REVIEW ARTICLE

MECHANISMS OF DISEASE

Acute Lymphoblastic Leukemia

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THE 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.

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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

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
			yr	%	
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 CBFα) (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 (CBFα plus CBFβ) 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 sequence[25] (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-

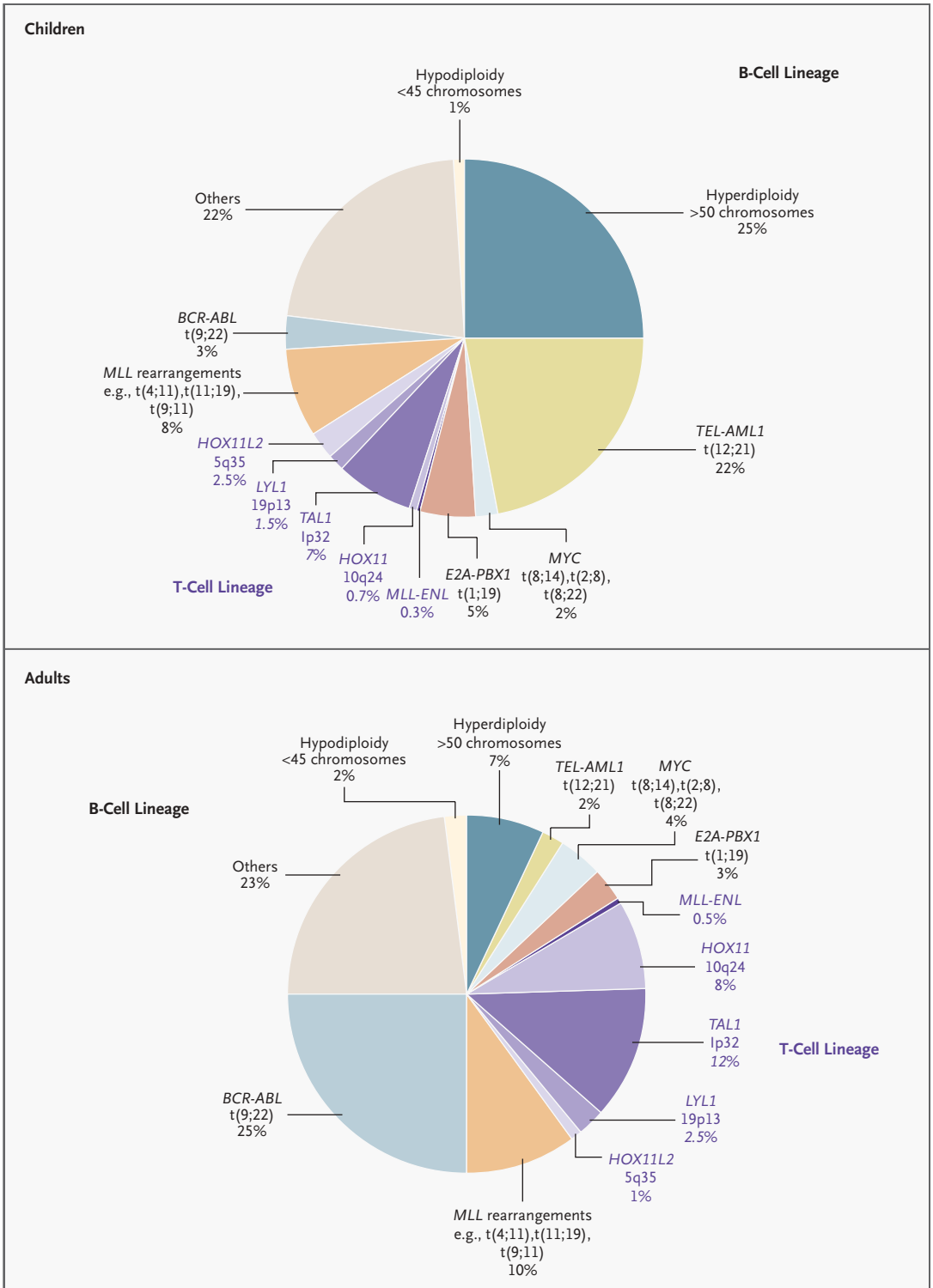

Figure 1. Estimated Frequency of Specific Genotypes of ALL in Children and Adults.

