

Acute lymphoblastic leukaemia

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Acute lymphoblastic leukaemia, a malignant disorder of lymphoid progenitor cells, affects both children and adults, with peak prevalence between the ages of 2 and 5 years. Steady progress in development of effective treatments has led to a cure rate of more than 80% in children, creating opportunities for innovative approaches that would preserve past gains in leukaemia-free survival while reducing the toxic side-effects of current intensive regimens. Advances in our understanding of the pathobiology of acute lymphoblastic leukaemia, fuelled by emerging molecular technologies, suggest that drugs specifically targeting the genetic defects of leukaemic cells could revolutionise management of this disease. Meanwhile, studies are underway to ascertain the precise events that take place in the genesis of acute lymphoblastic leukaemia, to enhance the clinical application of known risk factors and antileukaemic agents, and to identify treatment regimens that might boost the generally low cure rates in adults and subgroups of children with high-risk leukaemia.

Introduction

Addition of acute lymphoblastic leukaemia to the growing list of cancers that have succumbed to effective treatment is tempting. The decision would be easy to justify in view of data showing cure rates higher than 80% for children treated in modern centres, most of whom will lead healthy productive lives as long-term cancer survivors.^{1–3} Thus, the future management of acute lymphoblastic leukaemia might be viewed as simply tweaking existing protocols and devising alternative regimens for the fifth of patients who respond poorly to available agents. This scenario, however attractive, must be rejected on several grounds. It does not accommodate the poor prognosis for adults with acute lymphoblastic leukaemia or the complexity, expense, and toxic effects of contemporary multiagent treatments.¹⁴ Most importantly, it overlooks our rapidly increasing ability to analyse the genetic and epigenetic abnormalities of leukaemic cells and to translate them into enhanced diagnostic methods and molecularly targeted therapy.^{5,6} Although the molecular medicine approach is still in its investigative stage, with many new obstacles to overcome, it holds enormous promise. Put simply, we are about to enter an era in which leukaemia patients will probably receive individualised treatment based on the genetic features of their malignant cells and their own unique genetic make-up (so-called pharmacogenomics).⁷ Our intent in this Seminar is to review advances in both the fundamental understanding and clinical management of acute lymphoblastic leukaemia in children and adults.

Epidemiology and cause

The precise pathogenetic events leading to development of acute lymphoblastic leukaemia are unknown. Only a few cases (<5%) are associated with inherited, predisposing genetic syndromes, such as Down's syndrome, Bloom's syndrome, ataxia-telangiectasia, and Nijmegen breakage syndrome, or with ionising radiation or exposure to specific chemotherapeutic drugs. Although accumulating published work on high birthweight as a risk factor for childhood acute lymphoblastic leukaemia is becoming increasingly convincing,⁸ there exists an

extensive list of conflicting or isolated reports of factors purported to confer an increased risk for this disease, including parental occupation, maternal reproductive history, parental tobacco or alcohol use, maternal diet, prenatal vitamin use, exposure to pesticides or solvents, and exposure to the highest levels (>0.3 or 0.4 μ T) of residential, power-frequency magnetic fields.^{9,10}

Observations of a peak age of development of childhood acute lymphoblastic leukaemia of 2–5 years, an association of industrialisation and modern or affluent societies with increased prevalence of the disease, and the occasional clustering of childhood leukaemia cases (especially in new towns) have fuelled two parallel infection-based hypotheses by British investigators: Kinlen's population-mixing hypothesis and Greaves' delayed-infection hypothesis (figure 1).^{11,12} Kinlen's hypothesis predicts that clusters of childhood cases of acute lymphoblastic leukaemia result from exposure of susceptible (non-immune) individuals to common but fairly non-pathological infections after population-mixing with carriers. The delayed-infection hypothesis of Greaves is based on a minimal two-hits model and suggests that some susceptible individuals with a prenatally acquired preleukaemic clone had low or no exposure to common infections early in life because they lived in an affluent hygienic environment. Such infectious insulation predisposes the immune system of these individuals to aberrant or pathological responses after subsequent or delayed exposure to common infections at an age commensurate with increased lymphoid-cell proliferation.

Retrospective identification of leukaemia-specific fusion genes, hyperdiploidy, or clonotypic rearrangements of immunoglobulin or T-cell-receptor loci in

Search strategy and selection criteria

We searched Medline and PubMed for articles published in English dating from 2002, with the keywords "acute lymphoblastic leukemia", "acute lymphocytic leukemia", and "acute lymphoid leukemia". In some instances, review articles were selected over original articles because of space constraints.

archived neonatal blood spots (Guthrie cards) and studies of leukaemia in monozygotic twins indicate clearly a prenatal origin for some childhood leukaemias.^{12–15} Screening of neonatal cord-blood samples has revealed a putative leukaemic clone with the *TEL-AML1* fusion gene (also known as *ETV6-RUNX1*) in 1% of newborn babies, a frequency 100 times higher than the prevalence of acute lymphoblastic leukaemia defined by this fusion gene later in childhood.¹⁶ The variable incubation period and clinical outcome of such cases, and the 10% concordance rate of leukaemia in identical twins with this genotype, support the notion that additional postnatal events are needed for full leukaemic transformation.¹⁷ A recent study further established the presence of a preleukaemic clone with the *TEL-AML1* fusion.¹⁵

Investigations have also focused on the genetic variability in xenobiotic metabolism, DNA repair pathways, and cell-cycle checkpoint functions that might interact with environmental, dietary, maternal, and other external factors to affect development of acute lymphoblastic leukaemia. Although the number of investigations and sample sizes are limited, data exist to support a possible causal role for polymorphisms in genes encoding cytochrome P450, NAD(P)H quinone oxidoreductase, glutathione S-transferases, methyl-entetahydrofolate reductase, thymidylate synthase, serine hydroxymethyltransferase, and cell-cycle inhibitors.^{18–23} To date, however, no direct gene-environment interactions have been established convincingly.

