REVIEW ARTICLE

Acute Lymphoblastic Leukemia

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HE RATE OF SUCCESS IN THE TREATMENT OF ACUTE LYMPHOBLASTIC leukemia (ALL) has increased steadily since the 1960s. The five-year event-free survival rate is nearly 80 percent for children with ALL and approximately 40 percent for adults (Table 1).¹⁻⁹ If we include cases of relapsed ALL that respond well to so-called remission retrieval therapy, the rates of cure (defined by the absence of evidence of disease for at least 10 years) with the use of modern treatments are about 80 percent for children and 40 percent for adults.^{10,11} Attempts to boost cure rates further with the use of hematopoietic stem-cell transplantation have improved the outcome for some,^{7,12} but not all,¹³ subtypes of ALL, suggesting that intensification of existing treatments is unlikely to raise cure rates substantially and will instead increase treatment-related mortality and the risk of such life-threatening late sequelae as second cancers. The best hope for continued progress lies in a better understanding of the pathogenesis of ALL and the basis of resistance to chemotherapy. Here, we review current and emerging concepts of the pathobiology of ALL, emphasizing results likely to have the greatest influence on clinical management during the next decade.

MOLECULAR GENETIC ALTERATIONS

PRIMARY ABNORMALITIES

Molecular analysis of the common genetic alterations in leukemic cells has contributed greatly to our understanding of the pathogenesis and prognosis of ALL.^{10,14} Although the frequency of particular genetic subtypes differs in children and adults (Fig. 1),^{10,15} the general mechanisms underlying the induction of ALL are similar. They include the aberrant expression of proto-oncogenes, chromosomal translocations that create fusion genes encoding active kinases and altered transcription factors, and hyperdiploidy involving more than 50 chromosomes (Fig. 1). These genetic alterations contribute to the leukemic transformation of hematopoietic stem cells or their committed progenitors by changing cellular functions. They alter key regulatory processes by maintaining or enhancing an unlimited capacity for self-renewal, subverting the controls of normal proliferation, blocking differentiation, and promoting resistance to death signals (apoptosis) (Fig. 2A).¹⁶

Some genetic lesions primarily affect only one of these pathways, whereas others impinge on more than one. An example of the latter is the t(9;22) translocation, which underlies the BCR-ABL fusion protein. The proto-oncogene *ABL* encodes a tyrosine-specific protein kinase, whose activity is tightly regulated. By contrast, the BCR-ABL fusion protein is a constitutive protein kinase that alters signaling pathways that control the proliferation, survival, and self-renewal of hematopoietic stem cells.¹⁷ Another example of an altered signaling pathway involves *PTPN11*, which encodes the SHP-2 protein tyrosine phosphatase.¹⁸ Somatic missense mutations in *PTPN11* cause constitutive activation of SHP-2 and enhance signaling through the mitogen-associated protein (MAP) kinase pathways, which lie downstream of growth factor receptors. *PTPN11* mutations

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Table 1. Results of Selected Clinical Trials in Patients with ALL.*					
Patients and Study Group	Years of Study	No. of Patients	Age Range	5-Yr Event-free Survival	Reference
			γr	%	
Children					
ALL-BFM 90	1990–1995	2178	0–18	78±1.0	Schrappe et al. ¹
CCG-1800	1989–1995	5121	0-21	75±1.0	Gaynon et al.²
COALL-92	1992–1997	538	1–18	76.9±1.9	Harms and Janka-Schaub ³
DFC protocol 91-01	1991–1995	377	0–18	83±2	Silverman et al. ⁴
NOPHO ALL-92	1992–1998	1143	0–15	77.6±1.4	Gustafsson et al.⁵
SJCRH XIII	1991–1998	412	0–18	79.4±2.3	Pui et al. ⁶
Adults					
GMALL 02/84	1983–1987	562	15–65	39 (at 7 yr)†	Gökbuget and Hoelzer ⁷
MDACC	1992–1998	204	16–79	38†	Kantarjian et al. ⁸
UCSF 8707	1987–1998	84	16–59	48±13	Linker et al.9

* Plus-minus values are means ±SE. BFM denotes Berlin-Frankfurt-Münster, CCG Children's Cancer Group, COALL Cooperative Study Group of Childhood Acute Lymphoblastic Leukemia, DFC Dana-Farber Consortium, NOPHO Nordic Society of Pediatric Haematology and Oncology, SJCRH St. Jude Children's Research Hospital, GMALL German Acute Lymphoblastic Leukemia Study Group, MDACC M.D. Anderson Cancer Center, and UCSF University of California, San Francisco.

† The rate of continuous complete remission is shown; patients in whom induction therapy failed and those who died were excluded from the analysis.

occur in approximately 6 percent of children with ALL.¹⁸ Cancers with such a mutation have no other recognized genetic aberrations, suggesting that this change is a novel primary abnormality.

CHIMERIC TRANSCRIPTION FACTORS

Altered self-renewal and differentiation of hematopoietic stem cells can also result from chimeric transcription factors, which arise from genetic translocations that fuse portions of two different transcription factors. These chimeric transcription factors activate diverse transcriptional cascades that, at least in part, converge to modify the normal pattern of expression of members of the important family of *HOX* genes, which encode the HOX transcription factors (Fig. 2B).^{15,19} The HOX transcription factors bind to DNA and regulate genes involved in the differentiation of both the embryo and the hematopoietic stem cell; they are also important in the self-renewal and proliferation of hematopoietic stem cells.

The TEL-AML1 Fusion Gene

The t(12;21) translocation creates a fusion gene that includes the 5' portion of TEL, a member of the ETS family of transcription factor genes, and almost the

entire coding region of another transcription factor gene, AML1, which encodes the α subunit of core binding factor, a master regulator of the formation of definitive hematopoietic stem cells.^{20,21} The chimeric TEL-AML1 transcription factor retains an essential protein-protein interaction domain of TEL and the DNA-binding and transcriptional regulatory sequences of AML1 (also called CBF α) (Fig. 3).^{20,21} TEL is required for the homing of hematopoietic progenitor cells to the bone marrow,²² whereas AML1 is the DNA-binding component of the heterodimeric transcription factor (CBF α plus CBF β) called core-binding factor, which has a central role in hematopoiesis.^{21,23} The HOX genes probably operate downstream of the transcriptional cascade initiated by core-binding factor (Fig. 2B).19,24

A prominent effect of the TEL-AML1 fusion protein is inhibition of the transcriptional activity that is normally initiated when AML1 binds to a DNA region termed the core enhanced sequence²⁵ (Fig. 3A). The binding of AML1 to the core enhanced sequence recruits other transcription factors and coactivators to this region, and the resulting protein complex regulates transcription. This complex includes histone acetylases, which add acetyl groups to DNA-bound histones, thereby causing conforma-