Data were modified from Pui and Evans[10] to include recently described T-cell genotypes.[15] The genetic lesions that are exclusively seen in cases of T-cell–lineage leukemias are indicated in purple. All other genetic subtypes are either exclusively or primarily seen in cases of B-cell–lineage ALL.

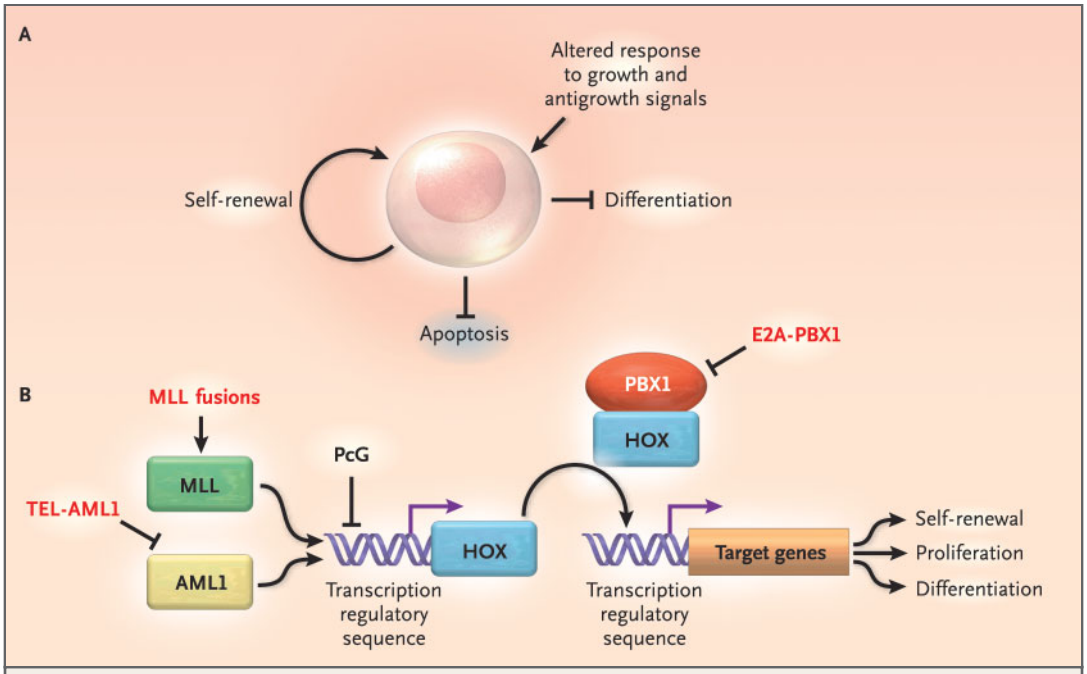

Figure 2. Transformation of Hematopoietic Cells in the Pathogenesis of ALL.

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-CBFβ 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 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 portion of 1 of more than 40 partners. This genetic

