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REVIEW ARTICLE

Pediatric acute lymphoblastic leukemia: state of the art and new trends

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Abstract:
Recently, much progress has been made in understanding the biology, genetics and therapy of acute lymphoblastic leukemia – a heterogeneous group of lymphoid disorders resulting from a monoclonal proliferation and expansion of immature B or T lymphocyte progenitor in the bone marrow, blood, and other organs. The disease is most common in children, but can occur in any age group. ALL in infants aged younger than 12 months is both rare and biologically different from ALL in older children. Children with ALL develop symptoms related to infiltration of blasts in the bone marrow, lymphoid system, and extramedullary sites, such as the central nervous system. In most patients, the cause of ALL is unknown. Diagnosis of ALL relies on an assessment of morphology, flow cytometry immunophenotyping, and identification of cytogenetic-molecular abnormalities. In ALL immunological markers give information of relevance to prognosis – the worst prognosis is seen in the small minority of cases showing a mature B phenotype. The subtypes of ALL may be further determined by genomic profiling. Recent genomewide analyses of DNA copynumber abnormalities have identified numerous recurring genetic alterations in ALL. Mutations of genes encoding transcriptional regulators of B lymphoid development, including PAX5, EBF1, and IKZF1, occur in more than 40% of patients with B-cell–progenitor ALL. Children whose ALL cells exhibit in vitro resistance to antileukemic agents have a substantially worse prognosis than children whose ALL cells are drug-sensitive. The currently accepted philosophy behind treatment of pediatric ALL is based on the recognition of the heterogeneity of the disease and the definition of specific cytogenetic-molecular abnormalities.

Keywords: acute lymphoblastic leukemia, children, diagnosis, therapy
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Inroduction

Acute lymphoblastic leukemia (ALL) is a heterogeneous group of lymphoid disorders that result from a monoclonal proliferation and expansion of immature B or T lymphocyte progenitor in the bone marrow, blood, and other organs [1, 2]. Proliferation and accumulation of leukemic cells result in the suppression of normal haematopoiesis and involves various extramedullary sites, especially the liver, spleen, lymph nodes, thymus, central nervous system and gonads. The disease is most common in children, but can occur in any age group. With a cure rate of childhood ALL of more than 80%, the evolution of therapies for this disease is one of the unqualified success stories in the history of modern medicine [2].

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Epidemiology

Although overall incidence is rare, leukemia is the most common type of childhood cancer, accounting for 30% of all cancers diagnosed in children younger than 15 years [3].
Within this population, acute lymphoblastic leukemia occurs approximately five times more frequently than

acute myelogenous leukemia (AML) and accounts for approximately three-quarters of all childhood leukemia diagnoses. In the United States, the incidence of childhood ALL increased between 1975 and 2002; however, this increase was not statistically significant [4].

The incidence rate of childhood ALL is now stable at three to four new cases per year per 100,000 children who are younger than 15 years, with a peak incidence at approximately 2–5 years of age. The incidence of ALL, particularly T-cell ALL, is slightly higher among boys than girls [4].

Etiology and risk factors

In most patients, the cause of ALL is unknown [1]. Because childhood leukemia is a rare occurrence, prospective studies are difficult to conduct, and therefore studies most frequently use a retrospective case-control design.

Epidemiological studies of acute leukemias in children have examined a number of possible risk factors (e.g., environmental, genetic, or infectious) in an effort to determine the etiology of the disease. Only two environmental risk factors (ionizing radiation and benzene) has been significantly linked with ALL; most environmental risk factors [e.g., electromagnetic fields, cigarette smoking] have been weakly or inconsistently associated with either form of childhood leukemia [4].

The precise pathogenetic events leading to development of acute lymphoblastic leukemia are unknown. Only a few cases (<5%) are associated with inherited, predisposing genetic syndromes [5]. A higher incidence of ALL has been noted among monozygotic and dizygotic twins of patients with ALL, reflecting possible genetic predisposition. Patients with trisomy 21, Klinefelter syndrome, and inherited diseases with excessive chromosomal fragility such as Fanconi anemia, Bloom syndrome, Nijmegen breakage syndrome and ataxia-telangiectasia have a higher risk of developing ALL [1, 5].

