progenitor acute lymphoblastic leukemia. *Blood* 115:5312-5321, 2010
43. Meijerink JP, den Boer ML, Pieters R: New genetic abnormalities and treatment response in acute lymphoblastic leukemia. *Semin Hematol* 46:16-23, 2009
44. Graux C, Stevens-Kroef M, Lafage M, et al: Heterogeneous patterns of amplification of the NUP214-ABL1 fusion gene in T-cell acute lymphoblastic leukemia. *Leukemia* 23:125-133, 2009
45. Coustan-Smith E, Mullighan CG, Onciu M, et al: Early T-cell precursor leukaemia: A subtype of very high-risk acute lymphoblastic leukaemia. *Lancet Oncol* 10:147-156, 2009
46. Mullighan CG, Su X, Zhang J, et al: Deletion of IKZF1 and prognosis in acute lymphoblastic leukemia. *N Engl J Med* 360:470-480, 2009
47. Den Boer ML, van Sleghenthorst M, De Menezes RX, et al: A subtype of childhood acute lymphoblastic leukaemia with poor treatment outcome: A genome-wide classification study. *Lancet Oncol* 10:125-134, 2009
48. Mullighan CG, Zhang J, Harvey RC, et al: JAK mutations in high-risk childhood acute lymphoblastic leukemia. *Proc Natl Acad Sci U S A* 106:9414-9418, 2009
49. Breit S, Stanulla M, Flohr T, et al: Activating NOTCH1 mutations predict favorable early treatment response and long-term outcome in childhood precursor T-cell lymphoblastic leukemia. *Blood* 108:1151-1157, 2006
50. Gutierrez A, Sanda T, Grebliunaite R, et al: High frequency of PTEN, PI3K, and AKT abnormalities in T-cell acute lymphoblastic leukemia. *Blood* 114:647-650, 2009
51. Mullighan CG, Miller CB, Radtke I, et al: BCR-ABL1 lymphoblastic leukaemia is characterized by the deletion of Ikaros. *Nature* 453:110-114, 2008
52. Hertzberg L, Vendramini E, Ganmore I, et al: Down syndrome acute lymphoblastic leukemia, a highly heterogeneous disease in which aberrant expression of CRLF2 is associated with mutated JAK2: A report from the International BFM Study Group. *Blood* 115:1006-1017, 2010
53. van Grotel M, Meijerink JP, van Wering ER, et al: Prognostic significance of molecular-cytogenetic abnormalities in pediatric T-ALL is not explained by immunophenotypic differences. *Leukemia* 22:124-131, 2008
54. Yang JJ, Bhojwani D, Yang W, et al: Genome-wide copy number profiling reveals molecular evolution from diagnosis to relapse in childhood acute lymphoblastic leukemia. *Blood* 112:4178-4183, 2008
55. Mullighan CG, Phillips LA, Su X, et al: Genomic analysis of the clonal origins of relapsed acute lymphoblastic leukemia. *Science* 322:1377-1380, 2008
56. Bhojwani D, Kang H, Moskowitz NP, et al: Biologic pathways associated with relapse in childhood acute lymphoblastic leukemia: A Children's Oncology Group study. *Blood* 108:711-717, 2006
57. Papaemmanuil E, Hosking FJ, Vijayakrishnan J, et al: Loci on 7p12.2, 10q21.2 and 14q11.2 are associated with risk of childhood acute lymphoblastic leukemia. *Nat Genet* 41:1006-1010, 2009
58. Treviño LR, Yang W, French D, et al: Germline genomic variants associated with childhood acute lymphoblastic leukemia. *Nat Genet* 2009 41:1001-1005, 2009
59. Yang W, Treviño LR, Yang JJ, et al: ARID5B SNP rs10821936 is associated with risk of childhood acute lymphoblastic leukemia in blacks and contributes to racial differences in leukemia incidence. *Leukemia* 24:894-896, 2010
60. Lane SW, Scadden DT, Gilliland DG: The leukemic stem cell niche: Current concepts and therapeutic opportunities. *Blood* 114:1150-1157, 2009
61. Gilliland DG, Jordan CT, Felix CA: The molecular basis of leukemia. *Am Soc Hematol Educ Program* 80-97, 2004
62. Wiemels J, Kang M, Greaves M: Backtracking of leukemic clones to birth. *Methods Mol Biol* 538:7-27, 2009
63. Weisser M, Haferlach C, Hiddemann W, et al: The quality of molecular response to chemotherapy is predictive for the outcome of AML1-ETO-positive AML and is independent of pretreatment risk factors. *Leukemia* 21:1177-1182, 2007
64. Mori H, Colman SM, Xiao Z, et al: Chromosome translocations and covert leukemic clones are generated during normal fetal development. *Proc Natl Acad Sci U S A* 99:8242-8247, 2002