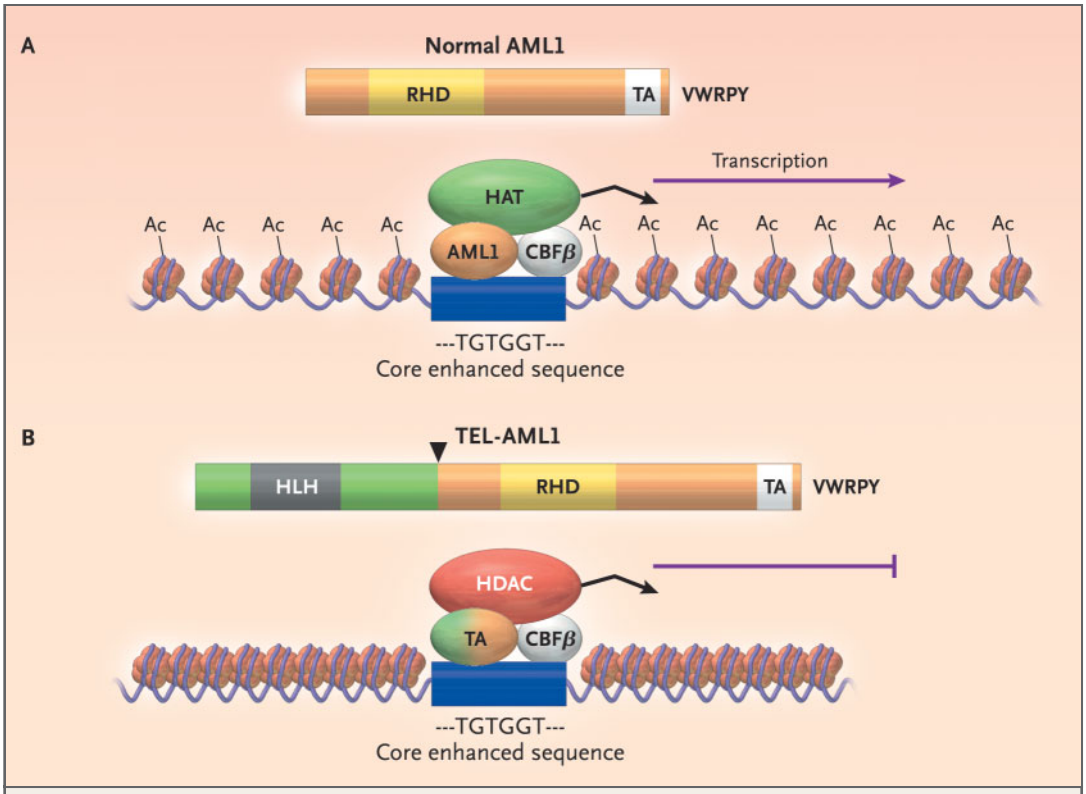

Figure 3. Mechanism of Transcriptional Repression by TEL-AML1.

Panel A shows the structure of AML1, including its central runt homology domain (RHD), which mediates DNA binding and heterodimerization with core binding factor β (CBFβ), a transcriptional activation domain (TA), and the C-terminal amino acids VWRPY (in single amino acid code), which mediate binding to the Groucho corepressors. AML1 binds to the core enhanced transcriptional regulatory sequences as a heterodimer with CBFβ, and together they recruit a transcriptional activation complex that includes proteins with histone acetyltransferase (HAT) activity. The HAT proteins acetylate (Ac) lysine residues in the core histones, which opens the chromatin structure and leads to transcriptional activation (purple arrow). Panel B shows the structure of the t(12;21)-generated TEL-AML1 fusion protein, in which the N-terminal helix–loop–helix (HLH) domain of TEL is fused to the nearly complete AML1 protein. An arrowhead indicates 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 CBFβ; 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 effects 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-

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 DNA-binding 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 $P14^{ARF}$ 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 $P16^{INK4a}$ and $P14^{ARF}$ 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.

