Acute lymphoblastic leukaemia

Hiroto Inaba, Mel Greaves, Charles G Mulighan

Acute lymphoblastic leukaemia occurs in both children and adults but its incidence peaks between 2 and 5 years of age. Caution is multifactorial and exogenous or endogenous exposures, genetic susceptibility, and chance have roles. Survival in paediatric acute lymphoblastic leukaemia has improved to roughly 90% in trials with risk stratification by biological features of leukaemic cells and response to treatment, treatment modification based on patients pharmacodynamics and pharmacogenomics, and improved supportive care. However, innovative approaches are needed to further improve survival while reducing adverse effects. Prognosis remains poor in infants and adults. Genome-wide profiling of germline and leukaemic cell DNA has identified novel submicroscopic structural genetic changes and sequence mutations that contribute to leukaemogenesis, define new disease subtypes, affect responsiveness to treatment, and might provide novel prognostic markers and therapeutic targets for personalised medicine.

Introduction

An estimated 6000 new cases (male:female prevalence of roughly 1:3:1) of acute lymphoblastic leukaemia are diagnosed yearly in the USA. Patients are mainly children; roughly 60% of cases occur in people aged younger than 20 years. Survival in childhood acute lymphoblastic leukaemia is approaching 90% (appendix), but treatment in infants (ie, children younger than 12 months) and adults needs improvement. We review advances in the epidemiology, pathobiology, and clinical management of acute lymphoblastic leukaemia.

Epidemiology

Acute lymphoblastic leukaemia, like cancer in general, probably arises from interactions between exogenous or endogenous exposures, genetic (inherited) susceptibility, and chance (figure 1). These factors account for the roughly 1 in 2000 risk of the disease in childhood (0–15 years). The challenge is to identify the relevant exposures and inherited genetic variants and decipher how and when these factors contribute to the multistep natural history of acute lymphoblastic leukaemia from initiation (usually in utero) through the largely covert evolution to overt disease. The rarity of the illness and the existence of biologically distinct subtypes that might not share common causative mechanisms complicates matters. For example, in infants acute lymphoblastic leukaemia is usually associated with MLL rearrangement, and the remarkably high concordance rate in monozygotic twins (approaching 100% in those with a single or mono-chorionic placenta) suggests that leukaemogenesis is largely complete at birth. By contrast, incidence of non-MLL-rearranged B lymphoblastic leukaemia peaks between 2 and 5 years and has a concordance rate of 10–15%, suggesting that, although initiation in utero usually occurs, other so-called promotional exposures are probably necessary for disease emergence.

Contributing exposures

Exposures and their roles remain contentious. More than 20 candidate exposures that contribute to childhood disease have been identified through epidemiological and case-control studies, but very few of these findings are based on reproducible data or are biologically plausible. Some of these candidate exposures are of public concern, especially ionising and non-ionising (eg, electromagnetic field) radiation. Ionising radiation is an established causal exposure for childhood acute lymphoblastic leukaemia, as evidenced by the effects of the 1945 atomic bombs in Japan and the slightly but significantly increased risks associated with x-ray pelvimetry during pregnancy. Such exposures are no longer relevant, although some scientists argue that background or natural radiation could be important.

Exposures to electromagnetic fields (eg, via power lines) have been particularly controversial, and concern or confusion might be exacerbated by uncritical news reporting. A meta-analysis suggests that high levels of electromagnetic field radiation (ie, magnetic flux densities >0.2 μT) are associated with slightly increased risk, but the reliability of this finding is uncertain. To prove that exposure to electromagnetic fields never causes acute lymphoblastic leukaemia is impossible, but at most such radiation might be implicated in only a few cases. Furthermore, the likelihood that exposure to electromagnetic field radiation is causal in promotional or late-stage leukaemia development is lessened by the absence of any biological mechanism or credible modelling in vitro or in vivo.