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The development of leukemia requires a hematopoietic stem cell or one of its committed progenitors to elude the normal mechanisms of homeostatic control that regulate growth-factor signaling, differentiation, apoptosis, and self-renewal (Panel A). A common pathway targeted by translocation-generated chimeric transcription factors, such as MLL fusion proteins, TEL-AML1, and E2A-PBX1, is the HOX gene-mediated transcriptional cascade (Panel B). The AML1-CBF β transcription factor complex (AML1) functions either directly or indirectly to regulate the transcription of specific members of the HOX gene family. The MLL protein is required to maintain this transcription, whereas members of the polycomb group (PcG) family of proteins repress HOX gene transcription. HOX proteins, in turn, collaborate with cofactors, including the PBX1 protein, to induce the transcription of downstream target genes, whose products influence self-renewal, proliferation, and differentiation of hematopoietic stem cells and their committed progenitors. The sites of action of three chimeric transcription factors linked to leukemic transformation are indicated in red.

tional changes in chromatin that enhance the transcription of target genes. Like AML1, the abnormal TEL-AML1 fusion protein can bind to the core enhanced sequence, but instead of activating transcription, it recruits histone deacetylases, which induce closure of the chromatin structure and, hence, inhibition of transcription (Fig. 3B). These changes in the normal AML1-mediated transcriptional cascade alter both the self-renewal capacity and the differentiation capacity of hematopoietic stem cells.21,26

The conversion by chromosomal translocation of a transcription factor from an activator to a repressor of genes is a recurrent pathogenic mechanism not only in ALL, but also in acute myeloid leukemias that form fusion proteins containing AML1 or the retinoic acid α receptor.^{27,28} Recently developed small-molecule inhibitors of the histone portion of 1 of more than 40 partners. This genetic

deacetylase enzymes can reverse transcriptional repression by chimeric transcription factors, thereby abolishing their oncogenic activity. Indeed, histone deacetylase inhibitors have shown activity in preclinical studies and are now in clinical trials.²⁹ These inhibitors appear to possess only limited activity when used alone, but in combination with other agents they could be beneficial.

Translocations Involving the MLL Gene

A second component of the HOX regulatory pathway is the mixed-lineage leukemia (MLL) protein,^{30,31} a nuclear protein that maintains the expression of particular members of the HOX family (Fig. 2B). Leukemia-associated translocations of MLL result in chimeric proteins consisting of the N-terminal portion of MLL fused to the C-terminal



the site of fusion between these segments. TEL-AML1 retains its ability to bind to the core enhanced sequence and to form a heterodimer with CBF β ; however, unlike the normal AML1 protein, it recruits a transcriptional corepressor complex that includes proteins with histone deacetylase activity (HDAC), which remove acetyl groups from histones, resulting in closure of the chromatin and repression of transcription.

alteration occurs in more than 80 percent of infants with ALL and in most therapy-induced leukemias caused by topoisomerase II inhibitors. The MLL fusion proteins have a dominant gain-of-function effect that enhances their transcriptional activity. This alteration disrupts the normal pattern of expression of *HOX* genes, causing a change in the self-renewal and growth of hematopoietic stem cells and committed progenitors. In mice, overexpression of an MLL fusion protein in hematopoietic cells enhances the self-renewal of early hematopoietic progenitors and eventually leads to leukemia.^{32,33} These ef-

fects depend on the presence of HOXA7 and HOXA9, suggesting that altered expression of these specific HOX family members is necessary for leukemogenesis.³⁴

Other HOX Genes and HOX Cofactors

Support for a critical role of altered *HOX* gene expression in leukemogenesis comes from other lines of investigation. In adult mice, forced expression of *HOXB4* induced the proliferation of hematopoietic stem cells,³⁵ whereas enforced expression of *HOXA10* induced leukemia directly.³⁶ In humans, in-

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creased expression of certain HOX genes and their DNA-binding cofactor MEIS1 is a consistent finding in leukemias with MLL rearrangements,37 in a subgroup of T-cell ALL,³⁸ and in specific subtypes of acute myeloid leukemia.39,40 Another HOX DNAbinding cofactor, PBX1, is targeted by the t(1;19) translocation, which occurs in approximately 25 percent of cases of pre-B-cell ALL (Fig. 2B). This translocation forms a fusion gene that encodes a chimeric transcription factor, E2A-PBX1, which disrupts both the expression of HOX genes and the targets of the E2A transcription factor.41 All these findings make components of the HOX regulatory pathway attractive targets for the development of novel therapeutic agents. Identification of the specific molecules best suited to serve as drug targets is likely to be a high priority in the coming years.

COOPERATIVE MUTATIONS

The oncogenic events triggered by chromosomal rearrangements are probably insufficient by themselves to cause leukemia. Instead, genetic alterations that impair differentiation, such as those described above, probably cooperate with a second class of mutations that alter the proliferation and survival of hematopoietic progenitors.²¹ Although this scheme is an oversimplification, it provides a framework for investigating other genes in the pathogenesis of ALL. We turn now to several genes involved in the second type of mutations.

The FLT-3 Receptor

Overexpression of FLT-3, a receptor tyrosine kinase important for the development of hematopoietic stem cells, occurs in cases of ALL with either MLL rearrangements or hyperdiploidy involving more than 50 chromosomes.^{37,42} Normally, the FLT-3 ligand triggers the tyrosine kinase activity of FLT-3,43 but in these subtypes of leukemia, the kinase is constitutively turned on by activating mutations, autocrine secretion of the FLT-3 ligand, or self-activation induced by the overexpression of FLT-3.^{44,45} Continuous signaling by the receptor contributes to the abnormal growth of leukemic cells, as demonstrated by the ability of small-molecule inhibitors of FLT-3 to block the in vitro growth of primary leukemic cells containing MLL rearrangements.44 These findings, and the remarkable success of a related tyrosine kinase inhibitor, imatinib mesylate, in the treatment of chronic myeloid leukemia,46 provide the impetus for clinical testing of inhibitors of the FLT-3 receptor kinase.

The Retinoblastoma Pathways

Another frequently altered regulatory network in ALL consists of the interrelated pathways controlled by the tumor suppressor retinoblastoma protein (RB), the related proteins p130 and p107, and p53 (Fig. 4). The principal role of RB is to control entry into the cell cycle.47 In its hypophosphorylated state, RB inhibits the ability of the E2F family of transcription factors to transcribe the genes necessary for entry into the S phase. Mitogenic signals induce the formation of active cyclin D-dependent kinase complexes that together with cyclin E-Cdk2 phosphorylate RB, thereby abrogating its ability to inhibit cell proliferation (Fig. 4). The activity of cyclin D-dependent kinases is in turn inhibited by the INK4 proteins (p16^{INK4a}, p15^{INK4b}, p18^{INK4c}, and p19^{INK4d}), thereby preventing the phosphorylation of RB. Despite the rarity of inactivating mutations or deletions of RB in ALL,48 functional inactivation of the RB pathway through the deletion or epigenetic silencing of P16^{INK4a} and P15^{INK4b} occurs in nearly all cases of childhood T-cell ALL and in a small proportion of cases of B-cell-lineage ALL.49 Alterations of these inhibitors of cyclin D-dependent kinase occur to a lesser extent in adult T-cell ALL.⁵⁰