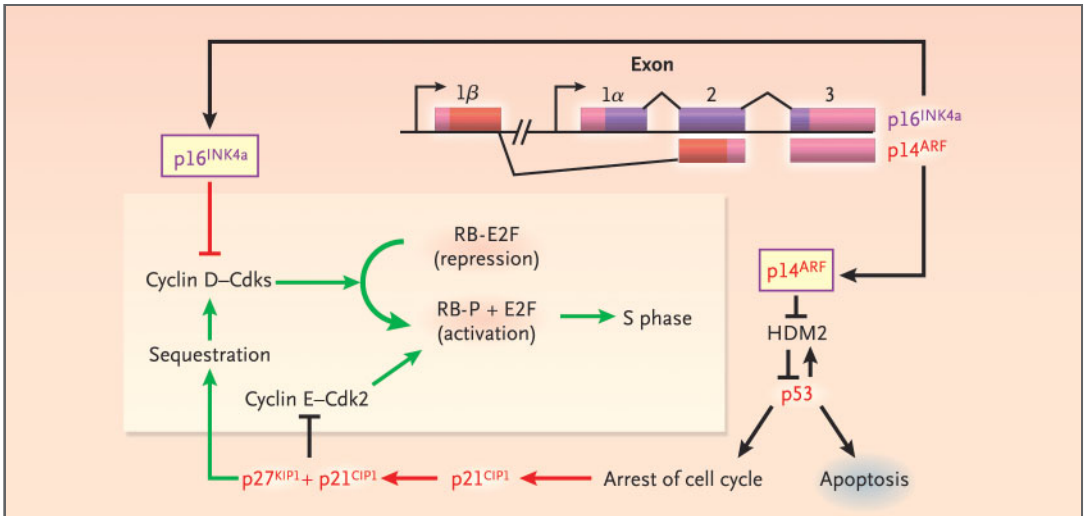

Figure 4. The Retinoblastoma (RB) and p53 Tumor-Suppressor Network.

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 (1α and 1β) 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

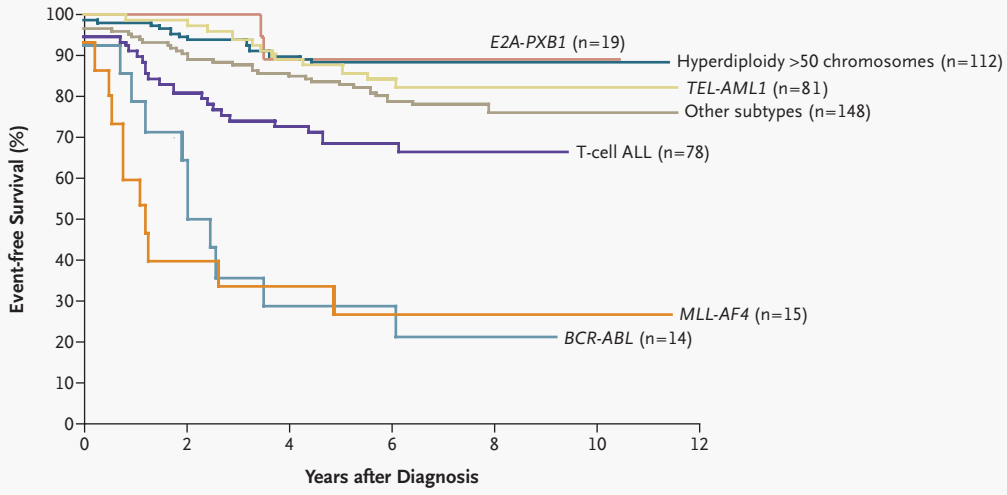

Figure 5. Kaplan–Meier Analysis of Event-free Survival According to the Subtype of Leukemia in 467 Children with ALL Who Were Enrolled in Three Consecutive Treatment Protocols at St. Jude Children's Research Hospital from 1991 to 1999.

Patients with t(1;19) leading to *E2A-PBX1* fusion, hyperdiploidy involving more than 50 chromosomes, or *TEL-AML1* fusion have a favorable treatment outcome, with mean (±SE) five-year event-free survival rates of 89.5±7.3 percent, 88.3±3.3 percent, and 87.5±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±11.4 percent and 28.6±10.8 percent, respectively. The prognosis is intermediate for patients with other B-cell–lineage ALL (83.6±3.3 percent) and T-cell ALL (68.6±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 cells[61] 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 percent[6] (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 presentation[12]; adults with this type of ALL have a dismal prognosis.[7] Among patients with *MLL*-rearranged 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 $P16^{INK4a}$ and $P14^{ARF}$, whereas cases involving *LYL1* are characterized by high levels of *N-MYC* expression and the deletion of as yet 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 leukemia[70] 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 adult[83] 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 chemotherapy[88-90] (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 wild-type alleles.[91] Importantly, such patients are at greater risk for radiation-induced brain tumors[96] 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