Infection was the first suggested causal exposure for childhood acute lymphoblastic leukaemia and remains the strongest candidate. Two specific hypotheses have

Search strategy and selection criteria

We searched Medline and PubMed with the keywords “acute lymphoblastic leukaemia”, “acute lymphocytic leukaemia”, and “acute lymphoid leukaemia” for articles published in English between Jan 1, 2007, and Nov 30, 2012. Additional information was obtained from abstracts presented to the American Society of Hematology and American Society of Clinical Oncology. We focused on publications from the past 5 years, but did not exclude commonly referenced and highly regarded older publications. We also searched the reference lists of articles identified by this search strategy and selected those we judged relevant. Review articles and book chapters are cited to provide readers with more details and more references than this Seminar can provide. Our reference list was modified on the basis of comments from peer reviewers.
been proposed, are often referred to by their eponymous titles, and are supported by epidemiological data (table 1).\textsuperscript{11,17,18} Both postulate that the disease results from an abnormal response to a common infection. The hypotheses differ in detail but are not mutually exclusive as explanations of rare time-space clusters of leukaemia\textsuperscript{10,22} or acute lymphoblastic leukaemia in the general community.\textsuperscript{10} A unique or single transforming virus,\textsuperscript{10} which has been noted in leukaemia in some animal species, has not been detected in acute lymphoblastic leukaemia.\textsuperscript{23} Rather, the illness is probably promoted indirectly by an abnormal or dysregulated immune response to one or more common infections (viral or bacterial) in susceptible individuals. Influenza viruses are plausible candidates.\textsuperscript{26} In such a scenario, susceptible children would have minimum previous exposure to infection during infancy, a persistent in-utero-generated preleukaemic clone,\textsuperscript{10} and a variable degree of genetic susceptibility. Exploration of the possible biological mechanisms of infectious promotion should continue,\textsuperscript{7} because it could lead to prophylactic interventions.

Inherited susceptibility

Very little evidence shows inherited predisposition via highly penetrant mutations in children or adults.\textsuperscript{29} The high concordance in identical twin children has a non-genetic explanation (blood cell chimaerism).\textsuperscript{9} Infants born with constitutive trisomy 21 or Down’s syndrome are, however, at substantially increased risk of acute lymphoblastic leukaemia (roughly 40 fold at age 0–4 years) and acute myeloid leukaemia.\textsuperscript{30} The seeming absence of familial clustering of acute lymphoblastic leukaemia or greatly raised sibling risk (maximum 2 fold) does not, however, argue against inherited susceptibility. Previous attempts to identify inherited genetic susceptibility to the disease have been based on a candidate gene approach. Such studies\textsuperscript{30} identified some noteworthy potential candidates implicated in, for example, folate metabolism and the immune response, but most were statistically underpowered or not consistently reproducible.

Genome-wide association studies of childhood acute lymphoblastic leukaemia compare the whole genome (usually remission-blood-derived DNA) in large series of patients to that in an ethnically matched control group and focus on single nucleotide polymorphisms in DNA sequences (with roughly 80% genome coverage).\textsuperscript{30–33} These studies need hundreds or thousands of patients and controls, and, in view of the thousands of pan-genome markers being compared, a p of less than 10\textsuperscript{−7} is generally needed to deem the result robust. Genome-wide association studies should be validated in a second, independent series of patients and independently confirmed by another research group. So far, common allelic variants in \textit{IKZF1, ARID5B, CEBPE,} and \textit{CDKN2A} have been significantly and consistently associated with childhood acute lymphoblastic leukaemia (appendix).\textsuperscript{30–33} Variants in other genes probably have odds ratios (or impact) less than 1–2, but can be identified only in very large cooperative studies (3000–5000 cases).

Gene variants have additive effects; someone inheriting one copy of a variant will have a roughly 50% increased risk whereas someone who inherits all four variants might also affect response to treatment.\textsuperscript{35} The products of the four genes implicated by genome-wide association studies do not impinge directly on