A popular, though not unchallenged, hypothesis is that fully transformed human cancer clones are arranged, like their normal tissue counterparts, in a hierarchical fashion and maintained by rare "tumor-propagating cells" (also referred to as cancer stem cells). Less is known about the crucial earliest first-hit events in cancer and the "precancerous" cells in which they occur. We have explored these precancerous cell hierarchies in the context of childhood precursor-B cell (pre-B cell) acute lymphoblastic leukemia (also known as common ALL, cALL) which is frequently associated with a chromosomal translocation creating the TEL-AML1 (ETV6-RUNX1) fusion gene. This fusion arises predominantly in utero, producing a persistent but clinically covert preleukemic clone that may convert to frank leukemia with acquisition of additional genetic changes [6].

Retrospective identification of leukemia-specific fusion genes, hyperdiploidy, or clonotypic rearrangements of immunoglobulin or T-cell-receptor loci in archived neonatal blood spots (Guthrie cards) and studies of leukemia in monozygotic twins indicate clearly a prenatal origin for some childhood leukemias. Screening of neonatal cord-blood samples has revealed a putative leukemic clone with the *TEL-AML1* fusion gene in 1% of newborn babies, a frequency 100 times higher than the prevalence of acute lymphoblastic leukemia defined by this fusion gene later in childhood. The variable incubation period and clinical outcome of such cases, and the 10% concordance rate of leukemia in identical twins with this genotype, support the notion that additional postnatal events are needed for full leukemic transformation. A recent study further established the presence of a preleukemic clone with the *TEL-AML1* fusion [5, 6].

The studies of Hong et al. suggest that a hierarchical structure, which has been demonstrated in frank leukemia, is also a feature of "early" or preleukemic populations. Understanding the nature of the preleukemic hierarchy is fundamental to understanding the function of the first-hit mutation and how it predisposes to leukemic transformation. These studies may also be relevant to cancer therapy where specific targeting of tumor propagating cells may be desirable. The observation that children in lengthy remission can relapse late with a novel leukemic clone, but which nonetheless appears to derive from the identical preleukemic clone that initiated the disease at presentation, suggests that the preleukemic stem cell compartment may persist even when the cells propagating the overt leukemia have been effectively eradicated [6].

Classification

The French-American-British Co-operative Group distinguishes three ALL groups (L1-L3) based on morphologic criteria (cell size, cytoplasm, nucleoli, basophilia, vacuolation) [7]. The morphologic distinction between L1 and L2 has lost its prognostic significance. The L3 morphology is associated with mature B-cell ALL and is characterized by a high rate of cell turnover, resulting in the "starry sky" pattern on bone marrow biopsy specimens. Blasts in ALL are negative for myeloperoxidase although low-level myeloperoxidase positivity (3%-5%) may occur in rare cases that otherwise lack expression of myeloid markers by flow cytometry [1].

The World Health Organization proposed new guidelines for the diagnosis of neoplastic diseases of hematopoietic

and lymphoid tissues or lymphomas [8]. In addition to lowering the blast count to 20% or greater as sufficient for a diagnosis of ALL, the distinction of L1, L2, and L3 morphologies was considered as no longer relevant. The French-American-British Co-operative Group and the World Health Organization classification systems rely strongly on morphologic assessment. Identification of the immunophenotype has become a major part of diagnosing ALL. Three broad groups can be distinguished: pre–B-cell ALL, mature B-cell ALL, and T-cell ALL (Table 1).

Table 1. Immunophenotypic classification of ALL [1].

B-cell lineage		T-cell lineage	
Blasts	Characterized by	Blasts	Characterized by
Pre-pre-B-cell ALL	CD19/CD79a/CD22	Pre–T-cell ALL	CD1a, CD2, CD5, CD7, CD8, cCD3
Common ALL	CD10 (CALLA)	Mature T-cell ALL	Surface CD3 (plus any other T-cell markers)
Pre–B-cell ALL	Cytoplasmic IgM		
Mature B-cell ALL	Cytoplasmic or surface Ig, κ or λ chains		

ALL - acute lymphoblastic leukemia; CALLA - common ALL antigen; Ig - immunoglobulins.