65. Balgobind BV, Raimondi SC, Harbott J, et al: Novel prognostic subgroups in childhood 11q23/MLL-rearranged acute myeloid leukemia: Results of an international retrospective study. *Blood* 114:2489-2496, 2009
66. Gupta M, Raghavan M, Gale RE, et al: Novel regions of acquired uniparental disomy discovered in acute myeloid leukemia. *Genes Chromosomes Cancer* 47:729-739, 2008
67. Raghavan M, Smith LL, Lillington DM, et al: Segmental uniparental disomy is a commonly acquired genetic event in relapsed acute myeloid leukemia. *Blood* 112:814-821, 2008
68. Radtke I, Mullighan CG, Ishii M, et al: Genomic analysis reveals few genetic alterations in pediatric acute myeloid leukemia. *Proc Natl Acad Sci U S A* 106:12944-12949, 2009
69. Walter MJ, Payton JE, Ries RE, et al: Acquired copy number alterations in adult acute myeloid leukemia genomes. *Proc Natl Acad Sci U S A* 106:12950-12955, 2009
70. Ho PA, Alonzo TA, Gerbing RB, et al: Prevalence and prognostic implications of CEBPA mutations in pediatric acute myeloid leukemia (AML): A report from the Children's Oncology Group. *Blood* 113:6558-6566, 2009
71. Kirstetter P, Schuster MB, Bereshchenko O, et al: Modeling of C/EBPalpha mutant acute myeloid leukemia reveals a common expression signature of committed myeloid leukemia-initiating cells. *Cancer Cell* 13:299-310, 2008
72. Crispino JD: GATA1 mutations in Down syndrome: Implications for biology and diagnosis of children with transient myeloproliferative disorder and acute megakaryoblastic leukemia. *Pediatr Blood Cancer* 44:40-44, 2005
73. Meshinchi S, Alonzo TA, Stirewalt DL, et al: Clinical implications of FLT3 mutations in pediatric AML. *Blood* 108:3654-3661, 2006
74. Schnittger S, Kohl TM, Haferlach T, et al: KIT-D816 mutations in AML1-ETO-positive AML are associated with impaired event-free and overall survival. *Blood* 107:1791-1799, 2006
75. Cairoli R, Beghini A, Grillo G, et al: Prognostic impact of c-KIT mutations in core binding factor leukemias: An Italian retrospective study. *Blood* 107:3463-3468, 2006
76. Nanri T, Matsuno N, Kawakita T, et al: Mutations in the receptor tyrosine kinase pathway are associated with clinical outcome in patients with acute myeloblastic leukemia harboring t(8;21)(q22;q22). *Leukemia* 19:1361-1366, 2005
77. Paschka P, Marcucci G, Ruppert AS, et al: Adverse prognostic significance of KIT mutations in adult acute myeloid leukemia with inv(16) and t(8;21): A Cancer and Leukemia Group B Study. *J Clin Oncol* 24:3904-3911, 2006
78. Paschka P, Du J, Schlenk RF, et al: Mutations in the *Fms-related tyrosine kinase 3 (FLT3)* gene independently predict poor outcome in acute myeloid leukemia (AML) with t(8;21): A study of the German-Austrian AML Study Group (AMLSG). *Am Soc Hematol Annual Meeting Abstracts* 114:340, 2009 (abstr 825)
79. Shih LY, Liang DC, Huang CF, et al: Cooperating mutations of receptor tyrosine kinases and Ras genes in childhood core-binding factor acute myeloid leukemia and a comparative analysis on paired diagnosis and relapse samples. *Leukemia* 22:303-307, 2008
80. Berman JN, Gerbing RB, Sung L, et al: Prevalence and clinical implications of N-RAS mutations in childhood AML: A report from the Children's Oncology Group. *Am Soc Hematol Annual Meeting Abstracts* 114:1211, 2009 (abstr 3115)
81. Scholl S, Fricke HJ, Sayer HG, et al: Clinical implications of molecular genetic aberrations in acute myeloid leukemia. *J Cancer Res Clin Oncol* 135:491-505, 2009
82. Brown P, McIntyre E, Rau R, et al: The incidence and clinical significance of nucleophosmin mutations in childhood AML. *Blood* 110:979-985, 2007
83. Hollink IH, Zwaan CM, Zimmermann M, et al: Favorable prognostic impact of NPM1 gene mutations in childhood acute myeloid leukemia, with emphasis on cytogenetically normal AML. *Leukemia* 23:262-270, 2009
84. Schnittger S, Kern W, Tschulik C, et al: Minimal residual disease levels assessed by NPM1