TP53

Like RB, the TP53 gene, which encodes the p53 transcription factor, is itself rarely altered in ALL; however, components of the p53 pathway are frequently mutated in ALL. As a tumor suppressor, p53 becomes activated in response to aberrant cellular proliferation, DNA damage, or hypoxia. The activated p53 triggers the arrest of the cell cycle or apoptosis, depending on the cellular context.⁵¹ The activity of p53 is harnessed by HDM2, a protein that binds to p53 and induces its degradation; HDM2, in turn, is inhibited by the p14^{ARF} tumor suppressor. Deletion or transcriptional silencing of P14ARF is a frequent event in ALL,52 whereas overexpression of HDM2 or silencing of the p53 transcriptional target p21^{CIP1} occurs in approximately 50 percent of cases of ALL.⁵³ P16^{INK4a} and P14^{ARF} are encoded by alternative reading frames in the same genetic locus.54 The high frequency of disabling homozygous deletions in P16INK4a and P14ARF thus suggests that alterations of the RB and p53 pathways collaborate in the pathogenesis of ALL. The central role of these pathways in both tumor suppression and the response of tumor cells to chemotherapy suggests that some components of these pathways are rational drug targets.



Figure 4. The Retinoblastoma (RB) and p53 Tumor-Suppressor Network.

Proliferative signals induce the expression of D-type cyclins (D1, D2, and D3), which interact with the cyclin-dependent kinases (Cdks) 4 and 6 to produce active enzymatic complexes that phosphorylate the RB tumor suppressor. Phosphorylation of RB in turn releases E2F transcription factors to stimulate the transcription of target genes required for a cell to enter the S phase of the cell cycle. One of these targets is cyclin E, which forms a complex with Cdk2 and further phosphorylates RB, creating a positive-feedback loop. Induction of the p53 tumor suppressor either induces apoptosis or arrests the cell cycle, depending on the cellular context. The p53-mediated arrest of the cell cycle results in part from the induction of the universal cyclin-dependent kinase inhibitor p21^{CIP1}, which together with the cyclin-dependent kinase inhibitor p27^{CIP1}, blocks the activity of cyclin E–Cdk2. The activity of p27^{CIP1} and p21^{CIP1} is normally regulated by their sequestration into cyclin D–Cdk complexes, which are resistant to their inhibitory activity. The single *INK4a* or *ARF* genomic locus encodes two key regulators of the RB and p53 pathways, p16^{INK4a} and p14^{ARF}, respectively. Alternative first exons (1 α and 1 β) are spliced to exon 2, which is translated in alternative reading frames, resulting in the production of two unrelated proteins from a single genomic region. The p16^{INK4a} protein directly inhibits cyclin D–Cdk complexes, whereas the p14^{ARF} protein interacts with HDM2 and inhibits the ability of the latter protein to induce the degradation of p53. The other INK4 proteins, P15^{INK4b}, P18^{INK4c}, and P19^{INK4d}, also inhibit cyclin D kinase complexes.

PROGNOSTIC FACTORS

SENSITIVITY TO CHEMOTHERAPY

The different clinical outcomes associated with the various subtypes of ALL can be attributed primarily to drug sensitivity or resistance of leukemic blasts harboring specific genetic abnormalities^{55,56} (Fig. 5). The unusual sensitivity of leukemic blast cells with a hyperdiploid karyotype to chemotherapy is an example. Although the mechanism of leukemic transformation in hyperdiploid ALL is unknown, patients with this abnormality have a favorable prognosis when they are treated with antimetabolite-based regimens. The exquisite sensitivity of these cells to chemotherapy correlates with their propensity to undergo spontaneous apoptosis when cultured in vitro and to have higher-than-average intracellular concentrations of methotrexate and its active polyglutamate metabolites after in vivo

treatment.⁵⁵ More than 97 percent of hyperdiploid blasts have three or four copies of chromosome 21, which harbors a gene encoding the transporter of methotrexate into cells.⁵⁷ The increase of this folate transporter resulting from an increase in gene dosage may account in part for the excessive accumulation of methotrexate polyglutamates in hyperdiploid leukemic cells.⁵⁷

Cases of ALL expressing the TEL-AML1 fusion protein also have a unique response to treatment. This subtype of ALL was initially thought to be associated with a relatively good prognosis; however, on further analysis, a favorable outcome was observed only in clinical trials featuring intensive chemotherapy, especially with asparaginase.²⁰ Interestingly, ALL cells that express TEL-AML1 are highly sensitive to asparaginase in vitro,⁵⁶ for reasons that remain unclear.⁵⁸

High-dose cytarabine has been credited with



fusion have a favorable treatment outcome, with mean (\pm SE) five-year event-free survival rates of 89.5 \pm 7.3 percent, 88.3 \pm 3.3 percent, and 87.5 \pm 4.0 percent, respectively, whereas those with t(4;11) leading to *MLL-AF4* fusion and t(9;22) leading to *BCR-ABL* fusion have a dismal prognosis, with five-year event-free survival rates of 26.7 \pm 11.4 percent and 28.6 \pm 10.8 percent, respectively. The prognosis is intermediate for patients with other B-cell–lineage ALL (83.6 \pm 3.3 percent) and T-cell ALL (68.6 \pm 5.9 percent).

improving the clinical outcome in infants and adults with t(4;11)-positive ALL, which involves the *MLL* gene — an advance now being tested in prospective clinical trials.⁵⁹ The increased sensitivity to cytarabine is apparently due to an increased level of hENT1, which transports cytarabine across the cell membrane.⁶⁰ Whether *hENT1* is a direct transcriptional target of MLL or lies downstream of the transcriptional activity of MLL fusion proteins is unclear.

Additional examples of subtype-specific effects of chemotherapy include the observation that T-cell–lineage blast cells form significantly fewer methotrexate polyglutamates than do B-cell–lineage blast cells⁶¹ and therefore require a very high dose of methotrexate (5 g per square meter of body-surface area) for an optimal response.⁶² ALL with the t(1;19)/*E2A-PBX1* fusion was classified as a high-risk subtype of leukemia when treatment consisted of standard antimetabolite-based regimens. The use of intensified chemotherapy for this form of ALL substantially improved the prognosis, so that disease-free survival rates now approach 90 percent⁶ (Fig. 5).

INFLUENCE OF AGE

There is a marked influence of age on the prognosis of certain genetic subtypes of ALL. For example, Philadelphia chromosome-positive ALL is generally associated with a poor prognosis in adolescents but a relatively favorable outcome in children one to nine years old who have a low leukocyte count at presentation¹²; adults with this type of ALL have a dismal prognosis.7 Among patients with MLLrearranged ALL, infants younger than one year of age fare considerably worse than older children.¹³ The basis for these differences may be related to some combination of secondary genetic events, the developmental stage of the target cell undergoing malignant transformation, and the pharmacogenetic or pharmacokinetic features of the patient (see below).