Coexpression of markers from more than 1 lineage is seen in 5% to 35% of children with ALL. With use of flow cytometry, lineage can be assigned in more than 95% of patients, and truly biphenotypic leukemias are rare. Coexpression of markers from more than 1 lineage have not been found to be prognostically important [1].

In ALL immunological markers give information of relevance to prognosis. The worst prognosis is seen in the small minority of cases showing a mature B phenotype (surface membrane immunoglobulin positive); many such cases can also be identified morphologically because they fall into the FAB L3 category, but some B-ALL have L2 morphology and therefore can only be recognised by immunological techniques. Among other B-lineage cases a pre-B phenotype was found in two studies to have a worse prognosis than other non-B, non-T lineage ALL but this was not observed in another study comparing the prognosis of pre-B ALL and common ALL [9].

Table 2. Cytogenetic-molecular abnormalities in ALL in children [1].

Category	Genes	Cytogenetics	Frequency (%)
Hyperdiploid			10-26
Hypodiploid			5-10
Pseudodiploid	*BCR-ABL*	t(9;22)(q34;q11)	2-6
	p16, p15	del(9)(p21-22)	20
	MLL	t(4;11),t(9;11), t(11;19),t(3;11)	<5
	ATM	del(11)(q22-23)	15*
	TEL-AML-1	t(12;21)(p12;q22)	20-25**
	E2A-PBX1, E2A-HLF	t(1;19),t(17;19)	<5
	TAL-1	t(1;14)(p32;q11)	5-10
	TAL-2	t(7;9)(q34;q32)	<1
	HOX11	t(10;14)(q24;q11)	<5
	HOX11L2	t(5;14)(q35;q32)	2-3
	TCR	t(1;14)(p32;q11)	20-25***
	miR15/miR16	del(13)(q14)	<5
	c-myc	(8;14),t(8;22),t(2;8)	2-5
	NR	+8	2
	NR	del(7p)	<5
	NR	del(5q)	<2
	NR	del(6q),t(6;12)	<5

NR – not reported

* Determined by loss of heterozygosity
** Determined by polymerase chain reaction
*** In T-cell ALL, overall incidence <10%

Recurrent cytogenetic-molecular abnormalities are common in ALL (Table 2). In addition to their prognostic significance, these abnormalities provide insights into the molecular events underlying the leukemic phenotype.

Oligonucleotide or complementary DNA microarray technologies are being investigated to identify previously unrecognized molecular ALL subtypes [1].

Biology

The antigen-expression profiles of leukemic lymphoblasts parallels the normal stages of B- and T-cell differentiation and maturation. The maturation of bone-marrow progenitor cells to mature B lymphocytes proceeds through stages that can be identified by the pattern of cellular immunoglobulin protein and cell-surface antigen expression [2].

Acute lymphoblastic leukemia is thought to originate from various important genetic lesions in blood-progenitor cells that are committed to differentiate in the T-cell or B-cell pathway, including mutations that impart the capacity for unlimited self-renewal and those that lead to precise stage-specific developmental arrest. Sometimes, the first mutation along the multistep pathway to overt acute lymphoblastic leukemia might arise in a haemopoietic stem cell possessing multilineage developmental capacity. The cells implicated in acute lymphoblastic leukemia have clonal rearrangements in their immunoglobulin or T-cell receptor genes and express antigen-receptor molecules and other differentiation-linked cell-surface glycoproteins that largely recapitulate those of immature lymphoid progenitor cells within the early developmental stages of normal T and B lymphocytes. Chromosomal translocations that activate specific genes are a defining characteristic of human leukemias and of acute lymphoblastic leukemia in particular. Gene-expression patterns studied in large series of newly diagnosed leukemias have substantiated the idea that specific chromosomal translocations identify unique subtypes of the disease. Usually, translocations activate transcription-factor genes, which in many cases can control cell differentiation (rather than cell division per se), are developmentally regulated, and frequently encode proteins at the apex of important transcriptional cascades [5]. About 80% of all cases of ALL express cell-surface markers indicative of a precursor B-cell lineage. Only 1% to 2% of cases express a phenotype typical of a mature B cell. T-cell ALL accounts for about 15% to 20% of cases [10].