In view of the scarcity of causal insights from large-scale epidemiological studies, some investigators have adopted a strategy that focuses on distinct subtypes of childhood acute lymphoblastic leukaemia. An important example is the study of infant acute lymphoblastic leukaemia with *MLL* rearrangement,²⁴ a genetic abnormality that has also been associated with secondary leukaemia after exposure to a topoisomerase II inhibitor.²⁵ Thus, dietary, medical, and environmental exposures to substances that inhibit topoisomerases, and the reduced ability of fetuses or their mothers to detoxify such agents, could lead to development of infant leukaemia.^{26,27}

Pathobiology

Acute lymphoblastic leukaemia is thought to originate from various important genetic lesions in blood-progenitor cells that are committed to differentiate in the T-cell or B-cell pathway, including mutations that impart the capacity for unlimited self-renewal and those that lead to precise stage-specific developmental arrest.^{6,28} In some cases, the first mutation along the multistep pathway to overt acute lymphoblastic leukaemia might arise in a haemopoietic stem cell possessing multilineage developmental capacity.²⁹ The cells implicated in acute lymphoblastic leukaemia have clonal rearrangements in their immunoglobulin or T-cell receptor genes and express antigen-receptor molecules and other

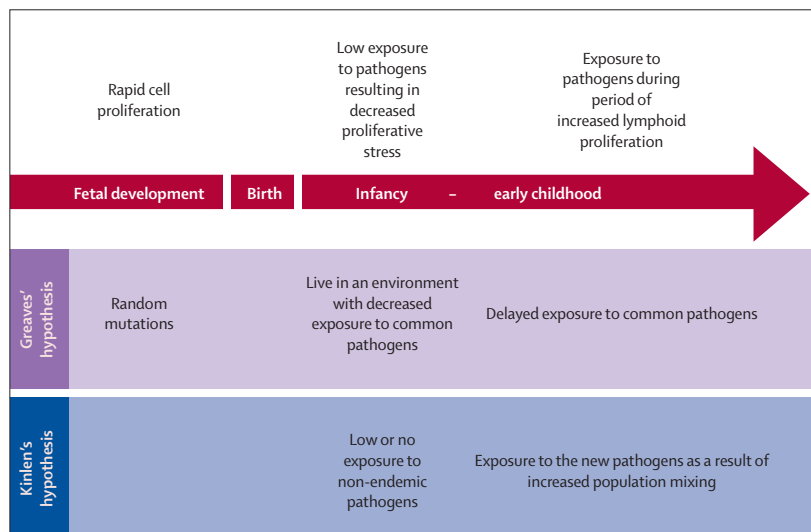


Figure 1: Infection-based models of leukaemia development

differentiation-linked cell-surface glycoproteins that largely recapitulate those of immature lymphoid progenitor cells within the early developmental stages of normal T and B lymphocytes.^{6,28,30} The dominant theme of contemporary research in pathobiology of acute lymphoblastic leukaemia is to understand the outcomes of frequently arising genetic lesions, in terms of their effects on cell proliferation, differentiation, and survival, and then to devise selectively targeted treatments against the altered gene products to which the leukaemic clones have become addicted.³¹

Chromosomal translocations

Chromosomal translocations that activate specific genes are a defining characteristic of human leukaemias and of acute lymphoblastic leukaemia in particular.^{6,28} Gene-expression patterns studied in large series of newly diagnosed leukaemias have substantiated the idea that specific chromosomal translocations identify unique subtypes of the disease.^{32–35} Usually, translocations activate transcription-factor genes, which in many cases can control cell differentiation (rather than cell division per se), are developmentally regulated, and frequently encode proteins at the apex of important transcriptional cascades.²⁸ These so-called master oncogenic transcription factors, which can exert either positive or negative control over downstream responder genes, are expressed aberrantly in leukaemic cells as one gene product or as a unique fusion protein combining elements from two different transcription factors.^{6,28}

About 25% of cases of B-cell precursor acute lymphoblastic leukaemia, the most frequent form of acute leukaemia in children, harbour the *TEL-AML1* fusion gene—generated by the t(12;21)(p13;q22) chromosomal translocation.⁶ Although the molecular pathogenesis of *TEL-AML1*-positive leukaemia remains unclear, findings in mice establish the *Tel* gene as an