GENE-EXPRESSION PROFILING

A relatively new technique, DNA microarrays, makes possible the simultaneous analysis of the expression of thousands of genes and not only accurately identifies known genotypic and phenotypic subtypes of ALL but also provides insights into their underlying biology and responses to antileukemic therapy.^{37,38,42,44,63}

Gene-expression studies can group virtually all cases of T-cell ALL according to multistep oncogenic pathways (Fig. 1): HOX11L2, LYL1 plus LMO2, TAL1 plus LMO1 or LMO2, HOX11, and MLL-ENL.15,38 Cases involving HOX11L2, TAL1, and HOX11 are characterized by high levels of MYC expression and the loss of P16INK4a and P14ARF, whereas cases involving LYL1 are characterized by high levels of N-MYC expression and the deletion of as vet unidentified genes on chromosomal arms 5q and 13q.^{15,38} That LMO1 and LMO2 are aberrantly expressed in association with the overexpression of either TAL1 or LYL1 is consistent with findings in transgenic animal models that LMO proteins act in concert with TAL1 and other members of the basic helix-loop-helix family of transcription factors in the development of T-cell ALL.38

Interestingly, among 11 children who received retrovirus-mediated gene therapy for X-linked severe combined immunodeficiency disease, the 2 youngest patients were found to have a T-cell acute leukemia-like syndrome in which the retrovirus vector was integrated near the promoter of the LMO2, leading to aberrant transcription and expression of the gene, almost three years later.⁶⁴ The long latency suggests that the development of leukemia involves a process of multiple mutations, and indeed, one of the children had an acquired mutation in TAL1.⁶⁴ Gene-expression profiling has prognostic significance in T-cell ALL. The cases involving HOX11 and MLL-ENL have a more favorable prognosis than do other subtypes.^{38,65} The prognostic significance of HOX11L2 depends largely on the type of treatment administered.38,66,67

Microarray analysis can also identify, within a given subtype of leukemia, previously unrecognized genes whose expression may have prognostic significance.⁴² Analysis of gene-expression profiles, or signatures, before and after treatment with methotrexate and mercaptopurine, alone or in combination, showed that leukemic cells of different molecular subtypes share a common pathway of genomic response to the same treatment and that changes in gene expression are treatment-specific.⁶³ The predictive power of these newly identified gene-expression signatures will require validation in prospective clinical trials. Gene profiling has identified unique leukemia-associated markers whose expression can be monitored by flow cytometry to enhance our ability to detect levels of residual disease that are undetectable with the use of standard methods.⁶⁸ This new capability is important because the identification of residual disease has independent prognostic significance and should facilitate early modifications in treatment.^{55,68} The evolving field of proteomics should further enhance our understanding of the biology of ALL and identify proteins that might serve as therapeutic targets or biologic markers.⁶⁹

MOLECULAR EPIDEMIOLOGY

IN UTERO DEVELOPMENT OF ALL

The concept that some cases of ALL originate in utero comes from elegant genetic studies of identical twins with concordant leukemia⁷⁰ and the detection of leukemia-specific fusion-gene sequences or clonotypic rearrangements of the immunoglobulin or T-cell–receptor loci in archived neonatal blood spots (Guthrie cards) of children in whom leukemia subsequently developed.^{71,72} Exposure to mutagens in utero may be an important initiating event in some cases, but the variable latency in the emergence of leukemia suggests that additional genetic alterations are required.

Another important insight has come from the realization that rearrangements of the MLL gene occur not only in most leukemias of infancy, but also in leukemias induced by drugs that inhibit topoisomerase II.59,73 Exposure of the fetus to substances that affect topoisomerase II could therefore be a leukemogenic event in leukemias with an MLL rearrangement. A variety of natural and synthetic compounds, including quinolone antibiotics, flavonoids in foods and drinks, catechins, podophyllin resin, benzene metabolites, and even estrogens can inhibit topoisomerase.59,72 Indeed, a recent international epidemiologic study has implicated transplacental exposure to DNA-damaging drugs, a nonsteroidal antiinflammatory drug (dipyrone), and a mosquitocidal agent, Baygon, in the development of leukemias of infancy involving MLL gene fusion.74 Although the leukemogenic effects of dietary, medical, and environmental exposures are much weaker than those of anticancer chemotherapy, the reduced ability of fetuses or their mothers to detoxify such agents could enhance the susceptibility of fetuses to ALL.55,59

AGE AT PRESENTATION

In children with leukemia of fetal origin, the age at presentation of overt disease varies widely and de-

pends on the leukemia subtype, suggesting important differences in pathogenesis. A concordance rate approaching 100 percent and a very short latency period (from a few weeks to a few months) are typical of infant twins with the t(4;11)/MLL-AF4 chimeric gene,75 suggesting that this fusion is leukemogenic by itself or could facilitate acquisition of additional cooperating mutations to cause leukemia. By contrast, the concordance rate in twins with the TEL-AML1 fusion gene is only about 10 percent, the incubation period is longer, and the presenting features and outcomes are variable, indicating that additional postnatal events are required for leukemic transformation.70,75 This interpretation is supported by the presence of rare cells expressing t(12;21)/TEL-AML1 fusion transcripts in approximately 1 percent of normal cord blood samples, a frequency 100 times the incidence of ALL defined by this fusion transcript.76

Clearly, not all cases of childhood ALL develop in utero, and most cases of adult ALL probably arise over a protracted time. Studies of archived neonatal blood spots and patterns of immunoglobulin and T-cell antigen receptor rearrangements of leukemic cells support a postnatal origin of ALL with t(1;19)/*E2A-PBX1* fusion.⁷⁷ Large-scale epidemiologic studies are under way in the United States and Britain to determine the role, if any, of exposure to chemicals, viruses, bacteria, or ionizing radiation in the genesis of ALL.⁷⁵ Exposure to residential magnetic fields has largely been excluded as an instigating factor.⁷⁸

GENETIC POLYMORPHISMS

Detoxifying Enzymes

Although several monogenic syndromes (e.g., ataxia telangiectasia and the Bloom syndrome) are associated with an increased risk of ALL, they explain only a small fraction of cases. For most leukemias, multiple subtle genetic polymorphisms of xenobiotic metabolizing enzymes may interact with environmental, dietary, maternal, and other external factors to affect the development of ALL. For example, inactivating polymorphisms of detoxifying enzymes (e.g., glutathione S-transferase, reduced nicotinamide adenine dinucleotide phosphate:quinone oxidoreductase) have been variously associated with the development of ALL.79-82 However, these findings need to be confirmed by larger studies with careful attention to ethnic and geographic diversity in the frequency of polymorphisms.

Folate-Metabolizing Enzymes

Low-penetrance polymorphisms of folate-metabolizing enzymes have also been associated with the development of ALL. First, polymorphic variants of methylenetetrahydrofolate reductase, which catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (the predominant circulating form of folate), have been linked to a decreased risk of adult⁸³ and pediatric^{84,85} ALL. This protective effect may be due to the greater availability of 5,10-methylenetetrahydrofolate and thymidine pools and to an increased fidelity of DNA synthesis. Second, polymorphisms of two other folate-related genes - serine hydroxymethyltransferase and thymidylate synthase - have been associated with a lower risk of adult ALL.⁸⁶ Third, a role for folate pathways in susceptibility to ALL has been suggested by an association between folate supplementation and a reduced risk of ALL in children,⁸⁷ but this finding needs confirmation.