Clinical presentation and diagnosis

Children with ALL develop symptoms related to infiltration of blasts in the bone marrow, lymphoid system, and extramedullary sites, such as the central nervous system. Common constitutional symptoms include fever (60%), fatigue (50%), pallor (25%), and weight loss (26%). Infiltration of blast cells in the marrow cavity and periosteum often lead to bone pain (23%) and disruption of normal hematopoiesis. Thrombocytopenia with platelet counts less than 100,000 are seen in about 75% of patients. Approximately 40% of patients with childhood ALL present with hemoglobin levels less than 7 g/dL. Although leukocyte counts greater than 50x10^9/L occur in 20% of cases, neutropenia (defined as an absolute neutrophil count less than 500) is common at presentation and is associated with an increased risk of infection [10].

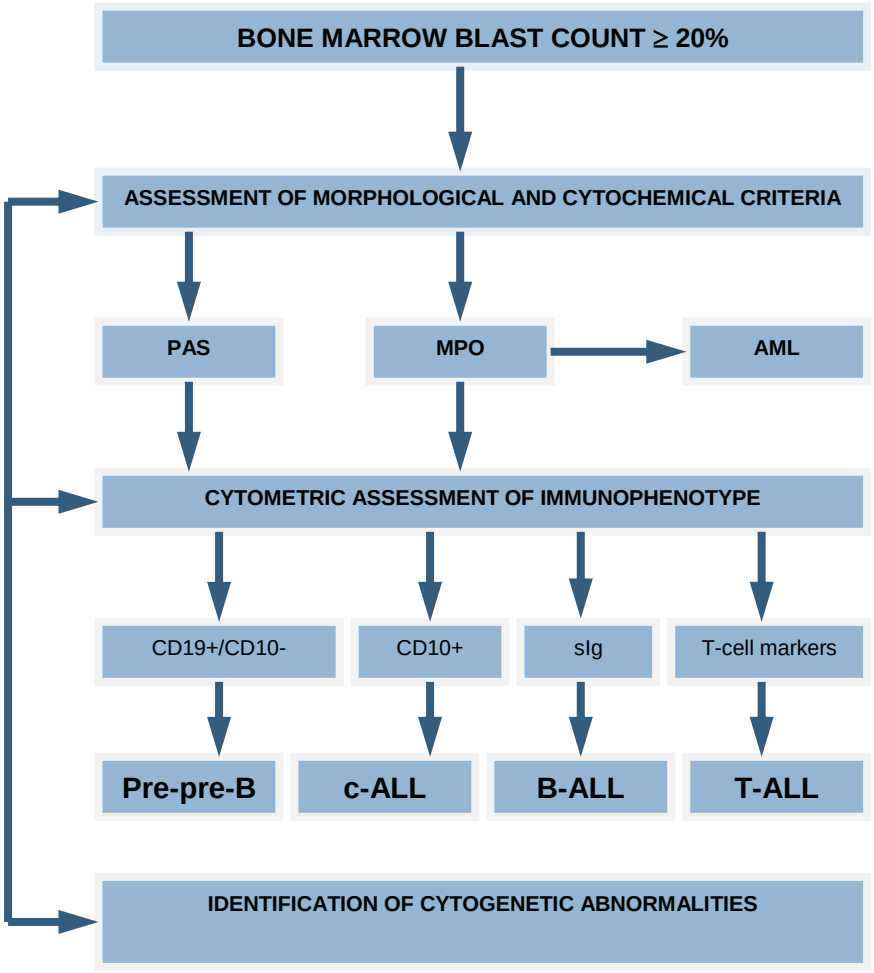
Diagnosis of ALL relies on an assessment of morphology, flow cytometry immunophenotyping, and identification of cytogenetic-molecular abnormalities (Figure 1) [1]. A lumbar puncture and cerebrospinal fluid examination are also performed to determine the presence of occult central nervous system involvement [10].

Acute lymphoblastic leukemia subtypes with different responses to therapy and prognosis, which are only partially discriminated by current diagnostic tools, may be further determined by genomic profiling [1].