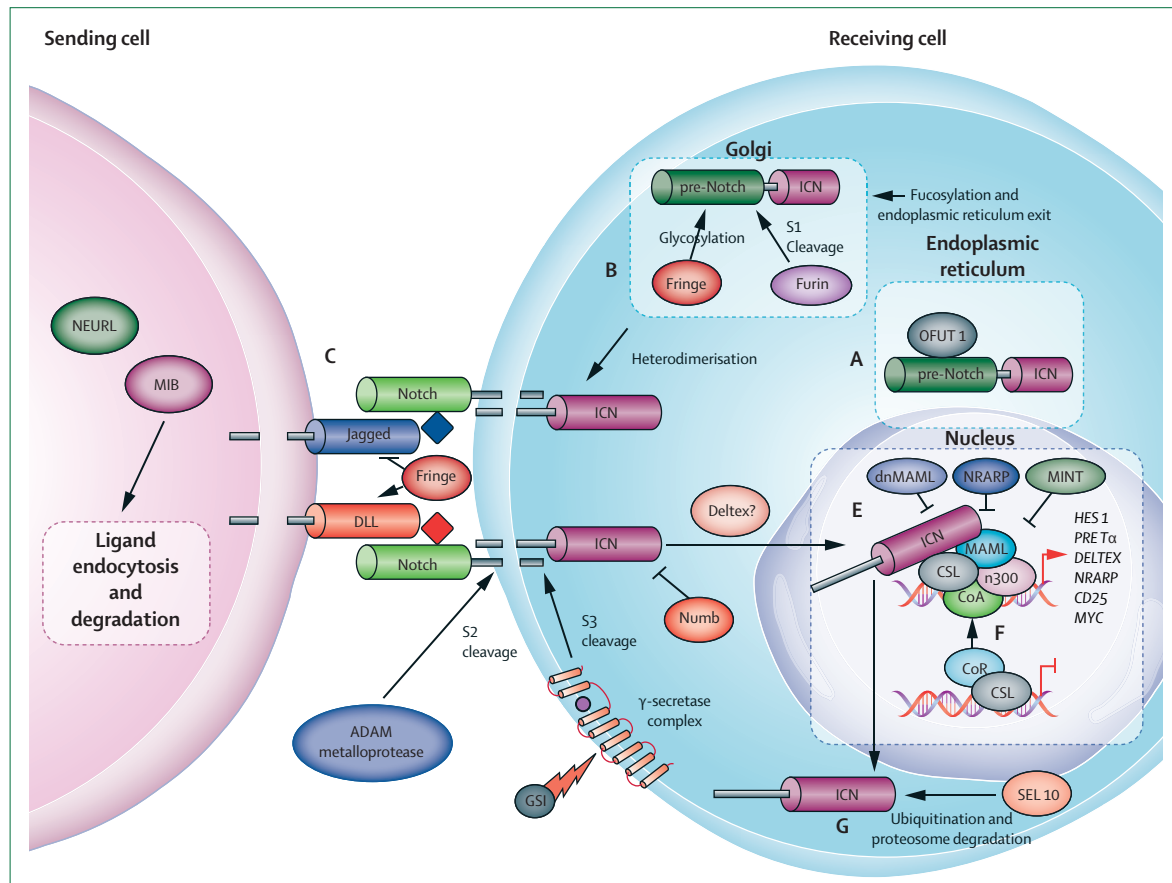


Figure 2: NOTCH signalling in normal thymocytes

The NOTCH signalling pathway is complex and involves the coordinated activities of many different molecules. Briefly, NOTCH is synthesised in the endoplasmic reticulum (A) as one protein consisting of an extracellular domain (pre-Notch) and an intracellular domain (ICN), which are transported in tandem to the Golgi (B), where several post-translational modifications take place, including a proteolytic cleavage (S1) that separates the two domains from each other. The resultant heterodimer is then transported to the cell membrane, where NOTCH interacts with ligands (C) and is cleaved twice (D) by the ADAM protease (S2) and a γ -secretase complex (S3), enabling the liberated ICN domain to translocate to the nucleus (E). Nuclear ICN forms a binding/activator complex with a group of cooperating proteins (F), resulting in transcriptional activation of several functionally important genes, including MYC and pre-T α . Hyperphosphorylation of ICN via interaction of its PEST domain (polypeptide enriched in proline, glutamate, serine, and threonine) with CDK8, MAML, and p300 facilitates ubiquitylation (G) by SEL1 family members, targeting ICN to the proteasome. ADAM=a disintegrin and metalloproteinase domain. DLL=Delta ligand. hes1=hairy/enhancer of split. GSI= γ -secretase inhibition. MAML=mastermind-like proteins. MIB=mindbomb. NEURL=neutralised-like. Nrarp= gene encoding NOTCH-regulated ankyrin-repeat protein. OFUT1=O-fucosyltransferase1. Pre-T α =pre-TCR α . Deltex=positive regulator of Notch signalling pathway. CSL=DNA binding component. CoA=co-activators. CoR=co-repressors. Fringe=regulator of Notch ligand. SEL10=positive regulator of Notch.

important regulator of haemopoietic-cell development, essential for definitive haemopoiesis.³⁶ Similarly, *Aml1* gene is essential for definitive embryonic haemopoiesis.^{37,38} Thus, the presence of the TEL-AML1 fusion protein in B-cell progenitors seems to lead to disordered early B-lineage lymphocyte development, a hallmark of leukaemic lymphoblasts. Analysis of TEL-AML1-induced cord blood cells suggests that the fusion gene serves as a first-hit mutation by endowing the preleukemic cell with altered self-renewal and survival properties.¹⁵

In adults, the most frequent chromosomal translocation is t(9;22), or the Philadelphia chromosome, which causes fusion of the BCR signalling protein to the ABL non-receptor tyrosine kinase, resulting in constitutive tyrosine kinase activity and complex interactions of this fusion protein with many other transforming elements,

such as the signalling pathway for RAS (GTP-binding protein that activates target genes involved in cell differentiation, proliferation, and survival).³⁹ As an activated kinase, BCR-ABL offers an attractive therapeutic target, and imatinib mesilate, a small-molecule inhibitor of the ABL kinase, has proven effective against leukaemias that express BCR-ABL.⁴⁰

More than 50% of cases of T-cell acute lymphoblastic leukaemia have activating mutations that involve NOTCH1,⁴¹ a gene encoding a transmembrane receptor that regulates normal T-cell development.⁴² NOTCH receptors become activated when ligands of the Delta-Serrate-Lag2 family of proteins bind to the extracellular portion of the transmembrane molecule. This interaction initiates a cascade of proteolytic cleavages, terminating in γ -secretase generation of intracellular

NOTCH1, which translocates to the nucleus and regulates by transcription a diverse set of responder genes, including the *MYC* oncogene (figure 2).^{43,44} The precise mechanisms by which aberrant NOTCH signalling (due to mutational activation) causes T-cell acute lymphoblastic leukaemia are still unclear but probably entail constitutive expression of oncogenic responder genes, such as *MYC*, and cooperation with other signalling pathways (pre-TCR [T-cell receptor for antigen] and RAS, for example). Interference with NOTCH signalling by small-molecule inhibition of γ -secretase activity has the potential to induce remission of T-cell acute lymphoblastic leukaemia.

Evidence suggests that the *MYC* oncoprotein is an important downstream mediator of the pro-growth effects of NOTCH1 signalling in developing thymocytes.^{28,45} However, results of retroviral insertional mutagenesis in murine models of transgenic T-cell acute lymphoblastic leukaemia show that *Notch1* mutations, with outcomes similar to those in primary human T-cell acute lymphoblastic leukaemias, can potentiate the effects of pre-existing *MYC* overexpression,^{42,46} suggesting that NOTCH1 must have important transformational targets other than *MYC*. Activating mutations in *NOTCH1* sufficient to produce constitutive NOTCH1 signalling can induce T-cell acute lymphoblastic leukaemia in experimental models and could be the instigating event in most human T-cell leukaemias.^{28,42} γ -secretase, a multicomponent membrane-associated enzyme, is needed for NOTCH1 signalling through mutant NOTCH receptors in T-cell acute lymphoblastic leukaemia, providing an attractive target for therapeutic intervention with newly developed γ -secretase inhibitors.^{28,47}

Cooperating mutations

Although chromosomal abnormalities are a hallmark of pathogenesis of acute lymphoblastic leukaemia, evidence suggests that they must act in concert with several other genetic lesions to induce overt leukaemia. A prime example is the biallelic deletion or epigenetic silencing of the cyclin-dependent kinase inhibitor 2A gene (*CDKN2A*), which encodes both the tumour suppressors p16^{INK4A} and p14^{ARF} and whose inactivation neutralises both the TP53 and retinoblastoma pathways in most cases of T-cell and many cases of B-cell precursor acute lymphoblastic leukaemia.⁶ In a genome-wide analysis of 242 cases of paediatric acute lymphoblastic leukaemia using high-resolution single nucleotide polymorphism arrays, deletions, amplifications, point mutations, and other structural rearrangements were identified in genes encoding regulators of B-lymphocyte development in 40% of cases of B-cell precursor acute lymphoblastic leukaemia.⁴⁸ The *PAX5* gene was the most frequent target of somatic mutation, being altered in almost a third of cases. Deletions were also detected in other B-cell developmental genes, such as *TCF3* (*E2A*), *EBF1* (*EBF*),

LEF1, *IKZF1* (*Ikaros*), and *IKZF3* (*Aiolos*). Finally, in T-cell acute lymphoblastic leukaemia, at least five multistep mutational pathways leading to frank leukaemia have been identified, and in some cases these pathways entail five or more documented genetic lesions.^{28,30,42}