- mutation-specific RQ-PCR provide important prognostic information in AML. *Blood* 114:2220-2231, 2009
85. Haferlach C, Mecucci C, Schnittger S, et al: AML with mutated NPM1 carrying a normal or aberrant karyotype show overlapping biologic, pathologic, immunophenotypic, and prognostic features. *Blood* 114:3024-3032, 2009
86. Ley TJ, Mardis ER, Ding L, et al: DNA sequencing of a cytogenetically normal acute myeloid leukaemia genome. *Nature* 456:66-72, 2008
87. Mardis ER, Ding L, Dooling DJ, et al: Recurring mutations found by sequencing an acute myeloid leukemia genome. *N Engl J Med* 361:1058-1066, 2009
88. Ho PA, Alonzo TA, Kopecky KJ, et al: Molecular alterations of the IDH1 gene in AML: A Children's Oncology Group and Southwest Oncology Group study. *Leukemia* 24:909-913, 2010
89. Thol F, Damm F, Wagner K, et al: Prognostic impact of IDH2 mutations in cytogenetically normal acute myeloid leukemia. *Blood* 116:614-616, 2010
90. Chou WC, Hou HA, Chen CY, et al: Distinct clinical and biologic characteristics in adult acute myeloid leukemia bearing the isocitrate dehydrogenase 1 mutation. *Blood* 115:2749-2754, 2010
91. Marcucci G, Mahary G, Wu YZ, et al: IDH1 and IDH2 Gene mutations identify novel molecular subsets within de novo cytogenetically normal acute myeloid leukemia: A Cancer and Leukemia Group B study. *J Clin Oncol* 28:2348-2355, 2010
92. Wagner K, Damm F, Gohring G, et al: Impact of IDH1 R132 mutations and an IDH1 single nucleotide polymorphism in cytogenetically normal acute myeloid leukemia: SNP rs11554137 is an adverse prognostic factor. *J Clin Oncol* 28:2356-2364, 2010
93. Wouters BJ, Löwenberg B, Delwel R: A decade of genome-wide gene expression profiling in acute myeloid leukemia: Flashback and prospects. *Blood* 113:291-298, 2009
94. Wouters BJ, Jorda MA, Keeshan K, et al: Distinct gene expression profiles of acute myeloid/T-lymphoid leukemia with silenced CEBPA and mutations in NOTCH1. *Blood* 110:3706-3714, 2007
95. Figueroa ME, Wouters BJ, Skrabanek L, et al: Genome-wide epigenetic analysis delineates a biologically distinct immature acute leukemia with myeloid/T-lymphoid features. *Blood* 113:2795-2804, 2009
96. Chen J, Odenike O, Rowley JD: Leukaemogenesis: More than mutant genes. *Nat Rev Cancer* 10:23-36, 2010
97. Figueroa ME, Lugthart S, Li Y, et al: DNA methylation signatures identify biologically distinct subtypes in acute myeloid leukemia. *Cancer Cell* 17:13-27, 2010
98. Golub T, Arcenci RJ: Acute myelogenous leukemia, in Pizzo P, Poplack G (eds): Principles and Practice of Pediatric Oncology. Philadelphia, PA, Lippincott Williams & Wilkins, 2006, pp 591-644
99. Arcenci RJ, Brown P: Myeloid leukemias and myelodysplastic syndromes, in Carroll W, Finlay J (eds): Cancer in Children: The Clinical Biology of Childhood Cancer. Boston, MA, Jones and Bartlett Publishers, 2010, pp 185-215
100. Stanulla M, Schrappe M: Treatment of childhood acute lymphoblastic leukemia. *Semin Hematol* 46:52-63, 2009
101. Pui CH, Jeha S: New therapeutic strategies for the treatment of acute lymphoblastic leukaemia. *Nat Rev Drug Discov* 6:149-165, 2007
102. Moghrabi A, Levy DE, Asselin B, et al: Results of the Dana-Farber Cancer Institute ALL Consortium Protocol 95-01 for children with acute lymphoblastic leukemia. *Blood* 109:896-904, 2007