Although peripheral blasts with anemia and thrombocytopenia are strongly suggestive of ALL, the definitive diagnosis of ALL is based on the bone marrow aspiration or biopsy demonstrating more than 25% lymphoblasts. In patients with large leukemia cell burden, serum chemistries may reveal signs of tumor lysis syndrome, including hyperkalemia, hypocalcemia, hyperphosphatemia, and lactic acidosis. Serum uric acid may be elevated due to increased cell turnover. Tumor lysis can be a metabolic emergency at the time of presentation and may be exacerbated by initiation of therapy [10].

Immunophenotyping of leukemic lymphoblasts by flow cytometry is essential to establish the correct diagnosis and define cell lineage. Although acute lymphoblastic leukemia can be readily subclassified according to the many steps of normal B-cell and T-cell differentiation, the only findings with therapeutic importance are T-cell, mature B-cell, and B-cell precursor phenotypes. Myeloid-associated antigen expression can be detected in as many as half the cases of acute lymphoblastic leukemia. However, with contemporary treatment, this so-called aberrant antigen expression has no prognostic implications but can be used to distinguish leukemic cells from normal progenitor cells, thereby enabling detection of minimal (ie, submicroscopic) residual leukemia [5].

Figure 1. Diagnosis of acute lymphoblastic leukemia.



Risk stratification

Risk classification was based on presenting characteristics of the patients and treatment response [11]. Careful assessment of the risk of relapse in individual patients ensures that very intensive treatment is given only to high-risk cases, thus sparing people at lower risk from undue toxic effects.

Age at diagnosis has a strong prognostic effect. Children aged 1–9 years had a better outcome than either infants or adolescents. Children younger than 6 months have an especially poor outcome.

Leukocyte count is a continuous prognostic variable, with increasing counts conferring a poorer outcome, especially in patients with B-cell precursor disease [5]. T cell ALL is generally considered to be high or very high risk, depending on the patients' response to induction therapy. For T cell ALL, age and leukocyte count have little clinical significance; however, a leukocyte count > 100 × 10^9/L at diagnosis is an indication for more intensive central nervous system-directed therapy. In T-cell acute lymphoblastic leukemia, a leukocyte count greater than 100x10^9/L is associated with an increased risk of relapse in the central nervous system.

Patients with extreme hyperleucocytosis (>400x10^9/L) are at high risk for early complications such as central nervous system hemorrhage and pulmonary and neurological events due to leucostasis.

In US cooperative group studies, black and Hispanic patients fared worse than similarly treated white individuals [5]. Male sex has generally been associated with a poor prognosis [12].

Table 3. Prognostic factors in childhood ALL [12].

Leukemia subtype	Favorable Prognostic Factors	Unfavorable Prognostic Factors
B cell precursor	Hyperdiploidy(>50 chromosomes); TEL-AML 1 fusion; Trisomies 4, 10 and 17	Poor early response; MLL rearrangement in infants; Philadelphia chromosome; Leukocyte count >50x10^9/L; Age >10 years at diagnosis
T cell	HOX 11 overexpression; t(11;19) with MLL-ENL fusion*	Poor early response; Low-dose intensity chemotherapy

* This rearrangement occurs more often in B cell precursor ALL among which it is an unfavorable feature, especially in infant cases.

Primary genetic abnormalities of leukemia cells have important prognostic significance. In B-cell precursor ALL, hyperdiploidy (more than 50 chromosomes per leukemia cell) and t(12;21) with the TEL-AML1 fusion gene, accounting for approximately 50 percent of childhood cases but only 10 percent of adult cases, confer a highly favorable prognosis [13]. Patients with the Philadelphia chromosome or the t(4;11)/MLL-AF4 fusion are considered to have very high risk ALL. There is a marked influence of age on the prognosis of patients with these genetic subtypes. In Philadelphia chromosome–positive ALL, the prognosis is generally dismal for adolescents, but is relatively favorable in children 1 to 9 years old with a low leukocyte count at diagnosis [12]. Prognostic factors in ALL are presented in Table 3.