Ongoing research to define the oncogenic contributions of various classes of genetic lesions relies heavily on animal models that accurately recapitulate the molecular pathogenesis of B-cell precursor or T-cell acute lymphoblastic leukaemia.⁴⁹ Most studies undertaken to date have used genetically engineered mice to elucidate the multistep transformation pathways leading to T-cell acute lymphoblastic leukaemia.⁵⁰ Such models depend on breeding strategies to combine one or more genetic lesions and show synergy in transformation,⁵¹ whereas some investigators have also capitalised on the use of retroviral insertional mutagenesis screens to uncover collaborating oncogenes.^{46,52} New models of T-cell acute lymphoblastic leukaemia in the zebrafish offer a powerful alternative vertebrate system for leukaemia research, whose unique advantages complement those of extant murine models.⁵³ Currently available zebrafish models of acute lymphoblastic leukaemia include a *myc* transgene-driven system, in which lymphoblasts faithfully reproduce the multistep oncogenic pathway noted in up to 60% of human T-cell acute lymphoblastic leukaemias,^{54,55} and a transgenic zebrafish model, in which the TEL-AML1 oncoprotein induces B-cell precursor leukaemia.⁵⁶

The challenge now is to understand how these cooperative genetic lesions and their affected pathways interact to alter the proliferation, differentiation, and survival of lymphocyte progenitors leading to their leukaemic conversion. This research will undoubtedly provide the molecular rationales needed to select new therapeutic targets and to develop interfering small molecules or antibodies with high levels of antileukaemic specificity and activity.⁵⁷ The table provides a partial list of molecularly targeted drugs now in clinical testing.

Diagnosis

Phenotype

Immunophenotyping of leukaemic lymphoblasts by flow cytometry is essential to establish the correct diagnosis and define cell lineage. Although acute lymphoblastic leukaemia can be readily subclassified according to the many steps of normal B-cell and T-cell differentiation, the only findings with therapeutic importance are T-cell, mature B-cell, and B-cell precursor phenotypes.^{26,58} Myeloid-associated antigen expression can be detected in as many as half the cases of acute lymphoblastic leukaemia. However, with contemporary treatment, this so-called aberrant antigen expression has no prognostic implications but can be used to distinguish leukaemic cells from normal progenitor cells, thereby enabling detection of minimal (ie, submicroscopic) residual leukaemia.^{6,26}

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

Table: Selected antileukaemic drugs being tested in clinical trials

Genotype

Although chromosomal analysis is still an integral component of initial work-up of acute lymphoblastic leukaemia, other highly specific and sensitive techniques—such as RT-PCR, fluorescence in-situ hybridisation, and flow cytometry—are increasingly used to detect specific fusion transcripts, gain or loss of cellular DNA content, or specific chromosomes with prognostic or therapeutic relevance.^{26,58} Although still a research technique, gene-expression profiling can not only identify accurately the major subtypes of acute lymphoblastic leukaemia but also implicate single genes or signalling pathways as important determinants of clinical outcome.^{32-34,59-61} Once this method has been refined and made cost effective, it will undoubtedly replace many current diagnostic techniques.

Pharmacogenetics

Genetic polymorphisms of drug transporters, receptors, targets, and drug-metabolising enzymes can affect the effectiveness and toxic effects of antineoplastic drugs.^{7,62}

Traditionally, pharmacogenetic studies have focused on single genes identified on the basis of their influence on the pharmacokinetics and pharmacological effects of anticancer drugs. Findings of global gene-expression profiling studies have identified a growing number of genomic determinants of treatment responses that could allow development of polygenic models for optimisation of treatment for acute lymphoblastic leukaemia.^{6,7,63}

Despite the promise of pharmacogenetic studies to enhance treatment outcome in acute lymphoblastic leukaemia, only polymorphisms and the activity of thiopurine methyltransferase—an enzyme that catalyses S-methylation (inactivation) of thiopurines such as mercaptopurine and thioguanine—have been useful in clinical practice.^{7,64,65} About 10% of the total population inherit one wild-type gene encoding thiopurine methyltransferase and one non-functional variant allele, resulting in intermediate enzyme activity, whereas 1 in 300 people inherit two non-functional variant alleles with no enzyme activity. When treated with conventional doses of thiopurines, up to half of patients with the heterozygous deficiency and all homozygous-deficient patients develop haemopoietic toxic effects, which can be fatal in the homozygous group.⁷ The enzyme deficiency also confers a high risk of developing therapy-related acute myeloid leukemia⁷ and radiation-induced brain tumours, in the context of intensive thiopurine treatment.⁶⁶ Conversely, patients with high levels of enzyme activity might be at greater risk of relapse owing to decreased exposure of leukaemic cells to active drug metabolites.⁶⁵ In most centres, studies of thiopurine methyltransferase activity are undertaken only in people with poor tolerance to antimetabolite-based therapy, and the result is used to guide reductions in drug dosage.⁶⁷ We use a fairly high dose of mercaptopurine and, thus, study this enzyme prospectively in all patients, lowering the dose of mercaptopurine in individuals with enzyme deficiency.⁶⁴

Risk assessment

Careful assessment of the risk of relapse in individual patients ensures that very intensive treatment is given only to high-risk cases, thus sparing people at lower risk from undue toxic effects. Although enhanced treatment has abolished the prognostic strength of many clinical and biological risk factors identified in the past, we would stress that even so-called low-risk patients need a certain degree of treatment intensification to avoid unacceptable rates of relapse. Findings have shown that adolescents and young adults who were treated on adult protocols fared significantly worse than the same age-groups treated on paediatric protocols.⁶⁸⁻⁷⁰ The superior outcome achieved with paediatric regimens has been attributed to more effective treatment and to better adherence by patients, parents, and doctors.⁶⁸⁻⁷² To understand the actual basis for this difference in

outcome, several combined adult and paediatric consortia are using common regimens to treat patients aged 1–50 years.