103. Berg SL, Blaney SM, Devidas M, et al: Phase II study of nelarabine (compound 506U78) in children and young adults with refractory T-cell malignancies: A report from the Children's Oncology Group. *J Clin Oncol* 23:3376-3382, 2005
104. Schrappe M, Zimmermann M, Mörcke A, et al: Dexamethasone in induction can eliminate one third of all relapses in childhood acute lymphoblastic leukemia (ALL): Results of an international randomized trial in 3655 patients (Trial AEIOP-BFM ALL 2000). *Am Soc Hematol Annual Meeting Abstracts* 112:9, 2008 (abstr 7)
105. Pieters R, Schrappe M, De Lorenzo P, et al: A treatment protocol for infants younger than 1 year with acute lymphoblastic leukaemia (Interfant-99): An observational study and a multicentre randomised trial. *Lancet* 370:240-250, 2007
106. Pui CH, Gaynon PS, Boyett JM, et al: Outcome of treatment in childhood acute lymphoblastic leukaemia with rearrangements of the 11q23 chromosomal region. *Lancet* 359:1909-1915, 2002
107. Brown P, Levis M, McIntyre E, et al: Combinations of the FLT3 inhibitor CEP-701 and chemotherapy synergistically kill infant and childhood MLL-rearranged ALL cells in a sequence-dependent manner. *Leukemia* 20:1368-1376, 2006
108. Stumpel DJ, Schneider P, van Roon EH, et al: Specific promoter methylation identifies different subgroups of MLL-rearranged infant acute lymphoblastic leukemia, influences clinical outcome, and provides therapeutic options. *Blood* 114:5490-5498, 2009
109. Schafer E, Irizarry R, Negi S, et al: Promoter hypermethylation in MLL-r infant acute lymphoblastic leukemia: Biology and therapeutic targeting. *Blood* 115:4798-4809, 2010
110. Pulte D, Gondos A, Brenner H: Improvement in survival in younger patients with acute lymphoblastic leukemia from the 1980s to the early 21st century. *Blood* 113:1408-1411, 2009
111. Stock W, La M, Sanford B, et al: What determines the outcomes for adolescents and young adults with acute lymphoblastic leukemia treated on cooperative group protocols? A comparison of Children's Cancer Group and Cancer and Leukemia Group B studies. *Blood* 112:1646-1654, 2008
112. Nachman JB, La MK, Hunger SP, et al: Young adults with acute lymphoblastic leukemia have an excellent outcome with chemotherapy alone and benefit from intensive postinduction treatment: A report from the children's oncology group. *J Clin Oncol* 27:5189-5194, 2009
113. Boissel N, Auclerc MF, Lheritier V, et al: Should adolescents with acute lymphoblastic leukemia be treated as old children or young adults? Comparison of the French FRALLE-93 and LALA-94 trials. *J Clin Oncol* 21:774-780, 2003
114. de Bont JM, Holt B, Dekker AW, et al: Significant difference in outcome for adolescents with acute lymphoblastic leukemia treated on pediatric vs adult protocols in the Netherlands. *Leukemia* 18:2032-2035, 2004
115. Pui CH, Pei D, Campana D, et al: Improved prognosis for older adolescents with acute lymphoblastic leukemia. *J Clin Oncol* (in press)
116. Goldstone AH, Richards SM, Lazarus HM, et al: In adults with standard-risk acute lymphoblastic leukemia, the greatest benefit is achieved from a matched sibling allogeneic transplantation in first complete remission, and an autologous transplantation is less effective than conventional consolidation/maintenance chemotherapy in all patients: Final results of the International ALL Trial (MRC UKALL XII/ECOG E2993). *Blood* 111:1827-1833, 2008