Recent genomewide analyses of DNA copynumber abnormalities have identified numerous recurring genetic alterations in ALL. Mutations of genes encoding transcriptional regulators of B lymphoid development, including PAX5, EBF1, and IKZF1, occur in more than 40% of patients with B-cell–progenitor ALL. Deletion of IKZF1, which encodes the lymphoid transcription factor IKAROS, is a very frequent event in BCR-ABL1–positive ALL and at the progression of chronic myeloid leukemia to lymphoid blast crisis.

IKAROS is a transcription factor with well established roles in lymphopoiesis and cancer. Normal IKAROS contains four N-terminal zinc fingers, which are required for DNA binding, and two C-terminal zinc fingers that mediate dimerization of IKAROS with itself and with other IKAROS family members.

The development of all lymphoid lineages requires IKAROS, and in mice that are heterozygous for a dominant-negative Ikzf1 mutation, aggressive T-lineage hematopoietic disease develops. Deletion or mutation of IKZF1 was significantly associated with an increased risk of relapse and adverse events. IKZF1 deletions were also associated with a poor outcome in patients with BCR-ABL1–negative ALL. Furthermore, alteration of IKZF1 had an independent association with outcome after adjusting for age, leukocyte count at presentation, and cytogenetic subtype [14].

Infant leukemia

Leukemias diagnosed in the first 12 months of life are characterized by an equal distribution of lymphoid and myeloid subtypes and account for 2.5% to 5% of acute lymphoblastic leukemias and 6% to 14% of acute myeloid leukemias of childhood. In contrast to an excess of boys among older children with leukemia, there is a slight female predominance among infants with this disease. Infant leukemias display unique biological and clinical features that have provided important insights into the mechanisms governing normal and aberrant hematopoiesis in the fetus and young children, as well as reasons for the increased rates of treatment failure in infants as compared with older children [15].

Acute lymphoblastic leukemia in infants aged younger than 12 months is both rare and biologically different from acute lymphoblastic leukemia in older children. In infants, this disease is characterized by a high frequency of abnormalities in chromosome 11q23 that affect the mixed lineage leukemia *(MLL)* gene; by a very immature B-cell phenotype (pro-B ALL) with no CD10 expression; and by a high tumor load at presentation [16]. ALL of infancy is associated with a high leukocyte count at presentation, hepatosplenomegaly, and central nervous system involvement [15].

Studies in various countries have reported long-term rates of event-free survival (EFS) of 28–45%. These rates are much lower than EFS rates for older children with acute lymphoblastic leukemia. Infant lymphoblasts are more resistant to chemotherapy than cells in older children [16]. Age, immunophenotype, and ALL1/MLL/HRX rearrangement reflect or cause differences in drug-resistance factors. These can be pharmacokinetic factors that determine the amount of drugs to which the leukemic cells are exposed or differences in cellular

Table 4. Selected antileukemic drugs being tested in clinical trials [5, 13].

Agent	Mechanism of action	Subtype of leukemia targeted
Clofarabine	Inhibits DNA polymerase and ribonucleotide reductase; disrupts mitochrondria membrane	ALL
Nelarabine	Inhibits ribonucleotide reductase and DNA synthesis	T-cell
Forodesine	Inhibits purine nucleoside phosphorylase	T-cell
γ-secretase inhibitors (PF-03084014, semagacestat LY450139)	Inhibit γ-secretase, an enzyme required for NOTCH1 signalling	T-cell
Rituximab	Anti-CD20 chimeric murine-human monoclonal antibody	CD20-positive
Epratuzumab	Anti-CD22 humanised monoclonal antibody	CD22-positive
Alemtuzumab	Anti-CD52 humanised monoclonal antibody	CD52-positive
Gemtuzumab ozogamicin	Anti-CD33 monoclonal antibody conjugated with calicheamicin	CD33-positive
Imatinib mesilate	ABL kinase inhibition	BCR-ABL-positive
Nilotinib	ABL kinase inhibition	BCR-ABL-positive
Dasatinib	BCR-ABL kinase inhibition	BCR-ABL-positive
MK-0457	Aurora kinase inhibition	BCR-ABL-positive
Lestaurtinib; midostaurin; tandutinib; sunitinib malate; IMC-EB10	FMS-like tyrosine kinase 3 inhibition	MLL-rearranged; hyperdiploid
Tipifarnib; lonafarnib	Farnesyltransferase inhibition	ALL
Azacytidine; decitabine; temozolomide	DNA methyltransferase inhibition	ALL
Romidepsin; vorinostat; valproic acid; MD-27-275; AN-9	Histone deacetylase inhibition	ALL
Sirolimus; temsirolimus; everolimus; AP-23573	Mammalian target-of-rapamycin inhibition	ALL
Bortzezomib	Inhibition of ubiquitin proteasome pathway	ALL
Flavopiridol	Serine-threonine cyclin-dependent kinase inhibition	ALL
Oblimersen	Downregulation of BCL2	ALL
17-AAG	Heat shock protein-90 inhibitor	BCR-ABL-positive; ZAP-70-positive
BMS-354825	ABL–SRC kinase inhibition	BCR-ABL-positive
AMN107	ABL kinase inhibition	BCR-ABL-positive
PKC412; MLN518; CEP701	FMS-like tyrosine kinase 3 inhibition	*MLL*-rearranged, hyperdiploid