Clinical factors

Age at diagnosis has a strong prognostic effect (figure 3). In work done at St Jude Children's Research Hospital, 847 children with acute lymphoblastic leukaemia were enrolled in four consecutive treatment protocols from 1991 to 2006. Children aged 1–9 years had a better outcome than either infants or adolescents.^{26,58} 5-year event-free survival estimates were 88% (SE 2) for children aged 1–9 years, 73% (4) for adolescents aged 10–15 years, 69% (7) for those older than 15 years, and 44% (11) for babies younger than 12 months. Babies younger than 6 months have an especially poor outcome.^{73,74} The outcome of treatment in adults worsens with increasing age. Indeed, in the past, patients older than 60 years were not even included in clinical trials owing to their many coexisting health problems, their heightened susceptibility to treatment-related toxic effects, and their high frequency of Philadelphia chromosome-positive acute lymphoblastic leukaemia.^{1,4,75} This practice has begun to change, partly because of enhanced supportive care now available for older adults and development of specific tyrosine kinase inhibitors for Philadelphia chromosome-positive acute lymphoblastic leukaemia (table).^{75,76}

Leucocyte count is a continuous prognostic variable, with increasing counts conferring a poorer outcome, especially in patients with B-cell precursor disease.^{58,76} In T-cell acute lymphoblastic leukaemia, a leucocyte count greater than $100 \times 10^9/L$ is associated with an increased risk of relapse in the CNS.⁷⁷ Patients with extreme hyperleucocytosis ($>400 \times 10^9/L$) are at high risk for early complications such as CNS haemorrhage and pulmonary and neurological events due to leucostasis.⁷⁸ A uniform risk-classification system, based on both age and leucocyte count, was devised to facilitate comparisons of treatment results in childhood acute lymphoblastic leukaemia.²⁶ Two-thirds of patients aged 1–9 years with a leucocyte count less than $50 \times 10^9/L$ were judged to have a standard (or low) risk of relapse, whereas the remaining third were classified as high risk. This system by itself has limited value because up to a third of the so-called standard-risk patients could relapse, and individuals at very high risk—who need allogeneic haemopoietic stem-cell transplantation—cannot be distinguished reliably from high-risk cases.²⁶ Moreover, risk criteria apply only to B-cell precursor acute lymphoblastic leukaemia and have little prognostic value in T-cell disease.

In US cooperative group studies, black and Hispanic patients fared worse than similarly treated white individuals.⁷⁹ The poor prognosis for black people could be related to their high frequency of T-cell acute lymphoblastic leukaemia and the t(1;19) chromosomal abnormality with *E2A-PBX1* fusion.⁸⁰ However, in single-institution studies,

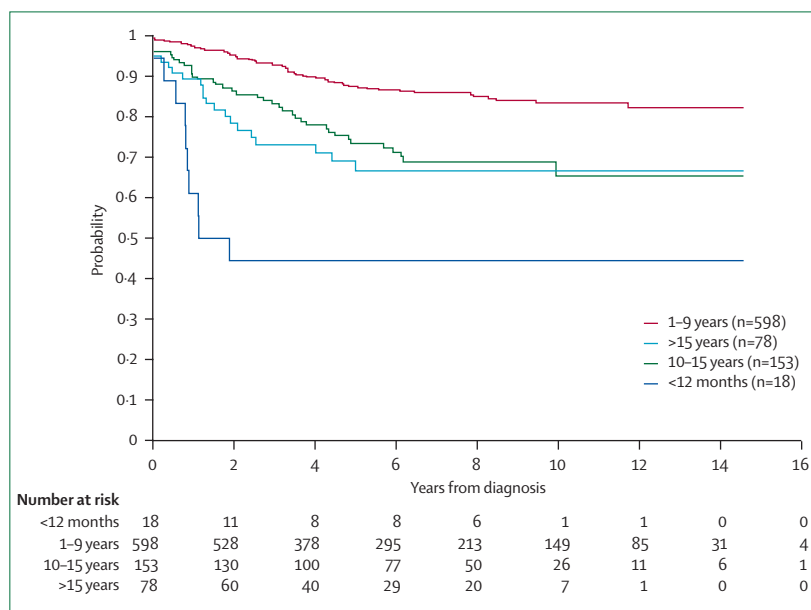


Figure 3: Kaplan-Meier estimates of event-free survival according to age at diagnosis of acute lymphoblastic leukaemia

black children had the same high cure rates as did white children when given equal access to effective treatment,⁸⁰ underscoring the over-riding prognostic importance of treatment. The adverse prognosis previously ascribed to male sex has also been abolished with enhanced treatment regimens.^{2,81}

Likewise, the effect of obesity on outcome of acute lymphoblastic leukaemia is also treatment dependent. A report by the Children's Oncology Group showed that overweight children with acute lymphoblastic leukaemia, aged 10 years or older, have a poor treatment outcome.⁸² By contrast, we noted no association between the body-mass index of patients with acute lymphoblastic leukaemia and clinical outcome, toxic effects, or the pharmacokinetics of several drugs tested.⁸³

Biological factors

T-cell and mature B-cell immunophenotypes, once associated with a poor outcome, have little prognostic importance in childhood acute lymphoblastic leukaemia and are actually favourable features in adult disease in the context of contemporary treatment.^{2,76,81,84} Although genetic abnormalities do not account entirely for treatment outcome they still provide indispensable prognostic information (figure 4). 841 children with acute lymphoblastic leukaemia and successful cytogenetic and immunophenotypic studies were enrolled in four consecutive treatment protocols at St Jude Children's Research Hospital from 1991 to 2006. Patients with hyperdiploidy (>50 chromosomes), *TEL-AML1* fusion, and t(1;19)/*E2A-PBX1* fusion had the most favourable outcome, whereas those with the t(9;22)/*BCR-ABL* fusion or t(4;11)/*MLL-AF4* fusion had a dismal prognosis. 5-year event-free

survival estimates were 91% (SE 3) for hyperdiploidy, 89% (3) for *TEL-AML1* fusion, 86% (7) for *E2A-PBX1* fusion, 82% (3) for other B-lineage disease, 73% (5) for T-cell acute lymphoblastic leukaemia, 37% (12) for *BCR-ABL* fusion, and 32% (12) for *MLL-AF4* fusion.