<table>
<thead>
<tr>
<th>Hypothesis</th>
<th>Key notions</th>
<th>Timing</th>
<th>Agent</th>
<th>Evidence</th>
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<tbody>
<tr>
<td>Kinlen population mixing hypothesis\textsuperscript{25,26}</td>
<td>Unusual demographic mixing of susceptible and infected individuals</td>
<td>Herd immunity\textsuperscript{40} Animal leukaemia precedents\textsuperscript{41}</td>
<td>Possibly perinatal</td>
<td>Possibly a single novel virus</td>
</tr>
<tr>
<td>Greaves delayed infection hypothesis\textsuperscript{29,31}</td>
<td>Delayed exposure to common infections in childhood because of underexposure as infants</td>
<td>Mismatch between evolutionary programming of immune system and modern (hygienic) lifestyle\textsuperscript{13} Two-step prenatal and postnatal natural history\textsuperscript{29}</td>
<td>Late promotional or triggering event\textsuperscript{32}</td>
<td>One or (more probably) several common infections (bacterial or viral)</td>
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Table 1: Infection-based hypotheses for childhood acute lymphoblastic leukaemia
Potential exposure pathways (eg, immune, liver detoxification), but rather are key regulators of blood cell development, proliferation, and differentiation. Acquired or somatic mutations of each of these genes have been detected in acute lymphoblastic leukaemia, suggesting that the inherited gene variants contribute to the intrinsic vulnerability of stem or precursor blood cells to transforming events either in utero at initiation or with subsequent postnatal promotion and clonal evolution, or both (figure 1). Evidence suggests that the risk-conferring gene variants have lowered expression of products, but functional aspects remain to be explored. These data provide valuable insights into the cause of childhood disease, but do not have enough predictive value to merit screening of all children.

Pathobiology

Genetic basis

High-resolution profiling of genetic alterations has transformed understanding of the genetic basis of acute lymphoblastic leukaemia. That most childhood cases harbour gross chromosomal alterations has been known for several decades (figure 2). In B-cell disease, these alterations include high hyperdiploidy with non-random gain of at least five chromosomes (including X, 4, 6, 10, 14, 17, 18, and 21); hypodiploidy with fewer than 44 chromosomes; and recurring translocations including t(12;21)(p13;q22) encoding ETV6–RUNX1, t(1;19)(q23;p13) encoding TCF3–PBX1, t(9;22)(q34;q11) encoding BCR–ABL1, rearrangement of MLL at 11q23 with a wide range of partner genes, and rearrangement of MYC into antigen receptor gene loci. Dysregulation of TALI, TLXI, TLX3, and LYL1, particularly by rearrangement into T-cell antigen receptor loci, often occurs in T lymphoblastic leukaemia. These changes are of key importance in both pathogenesis and clinical management (figure 3).

Many chromosomal rearrangements disrupt genes that regulate normal haemopoiesis and lymphoid development (eg, RUNXI, ETV6), activate oncogenes (eg, ABL1). Several are significantly associated with outcomes, particularly in B-cell disease, and are used in risk stratification. High hyperdiploidy and ETV6–RUNX1 rearrangement are associated with favourable outcome, whereas low hypodiploidy and MLL rearrangement (especially in infants and adults) are associated with poor prognosis in both children and adults.

However, many of these alterations alone do not induce leukaemia in experimental models, and no gross chromosomal alteration is noted in many cases, suggesting that additional submicroscopic genetic alterations contribute to leukaemogenesis. High-resolution microarray profiling of DNA copy number alterations (deletions and gains) and sequencing have led to identification of several novel structural genetic alterations and sequence mutations (some of which are being investigated as prognostic markers or therapeutic targets) that define new subtypes of B-cell disease.

Figure 2: Cytogenetic and molecular genetic abnormalities in childhood acute lymphoblastic leukaemia

Acute lymphoblastic leukaemia with rearrangement of CRLF2 but without the BCR–ABL1–like transcriptional profile rarely presents with other classifying karyotypic alterations, but can be noted with high hyperdiploidy. Dicentric cases might have a range of translocations, including classifying translocations (eg, ETV6–RUNX1).

iAMP21=intrachromosomal amplification of chromosome 21. ETP=early T-cell precursor.