PHARMACOGENETICS

The genes that encode drug-metabolizing enzymes, transporters, or drug targets (i.e., receptors or enzymes targeted by antileukemic drugs) can influence the efficacy and toxicity of chemotherapy⁸⁸⁻⁹⁰ (Fig. 6). For example, polymorphisms in the gene for thiopurine methyltransferase, an enzyme that catalyzes S-methylation (inactivation) of mercaptopurine, render the protein susceptible to degradation.94 Approximately 10 percent of patients carry at least one such variant allele for this gene, which leads to the accumulation of high levels of active metabolites of mercaptopurine (thioguanine nucleotides).88 Hence, when treated with standard doses of mercaptopurine, these patients have an increased risk of acute hematopoietic toxic effects (Fig. 6)95 and tend to have longer periods of leukemia-free survival than do patients with two wildtype alleles.⁹¹ Importantly, such patients are at greater risk for radiation-induced brain tumors96 and chemotherapy-induced acute myeloid leukemia.97 Fortunately, it is now possible to identify these patients with the use of simple genotyping tests.98

Thymidylate synthase, an essential enzyme in proliferating cells, is an important target of methotrexate. Homozygosity for a triple tandem-repeat polymorphism of the thymidylate synthase enhancer has been associated with increased enzyme expression and an inferior outcome of treatment in



Figure 6. Influence of Host Germ-Line and ALL Blast Genotypes on the Probability of Cure and of Adverse Events.

The clinical consequences of three host polymorphisms are indicated in the panels on the left. The genotype for thiopurine methyltransferase (TPMT) determines whether reductions in the mercaptopurine dose are needed (top left). Patients who are heterozygous for nonfunctional variant alleles have intermediate TPMT activity and require lower doses of mercaptopurine than do patients who are homozygous for the wildtype allele. Patients who are homozygous for a variant TPMT genotype have very low or undetectable TPMT activity and invariably require marked reductions in the dose of mercaptopurine.⁹¹ The C \rightarrow T polymorphism at position 677 in the methylenetetrahydrofolate reductase (MTHFR) gene affects the risk of mucositis after methotrexate therapy (center left). Patients who are homozygous for an MTHFR variant (TT) have a higher incidence of oral mucositis (as indicated by higher mucositis-index scores) than do those with a wild-type gene (CC).⁹² The repeat polymorphism of the enhancer region of thymidylate synthase (TS) affects the probability of event-free survival in ALL. Patients with a triple tandem-repeat polymorphism (3R/3R) have significantly higher levels of expression of TS and a poorer treatment outcome than do those with a double tandem-repeat polymorphism (2R/2R) or heterozygotes (2R/3R) (bottom left).⁹³ Examples of other genes subject to common functional polymorphisms that may affect the clinical outcome of ALL are shown in the center of the figure for both whites and blacks: the frequencies of the following are indicated by the various colors: homozygous for variant, purple; heterozygous for variant, dark pink; and homozygous for wild-type, light pink. GSTT1 denotes glutathione transferase theta 1, MDR1 multidrug resistance 1, RFC reduced folate carrier, CYP3A5 cytochrome P-450 3A5, NQO1 reduced nicotinamide adenine dinucleotide phosphate: quinone oxidoreductase, and CBS cystathionine beta-synthase.

children with ALL (Fig. 6).⁹³ However, this associ- drug.⁹⁹ Homozygosity for a polymorphism of methation was not found in another study in which pa- ylenetetrahydrofolate reductase (substitution of tients received higher doses of methotrexate, which T for C at position 677) correlates with an increased might have overcome the relative resistance to the risk of oral, gastrointestinal, or hepatic adverse ef-

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fects after low-dose methotrexate^{92,100} and with greater in vitro sensitivity of leukemic blasts to methotrexate.¹⁰¹

Cytochrome P-450 enzymes are involved in the activation of many anticancer drugs (e.g., epipodophyllotoxins and cyclophosphamide) or their inactivation (e.g., vincristine and glucocorticoids).⁸⁸ Levels of cytochrome P-450 enzymes are directly affected by drugs used in the supportive care of patients with leukemia. Specifically, phenytoin or phenobarbital, both used for the long-term treatment of seizures, increases the levels of these enzymes and may adversely affect the outcome of therapy for childhood ALL,¹⁰² whereas azole antifungal agents (e.g., fluconazole, voriconazole, itraconazole, and ketoconazole) inhibit these enzymes, increasing the toxicity of vincristine.¹⁰³

FUTURE DIRECTIONS

Progress in the molecular classification of ALL through use of DNA microarrays^{37,38,42,44} coupled with methods to assess the functional significance of newly discovered genes,¹⁰⁴ or through proteomic techniques⁶⁹ — will almost certainly lead to the identification of targets for specific treatments. A clear precedent is imatinib mesylate for the treatment of BCR-ABL–positive chronic myeloid leukemia.⁴⁶ This agent, which inhibits the BCR-ABL fusion protein and other constitutively active tyrosine kinases and which has induced transient remissions of BCR-ABL-positive ALL¹⁰⁵ and partial responses in other cancers,¹⁰⁶ is the forerunner of a new generation of molecularly targeted anticancer drugs. Other potentially useful agents that are under development include inhibitors of FLT-3 tyrosine kinases for use against leukemias characterized by activating mutations of this kinase44,107 and inhibitors of histone deacetylase for leukemias such as TEL-AML1-positive ALL.^{29,108} Further refinements in the molecular classification of ALL, together with the identification of genetic features that affect the efficacy and toxicity of antileukemic therapy, will afford unique opportunities to devise treatment plans for individual patients and thus to realize the elusive goal of cure in all patients, regardless of their presenting characteristics.

We are indebted to Adolfo A. Ferrando, M.D., and A. Thomas Look, M.D., for sharing the data on T-cell genotypes based on their studies of cases of childhood ALL from Dana–Farber Cancer Institute Consortium, Children's Oncology Group, and St. Jude Children's Research Hospital and of adult ALL from the Cancer and Leukemia Group B and the Eastern Cooperative Oncology Group; to John Cleveland, Ph.D., and William Evans, Pharm.D., for helpful comments; to Julie Groff for assistance with the figures; to John Gilbert for scientific editing; and to our many colleagues for their work.

Supported in part by grants (CA-21765, CA-51001, CA-36401, CA-78224, CA-71907, CA-60419, CA-71970, GM-61393 and GM-61374) from the National Institutes of Health, by a Center of Excellence grant from the State of Tennessee, and by the American Lebanese Syrian Associated Charities. Dr. Pui is the American Cancer Society–F.M. Kirby Clinical Research Professor.

REFERENCES

1. Schrappe M, Reiter A, Ludwig WD, et al. Improved outcome in childhood acute lymphoblastic leukemia despite reduced use of anthracyclines and cranial radiotherapy: results of trial ALL-BFM 90. Blood 2000;95: 3310-22.