In general, the Philadelphia chromosome, t(4;11) with *MLL-AF4* fusion, and hypodiploidy (<44 chromosomes per leukaemic cell) all confer a poor outcome, whereas hyperdiploidy (>50 chromosomes), *TEL-AML1* fusion, and trisomy 4, 10, and 17 are associated with favourable prognosis.^{6,85-87} About 2% of childhood cases were noted to have intrachromosomal amplification of chromosome 21, which is associated with a B-cell precursor immunophenotype, older age, low white-cell counts, and, more importantly, a threefold increase in risk of relapse.⁸⁸ The high frequency of unfavourable genetic features and low rate of favourable genetic abnormalities in adults with acute lymphoblastic leukaemia partly explain their inferior outcome compared with childhood cases.⁷⁶

Age affects the prognostic importance of genetic abnormalities for unknown reasons. In children with Philadelphia chromosome-positive acute lymphoblastic leukaemia, those aged 1–9 years fared better than did adolescents,⁸⁹ who in turn had a better prognosis than adults.^{90,91} In patients with *MLL-AF4* fusion, infants and adults have a worse prognosis than children.⁹¹⁻⁹³ The t(1;19) with *E2A-PBX1* fusion has no prognostic implications in childhood acute lymphoblastic leukaemia but is still associated with a poor prognosis in some adult cases.⁷⁶ Findings are scarce to suggest that activating mutations of the *NOTCH1* gene are associated with a

favourable prognosis in childhood T-cell acute lymphoblastic leukaemia⁹⁴ but an unfavourable outcome in adults.⁹⁵

Data of microarray analyses of leukaemic cells identified genes that affect the intracellular disposition of antileukaemic drugs and have shown distinct sets of genes that are associated with resistance to different classes of antileukaemic agents.^{59-61,96-99} Aberrant expression of some genes also seemed to have prognostic relevance.^{59,60} It is also noteworthy that numerical chromosomal abnormalities, depending on whether the affected chromosomes contain the wild-type or variant allele of the genes, can greatly affect the pharmacogenomics of cancer treatment and, thus, clinical outcome.¹⁰⁰

Response to treatment

Response to treatment is determined by the entire constellation of leukaemic-cell biological features (intrinsic drug sensitivity) in concert with the pharmacodynamics and pharmacogenomics of the host, the regimens administered, and treatment adherence. Not surprisingly, the degree of reduction of the leukaemic cell clone early during remission induction therapy has independent prognostic importance^{1,26,101-104} even in low-risk cases defined by clinical and biological features.¹⁰⁵ However, morphology-based methods traditionally used to assess treatment response are neither precise enough nor sensitive enough to measure this cytoreduction reliably.^{26,106} Molecular and flow-cytometric methods, which are at least 100-fold more sensitive than morphological determinations, now allow minimal residual leukaemia to be detected at very low levels (<0.01%), providing an useful means to identify patients at very low or high risk of relapse.^{1,26,101-106} Indeed, patients with 1% or more leukaemic cells at the end of 4–6 weeks of remission induction therapy fare almost as poorly as those who fail to achieve clinical remission by the accepted morphological standard ($\geq 5\%$ leukaemic cells), whereas people who achieve molecular or immunological remission (<0.01% leukaemic cells) have an excellent outcome.^{1,26} Findings of one study in adults showed that monitoring of minimal residual leukaemia can be used for early detection of impending relapse and hence for early treatment intervention.¹⁰⁷

Residual leukaemic T cells can be detected easily by their positivity for terminal deoxynucleotidyl transferase and cytoplasmic CD3. A simple and inexpensive assay for minimal residual leukaemia has been developed for B-cell precursor acute lymphoblastic leukaemia.¹⁰⁸ It is based on the rationale that normal, immature, CD19+ B-cell progenitors (those expressing CD10, CD34, or both) are exquisitely sensitive to corticosteroids and other antileukaemic drugs and are undetectable consistently in bone marrow after 2 weeks of remission induction treatment. Hence, any cells with this immunophenotype after 2 weeks of remission induction probably represent minimal residual leukaemia.¹⁰⁸

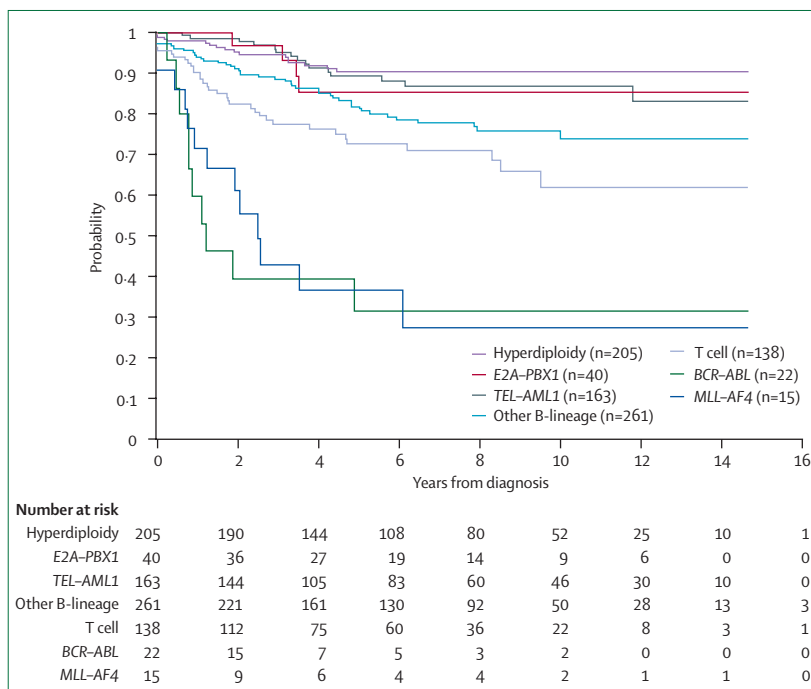


Figure 4: Kaplan-Meier analysis of event-free survival according to biological subtype of leukaemia

Treatment

With the exception of patients with mature B-cell acute lymphoblastic leukaemia, who are treated with short-term intensive chemotherapy (including high-dose methotrexate, cytarabine, and cyclophosphamide),^{109–111} treatment for acute lymphoblastic leukaemia typically consists of a remission-induction phase, an intensification (or consolidation) phase, and continuation therapy to eliminate residual disease. Treatment is also directed to the CNS early in the clinical course to prevent relapse attributable to leukaemic cells sequestered in this site.¹ The drugs currently in use for these phases were developed and tested between the 1950s and 1970s, but efforts to identify new antileukaemic agents have begun to intensify (table).