Figure 3: Genetic pathogenesis of B lymphoblastic leukaemia at diagnosis and relapse

| Genetic alterations that confer resistance to treatment (subclinical at diagnosis, or acquired) (eg, IKZF1; CREBBP, TP53) |
| Selective pressure (chemotherapy) |
| Relapse |
| Diagnosis |
| Genetic alterations that confer resistance to treatment (subclinical at diagnosis, or acquired) (eg, IKZF1; CREBBP, TP53) |
| Cooperating events |
| - Cell cycle and tumour suppressors (CDKN2A/CDKN2B [INK4/ARF], TP53, RB1) |
| - Cytokine receptor and kinases (CRLF2, JAK1, JAK2, ABL1, PDGFRB) |
| - Ras signalling (NRAS, KRAS, NF1, PTPN11) |
| - Lymphoid signalling (BTLA, TOX, ZAP70) |
| - Transcription factors, coregulators, coactivators |
| - Mutations in epigenetic regulators (CREBBP) |
| - Other |

Initiation
- Initiating lesion (eg, ETV6–RUNX1, MLL rearrangement) confers self-renewal
- Pre-B cell
- Pre-B cell
- Mature B cell

Lesion generation
- Aberrant RAG activity
- Developmental arrest

Cooperating events
- Cell cycle and tumour suppressors (CDKN2A/CDKN2B [INK4/ARF], TP53, RB1)
- Cytokine receptor and kinases (CRLF2, JAK1, JAK2, ABL1, PDGFRB)
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Figure 3: Genetic pathogenesis of B lymphoblastic leukaemia at diagnosis and relapse

Seminar
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Together drive establishment of the leukaemic clone.

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More than 50 regions of recurring DNA copy number alteration have been identified in acute lymphoblastic leukaemia. Deletions are more common than is amplification and are typically focal and often implicate only one gene. The nature and frequency of these alterations are associated with cell lineage and cytogenetic disease subtype. Notably, MLL-rearranged acute lymphoblastic leukaemia, which is typically aggressive and arises early in life, harbours, on average, roughly one additional genetic alteration per case, whereas diseases associated with ETV6–RUNX1 and BCR–ABL1 translocations manifest later in childhood and typically harbour at least six and eight additional genetic alterations. Many of the implicated genes encode regulators of lymphoid development or cell cycle, tumour suppressors, or lymphoid signalling molecules (figure 3, table 2). The most frequently altered genes are transcriptional regulators of B-cell lymphoid development (eg, PAX5, IKZF1, EBF1), which are affected in more than two-thirds of B-cell disease, and the CDKN2A/CDKN2B loci encoding the INK4/ARF tumour suppressors, which are implicated in more than 80% of T-cell cases. Although understanding of sequence mutations is incomplete, data show that several genes are changed by various mechanisms (including deletion or amplification, sequence mutation, and translocation) that are highly gene-dependent—eg, structural alterations are more common than are sequence mutations in PAX5 and IKZF1 in B-cell disease, whereas WTI, PHF6, and NOTCH1 are frequently targeted by sequence alteration in T-cell disease.

Several genetic alterations have well established roles in leukaemogenesis (eg, activating mutations in NOTCH1). The roles of many other genes remain unknown, partly because of the paucity of murine models that faithfully represent human acute lymphoblastic leukaemia. However, several alterations disrupt the activity of the encoded proteins in vitro or result in dominant negative isoforms, which are implicated in the onset of B lymphoblastic leukaemia in murine models. 48,49

A referenced version of this table is in the appendix. *Also mutated in non-Hodgkin lymphoma.