117. Ribera JM, Oriol A, Sanz MA, et al: Comparison of the results of the treatment of adolescents and young adults with standard-risk acute lymphoblastic leukemia with the Programa Espanol de Tratamiento en Hematologia pediatric-based protocol ALL-96. *J Clin Oncol* 26:1843-1849, 2008
118. DeAngelo DJ, Dahlberg S, Silverman LB, et al: A multicenter phase II study using a dose intensified pediatric regimen in adults with untreated acute lymphoblastic leukemia. *Am Soc Hematol Annual Meeting Abstracts* 110:181a, 2007 (abstr 587)
119. Storing JM, Minden MD, Kao S, et al: Treatment of adults with BCR-ABL negative acute lymphoblastic leukaemia with a modified paediatric regimen. *Br J Haematol* 146:76-85, 2009
120. Imai C, Iwamoto S, Campana D: Genetic modification of primary natural killer cells overcomes inhibitory signals and induces specific killing of leukemic cells. *Blood* 106:376-383, 2005
121. Triplett B, Handgretinger R, Pui CH, et al: KIR-incompatible hematopoietic-cell transplantation for poor prognosis infant acute lymphoblastic leukemia. *Blood* 107:1238-1239, 2006
122. Bargou R, Leo E, Zugmaier G, et al: Tumor regression in cancer patients by very low doses of a T cell-engaging antibody. *Science* 321:974-977, 2008
123. Veerman AJ, Kamps WA, van den Berg H, et al: Dexamethasone-based therapy for childhood acute lymphoblastic leukaemia: Results of the prospective Dutch Childhood Oncology Group (DCOG) protocol ALL-9 (1997-2004). *Lancet Oncol* 10:957-966, 2009
124. de Botton S, Coiteux V, Chevret S, et al: Outcome of childhood acute promyelocytic leukemia with all-trans-retinoic acid and chemotherapy. *J Clin Oncol* 22:1404-1412, 2004
125. Hollink IH, van den Heuvel-Eibrink MM, Zimmermann M, et al: Clinical relevance of Wilms tumor 1 gene mutations in childhood acute myeloid leukemia. *Blood* 113:5951-5960, 2009
126. Ho PA, Zeng R, Alonzo TA, et al: Prevalence and prognostic implications of WT1 mutations in pediatric acute myeloid leukemia (AML): a report from the Children's Oncology Group. *Blood* 116:702-710, 2010
127. Abrahamsson J, Clausen N, Gustafsson G, et al: Improved outcome after relapse in children with acute myeloid leukaemia. *Br J Haematol* 136:229-236, 2007
128. Ortega JJ, Madero L, Martin G, et al: Treatment with all-trans retinoic acid and anthracycline monotherapy for children with acute promyelocytic leukemia: A multicenter study by the PETHEMA Group. *J Clin Oncol* 23:7632-7640, 2005
129. Gregory J, Kim H, Alonzo T, et al: Treatment of children with acute promyelocytic leukemia: Results of the first North American Intergroup trial INT0129. *Pediatr Blood Cancer* 53:1005-1010, 2009
130. Sanz MA, Grimwade D, Tallman MS, et al: Management of acute promyelocytic leukemia: Recommendations from an expert panel on behalf of the European LeukemiaNet. *Blood* 113:1875-1891, 2009
131. de Botton S, Fawaz A, Chevret S, et al: Autologous and allogeneic stem-cell transplantation as salvage treatment of acute promyelocytic leukemia initially treated with all-trans-retinoic acid: A