Remission-induction phase

The goal of remission-induction treatment is to eradicate more than 99% of the initial leukaemic cell burden and to restore normal haemopoiesis and healthy performance status. This approach typically includes administration of a glucocorticoid (prednisone or dexamethasone), vincristine, and at least a third drug (asparaginase, anthracycline, or both). A three-drug induction regimen seems sufficient for most standard-risk cases provided they receive intensified post-remission treatment. Children with high-risk or very high-risk acute lymphoblastic leukaemia, and virtually all adult cases of the disease, are treated with four or more drugs for remission induction.¹ We measure levels of minimal residual leukaemia after 2 weeks of remission induction and we intensify treatment in patients with high amounts of residual blasts (>1%). Clinical remission can now be induced in 96–99% of children and 78–93% of adults.¹ Although no induction regimen is clearly superior to any others, addition of cyclophosphamide and intensive treatment with asparaginase are widely considered beneficial to patients with T-cell acute lymphoblastic leukaemia,^{2,76} and imatinib mesilate has greatly enhanced the remission-induction rate, duration of disease-free survival, and quality of life of patients with Philadelphia chromosome-positive acute lymphoblastic leukaemia.^{112–114} Whether the cure rate of this subtype of leukaemia can be raised with imatinib or the newly developed, more potent, tyrosine kinase inhibitors nilotinib and dasatinib remains unknown.^{115,116}

Presumably because of its longer half-life and increased penetration into the CNS, dexamethasone has been deemed more effective than either prednisone or prednisolone for treatment of acute lymphoblastic leukaemia.^{117,118} However, findings of a small randomised study showed that an augmented dose of prednisolone produced results comparable with those achieved with dexamethasone in the context of other intensive treatment.¹¹⁹ Similarly, the pharmacodynamics of asparaginase differ by formulation,¹²⁰ and in terms of leukaemia control, the dose intensity and duration of

asparaginase treatment (ie, the amount of asparagine depletion) are far more important than the type of asparaginase used.^{1,2} Compared with *Escherichia coli* asparaginase, *Erwinia* asparaginase was associated with inferior antileukaemic response but fewer toxic effects,^{121,122} a finding now attributed to use of inadequate doses of the *Erwinia* drug. In some current protocols, polyethylene glycol-conjugated asparaginase—a long-acting and less allergenic form—has replaced the native product in initial treatment.^{123–126} Many complications recorded during remission induction are attributable to the synergistic effects of corticosteroid and asparaginase. In the context of multiagent treatment, a fairly small increase in dose of dexamethasone or asparaginase can result in excessive toxic effects and death, especially in older children and adults.

Consolidation (intensification) treatment

With the restoration of normal haemopoiesis and body function, intensification treatment is generally used to eradicate drug-resistant residual leukaemic cells, thus reducing the risk of relapse. For example, patients with *TEL-AML1*-positive disease have an especially good outcome in clinical trials of intensive post-remission therapy with corticosteroids, vincristine, and asparaginase.^{81,127} Although the importance of this treatment phase is rarely disputed, consensus is scarce on the best regimens and duration of treatment. Frequently used strategies include high-dose methotrexate plus mercaptopurine, reinduction treatment with the same agent that was given initially, frequent pulses of vincristine and corticosteroid plus high-dose asparaginase for 20–30 weeks, and an augmented regimen consisting of reinduction treatment and additional doses of vincristine, asparaginase, and intravenous methotrexate during periods of myelosuppression.^{1–3,128} For patients with high-risk or very high-risk acute lymphoblastic leukaemia, incorporation of high-dose methotrexate plus mercaptopurine into a regimen based on intensive asparaginase treatment could be desirable. Findings of ongoing studies will establish if these approaches in children are effective and tolerable in adults.

Reinduction treatment has become an integral component of contemporary protocols. In one randomised study of intermediate-risk acute lymphoblastic leukaemia, double reinduction further enhanced treatment outcome, whereas additional pulses of vincristine and prednisone after one reinduction course were not beneficial, suggesting that the increased dose-intensity of other drugs—such as asparaginase—led to the noted improvement.¹²⁹ Although a standard intensification regimen for adult acute lymphoblastic leukaemia is absent, post-remission treatment with cytarabine, cyclophosphamide, anthracyclines, and methotrexate has improved outcome in some non-randomised studies.^{130–132}

The best dose of methotrexate depends on the leukaemic-cell genotype and phenotype and host pharma-

cogenetic and pharmacokinetic variables. Methotrexate at 1–2 g/m² is adequate for most patients with standard-risk acute lymphoblastic leukaemia, but a higher dose (eg, 5 g/m²) might benefit individuals with T-cell or high-risk B-cell precursor disease.^{133,134} The fairly low accumulation of methotrexate polyglutamates in blast cells with either *TEL-AML1* or *E2A-PBX1* fusion suggests that patients with these genotypes could also benefit from an increased dose of methotrexate.⁹⁸ However, mega doses of methotrexate (eg, 33·6 g/m²) do not seem necessary for patients with acute lymphoblastic leukaemia.¹³⁵ Finally, leucovorin rescue, although necessary after treatment with high-dose methotrexate, must not be given too early or at too high a dosage because it might counteract the antileukaemic effects of methotrexate.^{134–136}

Allogeneic haemopoietic stem-cell transplantation

Allogeneic haemopoietic stem-cell transplantation is the most intensive form of treatment for acute lymphoblastic leukaemia. Comparisons between this modality and intensive chemotherapy have yielded inconsistent results owing to the few patients studied and differences in case-selection criteria.^{137–140} Nonetheless, allogeneic transplantation clearly benefits several subgroups of patients with high-risk acute lymphoblastic leukaemia, such as individuals with Philadelphia chromosome-positive disease (even when treated with a tyrosine kinase inhibitor) and those with a poor initial response to treatment.^{89,113,137,140–142} It also improves the outcome of adults with the t(4;11) subtype of acute lymphoblastic leukaemia, but its benefits in infants with this genotype are controversial.^{74,92,143,144} Findings of studies suggest that matched unrelated-donor or cord-blood transplantation could produce results comparable with those obtained with matched related-donor transplantation.^{145,146} In view of the substantial morbidity and mortality associated with this procedure and the growing prospects for effective targeted therapy, the need for allogeneic transplantation should be reassessed continuously. Autologous transplantation, despite several practical advantages, has failed to enhance outcome in either adult or paediatric acute lymphoblastic leukaemia.^{138,147}

Continuation treatment

For reasons that (currently) remain elusive, patients with acute lymphoblastic leukaemia need continuation treatment to prevent or forestall relapse. Although about two-thirds of childhood cases can be treated successfully with only 12 months of therapy, they cannot be identified prospectively with any degree of certainty.¹⁴⁸ Hence, all patients receive chemotherapy for 2·0–2·5 years. Daily mercaptopurine and methotrexate every week constitute the backbone of continuation regimens. Many investigators advocate that drug dosages be adjusted to maintain leucocyte counts below 3×10⁹/L and neutrophil counts

between 0·5 and 1·5×10⁹/L to ensure adequate dose intensity during the continuation phase.¹

Since thioguanine is more potent than mercaptopurine in model systems and leads to higher concentrations of thioguanine nucleotides in cells and cytotoxic concentrations in cerebrospinal fluid,¹⁴⁹ several randomised trials have been done to compare the effectiveness of these two drugs.^{150–152} Thioguanine, given at a daily dose of 40 mg/m² or more, produced superior antileukaemic responses to mercaptopurine but was associated with profound thrombocytopenia, an increased risk of death in remission, and an unacceptably high rate (10–20%) of hepatic veno-occlusive disease.^{150–152} Although the lower activity of thiopurine methyltransferase is associated with thioguanine-related liver damage, this measure cannot identify reliably patients at risk.¹⁵³ Mercaptopurine, therefore, remains the drug of choice for acute lymphoblastic leukaemia, although thioguanine could still be given in short-term courses during the intensification phase of treatment.