Table 2: Key genetic alterations in B lymphoblastic leukaemia, by gene

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Pathway and consequences</th>
<th>Clinical relevance</th>
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<tr>
<td>PAX5</td>
<td>Focal deletions, translocations, sequence mutations 31.7%</td>
<td>Transcription factor needed for B-lymphoid development; mutations impair DNA binding and transcriptional activation</td>
</tr>
<tr>
<td>IKZF1</td>
<td>Focal deletions, sequence mutations 15% of paediatric cases and 66% of cases of chronic myeloid leukaemia in lymphoid blast crisis</td>
<td>Transcription factor needed for development of haemopoietic stem cells to lymphoid precursors; deletions and mutations result in loss of function or dominant negative isoforms</td>
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<tr>
<td>Inherited variants</td>
<td>–</td>
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<tr>
<td>JAK1, JAK2</td>
<td>Pseudokinase and kinase domain mutations 18–35% of Down’s-syndrome associated cases, 11% of high-risk BCR-ABL1-negative cases</td>
<td>Mutations cause constitutive JAK–STAT activation; transforms mouse Ba/F3-EpoR haemopoietic cell line</td>
</tr>
<tr>
<td>CRFL2 Focal deletion and sequence mutations 5–16% of paediatric and adult cases and &gt;50% of cases associated with Down’s syndrome</td>
<td>Associated with mutant JAK as in as much as 50% of cases; CRFL2 and JAK mutations cotransform in Ba/F3 cells, causing constitutive STAT activation</td>
<td>Associated with poor outcome</td>
</tr>
<tr>
<td>CREBBP Focal deletion and sequence mutations 19% of relapsed cases of B lymphoblastic leukaemia*</td>
<td>Impaired histone acetylation and transcriptional regulation</td>
<td>Mutations selected for at relapse and associated with glucocorticoid resistance</td>
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As much as 50% of BCR–ABL1-like cases have CRLF2 Rearrangements and JAK mutations. Next-generation sequencing, including transcriptome and whole-genome sequencing, has shown that the remaining cases harbour a diverse range of rearrangements, deletions, and sequence mutations that activate cytokine-receptor and kinase signalling (eg, those implicating ABL1, EPOR, IL7R, JAK2, and PDGFRB); several of these alterations transform in vitro and activate kinase signalling in primary leukaemic cells.58

Intrachromosomal amplification of chromosome 21 occurs in roughly 2% of cases of B lymphoblastic leukaemia (figure 2),19 and was originally defined by fluorescence in-situ hybridisation as a gain of at least three copies of RUNXI.64 Subsequent studies showed that the amplification region is typically large and complex and often accompanied by deletion of the subtelomeric regions of chromosome 21.64 The functional consequences of the amplification are largely unknown, but identification is important because this subtype is associated with poor outcomes with standard-risk regimens.59,62

Genetics of relapse and clonal heterogeneity
Cytogenetic44 and genomic profiling of samples collected at diagnosis and relapse shows substantial changes in the nature of genetic alterations during the course of most cases and that relapse often arises from the emergence of a minor subclone with genetic alterations distinct from those of the predominant clone at diagnosis (figure 3).42,67,68 In most cases, the relapse clone shares lesions with the predominant clone at diagnosis, suggesting a common preleukaemic origin, but other lesions are discordant. Sensitive assays specific for individual alterations show that the relapse clone is often present in low proportions at diagnosis, suggesting that relapse alterations confer resistance to treatment. Furthermore, several of the alterations that most often emerge at relapse are also associated with poor treatment outcomes when present at diagnosis—eg, deletions of IKZF1, CDKN2A/CDKN2B. Less frequently, the relapse clone seems identical or completely dissimilar to that at diagnosis. Similar findings were noted in candidate-gene-sequencing studies of relapsed disease, which identified enrichment of loss-of-function mutations of the transcriptional coactivator and acetyltransferase CREBBP (encoding CREB-binding protein) and TP53 (table 2, figure 3).52,65

Genome sequencing
Next-generation sequencing enables comprehensive identification of the genetic changes in leukaemia. Simultaneous sequencing of hundreds of thousands of nucleic acids (so-called massively parallel sequencing) might be used to identify sequence mutations and structural variants in the encoding portion of the genome (exome sequencing), the transcriptome (mRNA sequencing), or the entire genome. In 187 cases...
of high-risk B lymphoblastic leukaemia, 120 candidate genes and pathways targeted by DNA copy number alterations were sequenced; a high frequency of alterations targeting B lymphoid development (68%), the TP53–RB1 tumour suppressor pathway (54%), Ras signalling (50%), and Janus kinases (11%), and recurring mutations in genes including ETV6, TBL1XR1, CREBBP, MUC4, ASMTL, and ADARB2 were identified.25