2. Gaynon PS, Trigg ME, Heerema NA, et al. Children's Cancer Group trials in childhood acute lymphoblastic leukemia: 1983-1995. Leukemia 2000;14:2223-33.

3. Harms DO, Janka-Schaub GE. Co-operative study group for childhood acute lymphoblastic leukemia (COALL): long-term follow-up of trials 82, 85, 89 and 92. Leukemia 2000;14:2234-9.

4. Silverman LB, Gelber RD, Dalton VK, et al. Improved outcome for children with acute lymphoblastic leukemia: results of Dana-Farber Consortium Protocol 91-01. Blood 2001;97:1211-8.

5. Gustafsson G, Schmiegelow K, Forestier E, et al. Improving outcome through two decades in childhood ALL in the Nordic countries: the impact of high-dose methotrexate in the reduction of CNS irradiation. Leukemia 2000;14:2267-75.

6. Pui CH, Sandlund JT, Pei D, et al. Results

of therapy for acute lymphoblastic leukemia in black and white children. JAMA 2003; 290:2001-7.

 Gökbuget N, Hoelzer D. Recent approaches in acute lymphoblastic leukemia in adults. Rev Clin Exp Hematol 2002;6:114-41.

8. Kantarjian HM, O'Brien S, Smith TL, et al. Results of treatment with hyper-CVAD, a dose-intensive regimen, in adult acute lymphocytic leukemia. J Clin Oncol 2000;18:547-61.

9. Linker C, Damon L, Ries C, Navarro W. Intensified and shortened cyclical chemotherapy for adult acute lymphoblastic leukemia. J Clin Oncol 2002;20:2464-71.

10. Pui C-H, Evans WE. Acute lymphoblastic leukemia. N Engl J Med 1998;339:605-15.

11. Pui C-H, Cheng C, Leung W, et al. Extended follow-up of long-term survivors of childhood acute lymphoblastic leukemia. N Engl J Med 2003;349:640-9. [Erratum, N Engl J Med 2003;349:1299.]

12. Aricò M, Valsecchi MG, Camitta B, et al. Outcome of treatment in children with Philadelphia chromosome–positive acute lymphoblastic leukemia. N Engl J Med 2000; 342:998-1006.

13. Pui CH, Gaynon PS, Boyett JM, et al. Outcome of treatment in childhood acute lymphoblastic leukaemia with rearrangements of the 11q23 chromosomal region. Lancet 2002;359:1909-15.

 Gilliland DG, Tallman MS. Focus on acute leukemias. Cancer Cell 2002;1:417-20.
 Ferrando AA, Look AT. Gene expression profiling in T-cell acute lymphoblastic leukemia. Semin Hematol 2003;40:274-80.

16. Hanahan D, Weinberg RA. The hall-marks of cancer. Cell 2000;100:57-70.

17. Pane F, Intrieri M, Quintarelli C, Izzo B, Muccioli GC, Salvatore F. BCR/ABL genes and leukemic phenotype: from molecular mechanisms to clinical correlations. Oncogene 2002;21:8652-67.

18. Tartaglia M, Martinelli S, Cazzaniga G, et al. Genetic evidence for a lineage- and differentiation stage-related contribution of somatic *PTPN11* mutations to leukemogenesis in childhood acute leukemia. Blood (in press). (Available at http://www.bloodjournal.org.)

19. Buske C, Humphries RK. Homeobox

genes in leukemogenesis. Int J Hematol 2000;71:301-8.

20. Loh ML, Rubnitz JE. TEL/AML1-positive pediatric leukemia: prognostic significance and therapeutic approaches. Curr Opin Hematol 2002;9:345-52.

21. Speck NA, Gilliland DG. Core-binding factors in haematopoiesis and leukaemia. Nat Rev Cancer 2002;2:502-13.

22. Wang LC, Swat W, Fujiwara Y, et al. The TEL/ETV6 gene is required specifically for hematopoiesis in the bone marrow. Genes Dev 1998;12:2392-402.

23. Lorsbach RB, Downing JR. The role of the AML1 transcription factor in leukemogenesis. Int J Hematol 2001;74:258-65.

24. Canon J, Banerjee U. Runt and Lozenge function in Drosophila development. Semin Cell Dev Biol 2000;11:327-36.

25. Hiebert SW, Sun W, Davis JN, et al. The t(12;21) translocation converts AML-1B from an activator to a repressor of transcription. Mol Cell Biol 1996;16:1349-55.

26. Downing JR. The core-binding factor leukemias: lessons learned from murine models. Curr Opin Genet Dev 2003;13:48-54.

27. Hiebert SW, Lutterbach B, Amann J. Role of co-repressors in transcriptional repression mediated by the t(8;21), t(16;21), t(12;21), and inv(16) fusion proteins. Curr Opin Hematol 2001;8:197-200.

28. Lin RJ, Sternsdorf T, Tini M, Evans RM. Transcriptional regulation in acute promyelocytic leukemia. Oncogene 2001;20:7204-15.

29. Johnstone RW, Licht JD. Histone deacetylase inhibitors in cancer therapy: is transcription the primary target? Cancer Cell 2003;4: 13-8.

30. Ernst P, Wang J, Korsmeyer SJ. The role of MLL in hematopoiesis and leukemia. Curr Opin Hematol 2002;9:282-7.

31. Ayton PM, Cleary ML. Molecular mechanisms of leukemogenesis mediated by MLL fusion proteins. Oncogene 2001;20:5695-707.

32. Corral J, Lavenir I, Impey H, et al. An MII-AF9 fusion gene made by homologous recombination causes acute leukemia in chimeric mice: a method to create fusion oncogenes. Cell 1996;85:853-61.

33. Dobson CL, Warren AJ, Pannell R, et al. The mll-AF9 gene fusion in mice controls myeloproliferation and specifies acute myeloid leukaemogenesis. EMBO J 1999;18: 3564-74.

34. Ayton PM, Cleary ML. Transformation of myeloid progenitors by MLL oncoproteins is dependent on *Hoxa7* and *Hoxa9*. Genes Dev 2003;17:2298-307.

35. Antonchuk J, Sauvageau G, Humphries RK. *HOXB4*-induced expansion of adult hematopoietic stem cells ex vivo. Cell 2002;109: 39-45.

36. Thorsteinsdottir U, Sauvageau G, Hough MR, et al. Overexpression of HOXA10 in murine hematopoietic cells perturbs both myeloid and lymphoid differentiation and leads

to acute myeloid leukemia. Mol Cell Biol 1997;17:495-505.

37. Armstrong SA, Staunton JE, Silverman LB, et al. MLL translocations specify a distinct gene expression profile that distinguishes a unique leukemia. Nat Genet 2002; 30:41-7.

38. Ferrando AA, Neuberg DS, Staunton J, et al. Gene expression signatures define novel oncogenic pathways in T cell acute lymphoblastic leukemia. Cancer Cell 2002;1: 75-87.

39. Nakamura T, Largaespada DA, Lee MP, et al. Fusion of the nucleoporin gene NUP98 to HOXA9 by the chromosome translocation t(7;11)(p15;p15) in human myeloid leukaemia. Nat Genet 1996;12:154-8.