In a multicentre randomised trial, addition of six pulses of vincristine and dexamethasone during early continuation treatment failed to improve outcome of children with intermediate-risk acute lymphoblastic leukaemia.¹⁵⁴ Whether more intensive pulse therapy would enhance outcome in the context of contemporary therapy remains to be studied.

CNS-directed treatment

CNS relapse is a major obstacle to cure, accounting for 30–40% of initial relapses in some studies.^{117,129,155} Factors associated with an increased risk of CNS relapse include a T-cell immunophenotype, hyperleucocytosis, high-risk genetic abnormalities, and presence of leukaemic cells in cerebrospinal fluid (even from iatrogenic introduction due to a traumatic lumbar puncture).^{3,77,156–158} Polymorphisms in genes that code for proteins implicated in the pharmacodynamics of antileukaemic drugs have also been associated with risk of CNS relapse.⁶³

Because of its many associated acute and late complications,^{159,160} cranial irradiation is now administered to only 5–20% of patients at high risk for CNS relapse.⁷⁷ With effective systemic treatment, the radiation dose can be lowered to 12 Gy for most patients and to 18 Gy for those with CNS leukaemia at diagnosis.¹³³ In fact, 18 Gy irradiation was shown to be effective even in patients with late isolated CNS relapse, in the context of intensive systemic chemotherapy.¹⁶¹ We are testing the feasibility of omitting radiation for all patients with acute lymphoblastic leukaemia, reserving its use exclusively for remission retrieval therapy. Whether or not cranial radiation is used, the best regimen of intrathecal therapy should be administered.¹⁶² To avoid traumatic lumbar puncture from the repeated procedure and potential CNS seeding, we give intrathecal therapy with the very first diagnostic lumbar puncture, after the diagnosis of leukaemia has been established. Some investigators

recommend an Ommaya reservoir for this treatment in adults with acute lymphoblastic leukaemia.⁷⁶ In one randomised trial, triple intrathecal therapy with methotrexate, cytarabine, and hydrocortisone was more effective than intrathecal methotrexate in preventing CNS relapse, but it was associated with an increased frequency of bone marrow or testicular relapse.¹⁶³ One explanation for this seemingly paradoxical finding is that an isolated CNS relapse is, in fact, an early manifestation of systemic relapse, and the better CNS control secured with triple intrathecal therapy does not obviate overt leukaemic relapse in other sites. If so, more effective systemic chemotherapy is needed before the full benefit of triple intrathecal therapy can be realised. Indeed, systemic treatment has a substantial role in prevention of CNS relapse.^{117,118,122,152}

Remaining questions and the future

What are the major causative factors in the development of acute lymphoblastic leukaemia? Apart from isolated cases that can be attributed to inherited genetic syndromes or exposures to known leukaemogenic agents, identification of causal factors with a predictable effect on substantial numbers of children or adults has not been possible, impeding efforts to develop effective preventive measures against acute lymphoblastic leukaemia. In view of the failure of large-scale epidemiological studies to find such associations, future research in this area will probably restrict its focus to patients with a common primary genetic lesion, such as those with either *BCR-ABL*, *MLL-AF4*, or *TEL-AML1* fusion, or hyperdiploidy.

Assuming that molecular therapeutics will eventually replace standard combination chemotherapy and haemopoietic stem-cell transplantation in the management of patients with acute lymphoblastic leukaemia, which molecules implicated in disease pathogenesis are most likely to yield substantial clinical benefits? Experience to date shows that transient responses can be obtained by inhibition of certain key enzymes, such as tyrosine kinases, DNA methyltransferase, histone deacetylase, γ secretase, serine-threonine kinases, and proteasomes (table).¹⁶⁴ However, rapid development of drug resistance suggests that curative treatment will need alternative strategies. For example, short-lived remissions induced in *BCR-ABL*-positive acute lymphoblastic leukaemia by imatinib mesilate suggest a need to combine this drug with newly developed *ABL*-kinase inhibitors, agents whose mechanism of action differs from that of imatinib, or with specific inhibitors of pathways downstream of, or parallel, to the *BCR-ABL* pathway.^{39,115,116,165} A different situation arises when *NOTCH* signalling is interrupted. That is, proliferative intestinal crypt cells are destined to become post-mitotic goblet cells in the absence of *NOTCH* signals,^{47,166} raising the spectre of on-target toxic effects in human trials of γ -secretase inhibitors and other targeted therapeutics. In

the case of γ -secretase inhibitors, alleviation of adverse effects on gastrointestinal stem cells seems to be possible through an intermittent schedule that is still effective against leukaemic cells. This pitfall, and possible avoidance strategies based on drug scheduling, will loom especially large in leukaemia subtypes in which malignant cells have become addicted to signalling pathways that are also essential for maintenance and renewal of healthy tissues. As daunting as these challenges can seem, the payoff in terms of understanding the pathobiology of acute lymphoblastic leukaemia and devising novel effective treatments with few or no toxic effects could be enormous, making it our charge to bring this promise to fruition.

Are cancer stem cells likely to affect development of future targeted treatments for acute lymphoblastic leukaemia? Current evidence suggests that the stem-cell properties of certain human cancers could cause a resurgence of tumour unless the malignant stem cells are specifically targeted by treatment.¹⁶⁷ Findings show that transformation of committed haemopoietic progenitors by the *MLL-AF9* oncoprotein can impart stem-cell properties, especially a self-renewal-associated genetic programme.¹⁶⁸ Whether important subpopulations of leukaemic cells with stem-cell properties underlie some cases of relapsed acute lymphoblastic leukaemia remains to be determined and, therefore, they must be considered in the design of molecular therapeutics. Finally, increasing evidence suggests that the homing and engraftment properties of leukaemic stem cells differ from those of normal haemopoietic stem cells^{169,170} and that bone-marrow mesenchymal cells can protect leukaemic cells from the cytotoxic effects of chemotherapy.¹⁷¹ Possibly, enhanced understanding of the molecular interactions between leukaemic cells and the bone-marrow microenvironment will lead to treatment strategies that enhance the antileukaemic effects of chemotherapy.

Conflict of interest statement

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