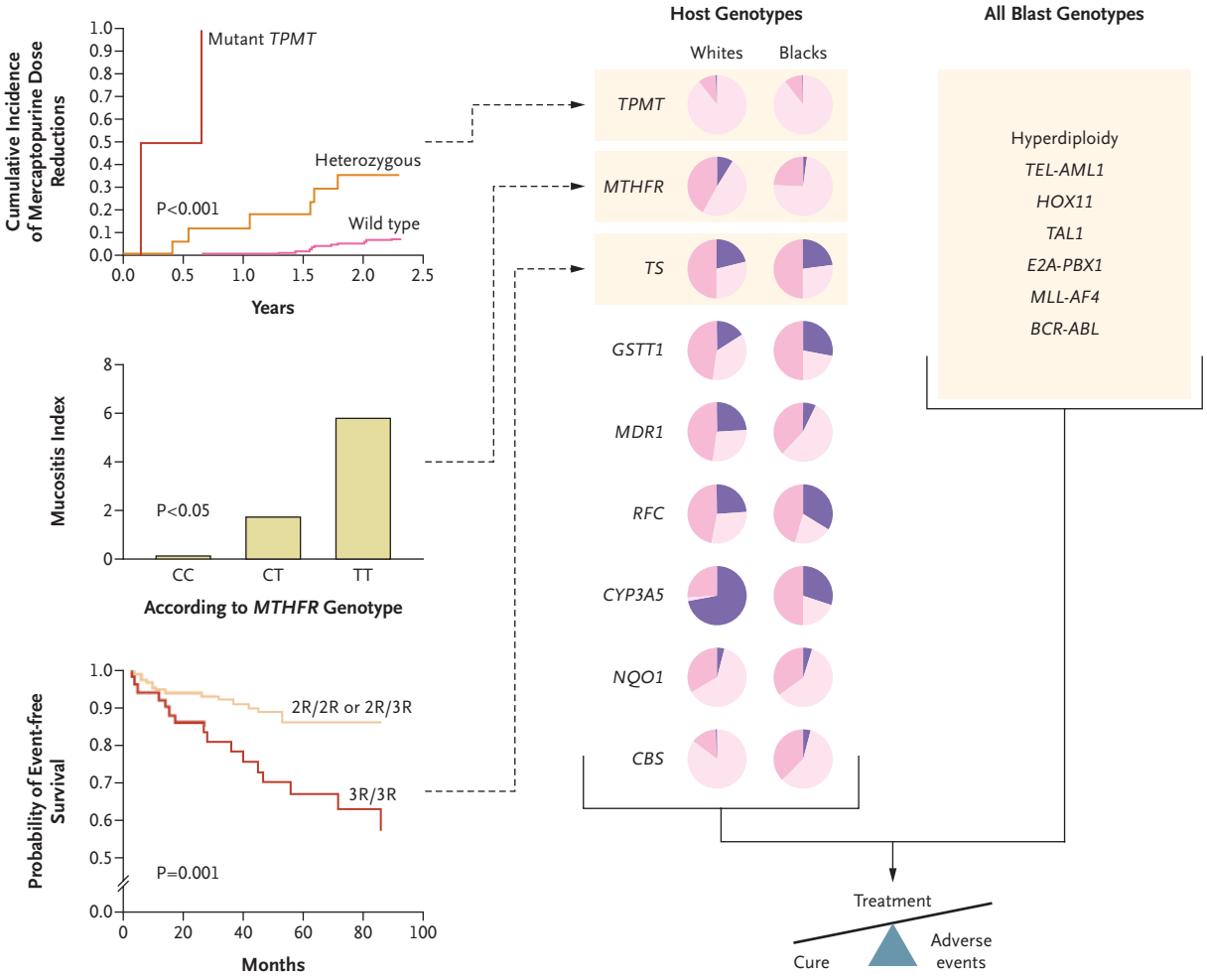

Figure 6. Influence of Host Germ-Line and ALL Blast Genotypes on the Probability of Cure and of Adverse Events.

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 wild-type 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→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 association was not found in another study in which patients received higher doses of methotrexate, which might have overcome the relative resistance to the drug.[99] Homozygosity for a polymorphism of methylenetetrahydrofolate reductase (substitution of T for C at position 677) correlates with an increased risk of oral, gastrointestinal, or hepatic adverse ef-

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 techniques[69] — 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 ALL[105] 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 kinase[44,107] 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.

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