pharmacodynamics that determine the sensitivity of the cells to the drugs. There are no data suggesting that pharmacokinetic resistance might explain the poorer outcome of infant ALL; infants simply do not show increased clearance of antileukemic drugs [15].

The results of treatment for infant ALL are still suboptimal. A variety of treatment regimens have been tested in infants, generally yielding event-free survival rates of 20% to 35%. In several recent clinical trials, high-dose methotrexate, high-dose cytarabine, and intensive consolidation/reinduction therapy appear to have improved clinical outcome, but these results should be viewed as preliminary because of the small numbers of patients enrolled, the lack of randomization, and the disproportionate numbers of cases with high-risk disease (ie, *ALL1/MLL/HRX-AF4* fusion). Moreover, the efficacy of any treatment component is affected by the overall therapeutic strategy [15].

Drug resistance

Children whose ALL cells exhibit in vitro resistance to antileukemic agents have a substantially worse prognosis than children whose ALL cells are drug-sensitive. However, little is known about the genetic basis of resistance to chemotherapy. Multidrug-resistance genes and genes involved in cell-cycle progression, DNA repair, drug metabolism, and apoptosis have been associated with the prognosis of ALL, but their role in determining the sensitivity of ALL cells to individual antileukemic agents is not known.

Gene products arising from rearrangements of the TEL-AML1, BCR-ABL, and MLL genes are also associated with prognosis and drug resistance, but for unknown reasons, many patients with a favorable genetic subtype (e.g., TEL-AML1) are not cured, whereas many with an unfavorable subtype (e.g., certain MLL rearrangements) are cured.

Although it is likely that multiple pathways and genes contribute to the sensitivity of ALL cells to specific agents, all studies to date have focused on a small number of candidate genes instead of taking advantage of the genomic survey that is possible with the use of gene-expression profiling [17]. Cellular resistance to drugs, as measured with an in vitro drug cytotoxicity assay, is associated with unfavorable risk factors, such as age more than 10 years, proB- and T-cell ALL immunophenotype, and presence of chromosomal abnormalities, such as 11q23/MLL gene rearrangements and Ph chromosomes [18].

Minimal residual disease

Minimal residual disease (MRD) is the presence of disease below the threshold of detection by conventional methods (light microscopy and cytochemical stains). Different methods are available to detect and monitor MRD, including fluorescence in situ hybridization, multicolor flow cytometry, and polymerase chain reaction (PCR) assays, especially realtime quantitative PCR. Multicolor flow cytometry and PCR techniques take advantage of either fusion transcripts resulting from chromosome abnormalities (e.g., *BCR-ABL*, *MLLAF4*, *TEL-AML1*) or patient-specific junctional regions of rearranged immunoglobulin and T-cell receptor genes [1].

In children with acute leukemia, measurements of minimal residual disease (MRD) provide unique information on treatment response and have become a crucial component of contemporary treatment protocols. In acute lymphoblastic leukemia, the most useful MRD assays are based on polymerase chain reaction (PCR) amplification of antigen receptor genes, and on flow cytometric detection of abnormal immunophenotypes [19].