Early T-cell precursor acute lymphoblastic leukaemia is an aggressive leukaemia characterised by an immature immunophenotype reminiscent of the murine thymic early T-cell precursor,26 aberrant expression of myeloid and stem cell markers, a distinct gene expression profile, and dismal outcomes.27 Whole-genome sequencing of 12 cases, and mutational-recurrence testing in an additional 94 patients with T lymphoblastic leukaemia (52 of whom had the early T-cell precursor form) showed that three pathways frequently mutated in acute myeloid leukaemia were mutated at high frequency in early T-cell precursor disease—specifically, inactivating mutations targeting haemopoietic and lymphoid development (including GATA3, ETV6, RUNX1, and IKZF1), mutations driving aberrant cytokine receptor and Ras signalling (NRAS, KRAS, FLT3, JAK1, JAK3, and IL7R), and deleterious mutations in chromatin-modifying genes, most notably components of polycomb repressor complex 2 (EZH2, EED, and SUZ12). Polycomb repressor complex 2 normally mediates trimethylation of H3K27, resulting in transcriptional repression; thus, these mutations are predicted to derepress transcription. These results extend findings of PHF6 mutations in T-cell disease, which were obtained by sequencing X chromosome genes and explained the increased incidence in men and boys.71

Whole-genome sequencing of all subtypes is needed to identify all genetic alterations contributing to leukaemogenesis. Additionally, studies of the nature of non-coding genetic mutations and the interaction of genetic, epigenetic, and transcriptional factors will be of great interest.

Diagnosis
Morphological identification of lymphoblasts by microscopy and immunophenotypic assessment of lineage commitment and developmental stage by flow cytometry are essential for diagnosis.7 Chromosomal analysis still has an important role in the initial cyogenetic work-up. Reverse transcriptase PCR, fluorescence in-situ hybridisation or multiplex ligation-dependent probe amplification, and flow cytometry are used to identify leukaemia-specific translocations, submicroscopic chromosomal abnormalities, and cellular DNA content, respectively. When genome-wide analysis becomes time effective and cost effective, it might replace many diagnostic techniques. Tests that have prognostic and therapeutic implications are listed in the appendix.

Risk assignment
Clinical and biological factors
Age (infant or ≥10 years old), presenting leucocyte count (≥50 × 10⁹/L), race (Hispanic or black), male sex, and T-cell immunophenotype are adverse clinical prognostic factors in children, although their effect is diminished by contemporary risk-adapted treatment and improved supportive care.3,4 Infants with MLL rearrangements, especially those younger than 6 months old with more than 300 × 10⁹ leucocytes per L at diagnosis, still have a dismal prognosis.7

Racial differences in prognosis have been linked not only to socioeconomic factors but also to differences in genomic variarions.5,24,25 For example, germline single nucleotide polymorphisms of PDE4B and ARID5B are associated with Native American genetic ancestry,7,26 and somatic CRLF2 rearrangements in acute lymphoblastic leukaemia blasts25 were over-represented in children from a Hispanic background; these alterations contributed to inferior outcomes in Hispanic children. Adverse prognosis conferred by genetic ancestry was mitigated by adding a course of delayed intensification therapy.74

Adolescents and adults have a greater prevalence of biologically high-risk leukaemia (eg, BCR–ABL1, MLL rearrangement), a lower incidence of favourable subtypes (eg, ETV6–RUNXI, hyperdiploidy), and poorer adherence to, and tolerance of, treatment than do pre-adolescent children.6 Older age (especially ≥60 years) and high presenting leucocyte count are poor prognostic factors in adults (appendix). Adolescents and adults seem to have better outcomes with paediatric than with adult regimens.25–28 Typically, paediatric regimens provide higher doses of non-myelosuppressive drugs, early and frequent intrathecal therapy, reinduction and long maintenance phases, and strict oversight of adherence.

Response to treatment
Early response is predictive of the risk of relapse and is used to assign patients to subsequent risk-adapted treatment.59 Methods that track residual leukaemic cells by flow cytometry (for aberrant immunophenotypes) and PCR amplification (for leukaemia-specific immunoglobulin and T-cell receptor genes or fusion transcripts) allow detection at proportions less than those detectable by microscopic morphological assessment—ie, minimal residual disease (MRD). MRD is the most powerful prognostic indicator in childhood and adult disease, even in patients with low-risk features at presentation.56–58 The kinetics of MRD clearance in response to identical remission-induction chemotherapy differs between disease subtypes; negative MRD on day 33 (after administration of four drugs) was the strongest prognostic factor in B-cell disease,56 whereas negative MRD on day 78 (after seven drugs) was also predictive in T-cell disease, irrespective of positive MRD status on day 33.59