40. Borrow J, Shearman AM, Stanton VP Jr, et al. The t(7;11)(p15;p15) translocation in acute myeloid leukaemia fuses the genes for nucleoporin NUP98 and class I homeoprotein HOXA9. Nat Genet 1996;12:159-67.

41. Aspland SE, Bendall HH, Murre C. The role of E2A-PBX1 in leukemogenesis. Oncogene 2001;20:5708-17.

42. Yeoh EJ, Ross ME, Shurtleff SA, et al. Classification, subtype discovery, and prediction of outcome in pediatric acute lymphoblastic leukemia by gene expression profiling. Cancer Cell 2002;1:133-43.

43. Gilliland DG, Griffin JD. The roles of FLT3 in hematopoiesis and leukemia. Blood 2002;100:1532-42.

44. Armstrong SA, Kung AL, Mabon ME, et al. Inhibition of FLT3 in MLL: validation of a therapeutic target identified by gene expression based classification. Cancer Cell 2003; 3:173-83.

45. Armstrong SA, Mabon ME, Silverman LB, et al. FLT3 mutations in childhood acute lymphoblastic leukemia. Blood (in press). (Available at http://www.bloodjournal.org.)
46. Hughes TP, Kaeda J, Branford S, et al. Frequency of major molecular responses to imatinib or interferon alfa plus cytarabine in newly diagnosed chronic myeloid leukemia. N Engl J Med 2003;349:1423-32.

47. Sherr CJ, McCormick F. The RB and p53 pathways in cancer. Cancer Cell 2002;2:103-12.

48. Krug U, Ganser A, Koeffler HP. Tumor suppressor genes in normal and malignant hematopoiesis. Oncogene 2002;21:3475-95.
49. Omura-Minamisawa M, Diccianni MB, Batova A, et al. Universal inactivation of both p16 and p15 but not downstream components is an essential event in the pathogenesis of T-cell acute lymphoblastic leukemia. Clin Cancer Res 2000;6:1219-28.

50. Stock W, Tsai T, Golden C, et al. Cell cycle regulatory gene abnormalities are important determinants of leukemogenesis and disease biology in adult acute lymphoblastic leukemia. Blood 2000;95:2364-71.

51. Vousden KH, Lu X. Live or let die: the cell's response to p53. Nat Rev Cancer 2002; 2:594-604.

52. Calero Moreno TM, Gustafsson G, Garwicz S, et al. Deletion of the Ink4-locus (the

p16ink4a, p14ARF and p15ink4b genes) predicts relapse in children with ALL treated according to the Nordic protocols NOPHO-86 and NOPHO-92. Leukemia 2002;16:2037-45.

53. Roman-Gomez J, Castillejo JA, Jimenez A, et al. 5' CpG island hypermethylation is associated with transcriptional silencing of the p21(CIP1/WAF1/SDI1) gene and confers poor prognosis in acute lymphoblastic leukemia. Blood 2002;99:2291-6.

54. Sherr CJ. The INK4a/ARF network in tumour suppression. Nat Rev Mol Cell Biol 2001;2:731-7.

55. Pui CH, Campana D, Evans WE. Childhood acute lymphoblastic leukaemia — current status and future perspectives. Lancet Oncol 2001:2:597-607.

56. Ramakers-van Woerden NL, Pieters R, Loonen AH, et al. TEL-AML1 gene fusion is related to in vitro drug sensitivity for L-asparaginase in childhood acute lymphoblastic leukemia. Blood 2000;96:1094-9.

57. Belkov VM, Krynetski EY, Schuetz JD, et al. Reduced folate carrier expression in acute lymphoblastic leukemia: a mechanism for ploidy but not lineage differences in methotrexate accumulation. Blood 1999;93:1643-50.

58. Stams WA, den Boer ML, Beverloo HB, et al. Sensitivity to L-asparaginase is not associated with expression levels of asparagine synthetase in t(12;21)⁺ pediatric ALL. Blood 2003;101:2743-7.

59. Biondi A, Cimino G, Pieters R, Pui CH. Biological and therapeutic aspects of infant leukemia. Blood 2000;96:24-33.

60. Stam RW, den Boer ML, Meijerink JP, et al. Differential mRNA expression of Ara-C-metabolizing enzymes explains Ara-C sensitivity in MLL gene-rearranged infant acute lymphoblastic leukemia. Blood 2003;101: 1270-6.

61. Pui CH, Relling MV, Evans WE. Role of pharmacogenomics and pharmacodynamics in the treatment of acute lymphoblastic leukaemia. Best Pract Res Clin Haematol 2003;15:741-56.

62. Pui CH, Sallan S, Relling MV, Masera G, Evans WE. International Childhood Acute Lymphoblastic Leukemia Workshop: Sausalito, CA, 30 November–1 December 2000. Leukemia 2001;15:707-15.

63. Cheok MH, Yang W, Pui CH, et al. Treatment-specific changes in gene expression discriminate *in vivo* drug response in human leukemia cells. Nat Genet 2003;34:85-90. [Erratum, Nat Genet 2003;34:231.]

64. McCormack MP, Rabbitts TH. Activation of the T-cell oncogene *LMO2* after gene therapy for X-linked severe combined immunodeficiency. N Engl J Med 2004;350: 913-22.

65. Pui CH, Chessells JM, Camitta B, et al. Clinical heterogeneity in childhood acute lymphoblastic leukemia with 11q23 rearrangements. Leukemia 2003;17:700-6.

66. Ballerini P, Blaise A, Busson-Le Coniat M, et al. HOX11L2 expression defines a clin-

MECHANISMS OF DISEASE

ical subtype of pediatric T-ALL associated with poor prognosis. Blood 2002;100:991-7. **67**. Cavé H, Suciu S, Preudhomme C, et al. Clinical significance of HOX11L2 expression linked to t(5;14)(q35;q32), of HOX11 expression, and of SIL-TAL fusion in childhood T-cell malignancies: results of EORTC studies 58881 and 58951. Blood 2004;103: 442-50.

68. Campana D. Determination of minimal residual disease in leukaemia patients. Br J Haematol 2003;121:823-38.

69. MacBeath G. Protein microarrays and proteomics. Nat Genet 2002;32:Suppl:526-32.

70. Greaves MF, Maia AT, Wiemels JL, Ford AM. Leukemia in twins: lessons in natural history. Blood 2003:102:2321-33.

71. Taub JW, Konrad MA, Ge Y, et al. High frequency of leukemic clones in newborn screening blood samples of children with B-precursor acute lymphoblastic leukemia. Blood 2002;99:2992-6.

72. Greaves MF, Wiemels J. Origins of chromosome translocations in childhood leukaemia. Nat Rev Cancer 2003;3:639-49.

73. Pui CH, Relling MV. Topoisomerase II inhibitor-related acute myeloid leukaemia. Br J Haematol 2000;109:13-23.

74. Alexander FE, Patheal SL, Biondi A, et al. Transplacental chemical exposure and risk of infant leukemia with MLL gene fusion. Cancer Res 2001;61:2542-6.