Minimal residual disease (MRD) has evolved as an independent prognostic parameter in childhood acute lymphoblastic leukemia and is currently used in several clinical trials for treatment stratification.

In vitro measurement of drug resistance in leukemic cells obtained at diagnosis has also shown promise in the prediction of clinical outcome in selected groups of patients, but only two small retrospective studies have investigated the relationship between in vitro drug resistance and MRD.

Three independent studies have shown that drug resistance profiles can identify patients at higher risk of early treatment failure but are less able to predict late relapses. This fits well with the present finding that drug sensitivity correlates to the clearance of malignant cells from the bone marrow during induction therapy. Late relapses may develop from leukemic cells hidden in sanctuary sites or from cells unresponsive to chemotherapy by dormancy, and other factors than drug sensitivity might then be of importance, e.g. the ability of the immune system to recognise and eradicate these cells.

The study of Lönnerholm et al. shows a significant correlation between in vitro cellular drug sensitivity at diagnosis and cell kill during induction therapy as measured by MRD day 29. Further studies are needed to clarify whether or not drug testing adds prognostic information to what is achieved from analysis of MRD [20].

The studies of Kang et al. provide evidence that improved outcome prediction and risk classification can be achieved in ALL through the development of gene expression classifiers. The application of gene expression classifiers

allows for the prospective identification of a significant subgroup of ALL patients with little chance for cure on contemporary chemotherapeutic regimens. Further analysis of these expression profiles, coupled with other comprehensive genomic studies, will hopefully lead to the continued identification of novel targets and more effective therapies for these children [21].

Treatment

The currently accepted philosophy behind treatment of pediatric ALL is based on the recognition of the heterogeneity of the disease. Although it is no longer acceptable to treat all cases of childhood ALL with a single treatment regimen, there are 4 basic phases in all treatment protocols. These phases include remission induction, treatment of clinical or occult central nervous system disease (consolidation), intensification, and maintenance therapy. The goals of induction therapy are to attain remission, defined as the absence of evidence of leukemia, which includes the absence of central nervous system or testicular disease, and to have bone marrow examination showing normal cellularity with fewer than 5% lymphoblasts. The 3-drug combination of vincristine, a glucocorticoid, and L-asparaginase successfully induces remission in 95% of children with ALL [10].

With the exception of patients with mature B-cell acute lymphoblastic leukemia, who are treated with short term intensive chemotherapy (including high-dose methotrexate, cytarabine, and cyclophosphamide), treatment for acute lymphoblastic leukemia typically consists of a remission-induction phase, an intensification (or consolidation) phase, and continuation therapy to eliminate residual disease. Treatment is also directed to the central nervous system early in the clinical course to prevent relapse attributable to leukemic cells sequestered in this site. The drugs currently in use for these phases were developed and tested between the 1950s and 1970s, but efforts to identify new antileukemic agents have begun to intensify (Table 4) [5]. Although most of the conventional drugs used to treat ALL either target DNA directly or inhibit nucleic acid synthesis, some block protein synthesis by hydrolysing an amino acid essential for leukemic cell growth or by interfering with the mitotic spindle apparatus. Because these are nonspecific, such drugs have a narrow therapeutic index and often produce adverse cytotoxic effects in various normal tissues. Therefore, although molecular therapeutics holds promise for the future, efforts to improve the effectiveness of commonly used antileukemic drugs continue at a substantial pace [2].

Prophylactic cranial irradiation has been a standard treatment in children with acute lymphoblastic leukemia who are at high risk for central nervous system relapse. The study of Pui et al. showed that with effective risk-adjusted chemotherapy, prophylactic cranial irradiation can be safely omitted from the treatment of childhood ALL. They relied on high-dose methotrexate, intensive asparaginase, dexamethasone, and optimal intrathecal therapy to control central nervous system leukemia. Intrathecal therapy was intensified in patients with blasts in the cerebrospinal fluid, even if the blasts were from traumatic lumbar puncture, which has been associated with poor outcome [11].

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