retrospective analysis of the European acute promyelocytic leukemia group. *J Clin Oncol* 23:120-126, 2005

132. Thirugnanam R, George B, Chendamarai E, et al: Comparison of clinical outcomes of patients with relapsed acute promyelocytic leukemia induced with arsenic trioxide and consolidated with either an autologous stem cell transplant or an arsenic trioxide-based regimen. *Biol Blood Marrow Transplant* 15:1479-1484, 2009

133. Oliansky DM, Rizzo JD, Aplan PD, et al: The role of cytotoxic therapy with hematopoietic stem cell transplantation in the therapy of acute myeloid leukemia in children: An evidence-based review. *Biol Blood Marrow Transplant* 13:1-25, 2007

134. Bernstein J, Dastugue N, Haas OA, et al: Nineteen cases of the t(1;22)(p13;q13) acute megakaryoblastic leukaemia of infants/children and a review of 39 cases: Report from a t(1;22) study group. *Leukemia* 14:216-218, 2000

135. Duchayne E, Fenneteau O, Pages MP, et al: Acute megakaryoblastic leukaemia: A national clinical and biological study of 53 adult and childhood cases by the Groupe Francais d'Hematologie Cellulaire (GFHC). *Leuk Lymphoma* 44:49-58, 2003

136. Pratz KW, Sato T, Murphy KM, et al: FLT3-mutant allelic burden and clinical status are predictive of response to FLT3 inhibitors in AML. *Blood* 115:1425-1432, 2010

137. Meshinchi S, Arceci RJ, Sanders JE, et al: Role of allogeneic stem cell transplantation in FLT3/ITD-positive AML. *Blood* 108:400-401, 2006

138. Pollard JA, Alonzo TA, Gerbing RB, et al: Prevalence and prognostic significance of KIT mutations in pediatric patients with core binding factor AML enrolled on serial pediatric cooperative trials for de novo AML. *Blood* 115:2372-2379, 2010

139. Sievers EL, Lange BJ, Alonzo TA, et al: Immunophenotypic evidence of leukemia after induction therapy predicts relapse: Results from a prospective Children's Cancer Group study of 252 acute myeloid leukemia patients. *Blood* 101:3398-3406, 2003

140. Gamis AS: Acute myeloid leukemia and Down syndrome evolution of modern therapy: State of the art review. *Pediatr Blood Cancer* 44:13-20, 2005

141. O'Brien MM, Taub JW, Chang MN, et al: Cardiomyopathy in children with Down syndrome treated for acute myeloid leukemia: A report from the Children's Oncology Group Study POG 9421. *J Clin Oncol* 26:414-420, 2008

142. Tallman MS: The expanding role of arsenic in acute promyelocytic leukemia. *Semin Hematol* 45:S25-S29, 2008

143. Powell BL: Effect of consolidation with arsenic trioxide (As₂O₃) on event-free survival (EFS) and overall survival (OS) among patients with newly diagnosed acute promyelocytic leukemia (APL): North American Intergroup Protocol C9710. *J Clin Oncol* 25:1s, 2007 (abstr 2)

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)

145. Adès L, Chevret S, Raffoux E, et al: Is cytarabine useful in the treatment of acute promyelocytic leukemia? Results of a randomized trial from the European Acute Promyelocytic Leukemia Group. *J Clin Oncol* 24:5703-5710, 2006

146. Estey EH, Giles FJ, Beran M, et al: Experience with gemtuzumab ozogamycin ("mylotarg") and all-trans retinoic acid in untreated acute promyelocytic leukemia. *Blood* 99:4222-4224, 2002

147. Lo Coco F, Ammatuna E, Noguera N: Treatment of acute promyelocytic leukemia with gemtuzumab ozogamycin. *Clin Adv Hematol Oncol* 4:57-62, 76-77, 2006

148. Ishii E, Oda M, Kinugawa N, et al: Features and outcome of neonatal leukemia in Japan: Experience of the Japan infant leukemia study group. *Pediatr Blood Cancer* 47:268-272, 2006

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

150. Robinson BW, Behling KC, Gupta M, et al: Abundant anti-apoptotic BCL-2 is a molecular target in leukaemias with t(4;11) translocation. *Br J Haematol* 141:827-839, 2008

151. Yang JJ, Cheng C, Yang W, et al: Genome-wide interrogation of germline genetic variation associated with treatment response in childhood acute lymphoblastic leukemia. *JAMA* 301:393-403, 2009

152. Treviño LR, Shimasaki N, Yang W, et al: Germline genetic variation in an organic anion transporter polypeptide associated with methotrexate pharmacokinetics and clinical effects. *J Clin Oncol* 27:5972-5978, 2009