75. Greaves M. Childhood leukaemia. BMJ 2002;324:283-7.

76. 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 2002; 99:8242-7.

77. Wiemels JL, Leonard BC, Wang Y, et al. Site-specific translocation and evidence of postnatal origin of the t(1;19) E2A-PBX1 fusion in childhood acute lymphoblastic leukemia. Proc Natl Acad Sci U S A 2002;99: 15101-6.

78. UK Childhood Cancer Study Investigators. Childhood cancer and residential proximity to power lines. Br J Cancer 2000;83: 1573-80.

79. Chen C-L, Liu Q, Pui CH, et al. Higher frequency of glutathione S-transferase deletions in black children with acute lymphoblastic leukemia. Blood 1997;89:1701-7.

80. Krajinovic M, Labuda D, Richer C, Karimi S, Sinnett D. Susceptibility to childhood acute lymphoblastic leukemia: influence of CYP1A1, CYP2D6, GSTM1, and GSTT1 genetic polymorphisms. Blood 1999;93:1496-501.

81. Davies SM, Bhatia S, Ross JA, et al. Glutathione S-transferase genotypes, genetic susceptibility, and outcome of therapy in childhood acute lymphoblastic leukemia. Blood 2002;100:67-71. **82.** Smith MT, Wang Y, Skibola CF, et al. Low NAD(P)H:quinone oxidoreductase activity is associated with increased risk of leukemia with MLL translocations in infants and children. Blood 2002;100:4590-3.

83. Skibola CF, Smith MT, Kane E, et al. Polymorphisms in the methylenetetrahydrofolate reductase gene are associated with susceptibility to acute leukemia in adults. Proc Natl Acad Sci U S A 1999;96:12810-5.
84. Franco RF, Simões BP, Tone LG, Gabellini SM, Zago MA, Falcao RP. The methylenetetrahydrofolate reductase C677T gene polymorphism decreases the risk of childhood acute lymphocytic leukaemia. Br J Haematol 2001;115:616-8.

85. Wiemels JL, Smith RN, Taylor GM, et al. Methylenetetrahydrofolate reductase (MTHFR) polymorphisms and risk of molecularly defined subtypes of childhood acute leukemia. Proc Natl Acad Sci U S A 2001;98: 4004-9.

86. Skibola CF, Smith MT, Hubbard A, et al. Polymorphisms in the thymidylate synthase and serine hydroxymethyltransferase genes and risk of adult acute lymphocytic leukemia. Blood 2002;99:3786-91.

87. Thompson JR, Gerald PF, Willoughby ML, Armstrong BK. Maternal folate supplementation in pregnancy and protection against acute lymphoblastic leukaemia in childhood: a case-control study. Lancet 2001; 358:1935-40.

88. Relling MV, Dervieux T. Pharmacogenetics and cancer therapy. Nat Rev Cancer 2001;1:99-108.

89. Weinshilboum R. Inheritance and drug response. N Engl J Med 2003;348:529-37.

90. Evans WE, McLeod HL. Pharmacogenomics — drug disposition, drug targets, and side effects. N Engl J Med 2003;348:538-49.
91. Relling MV, Hancock ML, Boyett JM, Pui CH, Evans WE. Prognostic importance of 6-mercaptopurine dose intensity in acute lymphoblastic leukemia. Blood 1999;93: 2817-23.

92. Ulrich CM, Yasui Y, Storb R, et al. Pharmacogenetics of methotrexate: toxicity among marrow transplantation patients varies with the methylenetetrahydrofolate reductase C677T polymorphism. Blood 2001; 98:231-4.

93. Krajinovic M, Costea I, Chiasson S. Polymorphism of the thymidylate synthase gene and outcome of acute lymphoblastic leukaemia. Lancet 2002;359:1033-4.

94. Tai HL, Krynetski EY, Schuetz EG, Yanishevski Y, Evans WE. Enhanced proteolysis of thiopurine S-methyltransferase (TMPT) encoded by mutant alleles in humans (TPMT*3A, TPMT*2): mechanisms for the genetic polymorphism of TPMT activity. Proc Natl Acad Sci U S A 1997;94:6444-9.
95. Relling MV. Hancock ML. Rivera GK. et

95. Relling MV, Hancock ML, Rivera GK, et al. Mercaptopurine therapy intolerance and

heterozygosity at the thiopurine S-methyltransferase gene locus. J Natl Cancer Inst 1999;91:2001-8.

96. Relling MV, Rubnitz JE, Rivera GK, et al. High incidence of secondary brain tumours after radiotherapy and antimetabolites. Lancet 1999;354:34-9.

97. Bo J, Schrøder H, Kristinsson J, et al. Possible carcinogenic effect of 6-mercaptopurine on bone marrow stem cells: relation to thiopurine metabolism. Cancer 1999;86:1080-6.
98. Yates CR, Krynetski EY, Loennechen T, et al. Molecular diagnosis of thiopurine S-methyltransferase deficiency: genetic basis for azathioprine and mercaptopurine intolerance. Ann Intern Med 1997;126:608-14.
99. Lauten M, Asgedom G, Welte K, Schrappe M, Stanulla M. Thymidylate synthase gene polymorphism and its association with relapse in childhood B-cell precursor acute lymphoblastic leukemia. Haematologica 2003;88:353-4.

100. Urano W, Taniguchi A, Yamanaka H, et al. Polymorphisms in the methylenetetrahydrofolate reductase gene were associated with both the efficacy and the toxicity of methotrexate used for the treatment of rheumatoid arthritis, as evidenced by single locus and haplotype analyses. Pharmacogenetics 2002; 12:183-90.

101. Taub JW, Matherly LH, Ravindranath Y, Kaspers GJ, Rots MG, Zantwijk CH. Polymorphisms in methylenetetrahydrofolate reductase and methotrexate sensitivity in childhood acute lymphoblastic leukemia. Leukemia 2002;16:764-5.

102. Relling MV, Pui CH, Sandlund JT, et al. Adverse effect of anticonvulsants on efficacy of chemotherapy for acute lymphoblastic leukaemia. Lancet 2000;356:285-90.

103. Venkatakrishnan K, von Moltke LL, Greenblatt DJ. Effects of the antifungal agents on oxidative drug metabolism: clinical relevance. Clin Pharmacokinet 2000;38:111-80.
104. McManus MT, Sharp PA. Gene silencing in mammals by small interfering RNAs. Nat Rev Genet 2002;3:737-47.

105. Druker BJ, Sawyers CL, Kantarjian H, et al. Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. N Engl J Med 2001;344:1038-42. [Erratum, N Engl J Med 2001;345:232.]
106. Druker BJ. STI571 (Gleevec) as a paradigm for cancer therapy. Trends Mol Med 2002;8:Suppl:S14-S18.

107. Levis M, Small D. FLT3: ITDoes matter in leukemia. Leukemia 2003;17:1738-52.

108. Batova A, Shao LE, Dicciani MB, et al. The histone deacetylase inhibitor AN-9 has selective toxicity to acute leukemia and drugresistant primary leukemia and cancer cell lines. Blood 2002;100:3319-24.

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