PCR is typically more sensitive than is flow cytometry for measurement of MRD (roughly 0·001% vs roughly
increasingly used. However, the optimum doses and schedule of glucocorticoids are associated with infection, osteonecrosis, fracture, psychosis, and myopathy; the incidence of such side-effects is generally higher with dexamethasone than with prednisone. Thus, high-dose dexamethasone (eg, 10 mg/m² per day) is not recommended for adolescents with B lymphoblastic leukaemia during remission-induction therapy.  

Three preparations of asparaginase are available. One is derived from *Escherichia coli* and one from *Erwinia caratovora*; the third is a monoethoxypolyethylene glycol succinimidyl conjugate of *E coli* L-asparaginase (PEG-asparaginase). These formulations have different half-lives (PEG-asparaginase > E-coli-derived > *E caratovora*-derived), and maintenance of asparaginase depletion by optimising dose intensity and schedule is crucial. PEG-asparaginase has largely replaced the native *E coli* product, because it provides at least 2 weeks of therapeutic activity after a single dose and induces antibodies less frequently. Native-*E coli*-asparaginase and PEG-asparaginase activity could be inversely related to titres of anti-*E coli*-asparaginase antibodies, although PEG-asparaginase was inhibited only at high titres. Therefore, PEG-asparaginase might be used when antibody titres are low or intermediate, and *E caratovora* asparaginase when titres are high. Glucocorticoids and asparaginase have a noteworthy pharmacokinetic interaction. High systemic exposure to asparaginase was associated with high exposure to dexamethasone, presumably because of impaired hepatic synthesis of proteins implicated in dexamethasone clearance. Thus, anti-asparaginase antibodies can reduce exposure to both drugs and might increase relapse risk.

Patients with BCR–ABL1-positive disease have poor prognoses but benefit from early treatment with tyrosine-kinase inhibitors (eg, imatinib, dasatinib). When tyrosine-kinase inhibitors are added to multidrug chemotherapy, complete remission rates are more than 90% and event-free survival is superior to that in historical controls. Unlike imatinib, dasatinib targets both ABL1 and Src kinases; it also has more potent activity against BCR–ABL1, is active against imatinib-resistant BCR–ABL1 (except that with T315I mutation), and has better CNS penetration.

**Intensification (consolidation) therapy**

Intensification (consolidation) therapy is given after remission-induction treatment, and eradicates residual leukaemic cells. High-dose (ie, 1–8 g/m²) methotrexate with mercaptopurine is often given, as are frequent pulses of vincristine and glucocorticoids, uninterrupted asparaginase for 20–30 weeks, and reinduction therapy with drugs similar to those used during remission-induction therapy.

The accumulation of the active metabolites of methotrexate—methotrexate polyglutamates (MTXPG₃–₇)—in leukaemic cells is associated with antileukaemic activity, which can be affected by somatic and germline genetic factors, dose and duration of methotrexate, and folic acid rescue. Functional enzyme and somatic genetic studies show that MTXPG₃, accumulation varies widely between subtypes of acute lymphoblastic leukaemia. Accumulation is low in *TCF3–PBX1*, T-cell, and *ETV6–RUNX1* disease (and thus patients might benefit from high doses of
methotrexate), and high in hyperdiploid B-cell disease, especially that with gain of chromosomes 18 or 10.\textsuperscript{107–109} Germline single nucleotide polymorphisms of the organic anion transporter polyprotein SLC01B1 are associated with high methotrexate clearance.\textsuperscript{108,111} In patients with high-risk disease high-dose methotrexate (ie, 5 g/m\textsuperscript{2} every 14 days for four doses) and mercaptopurine were more effective than were escalating-dose methotrexate (ie, initial dose of 100 mg/m\textsuperscript{2}, increasing by 50 mg/m\textsuperscript{2} every 10 days for five doses) plus PEG-asparaginase, and were not associated with increased acute toxic effects.\textsuperscript{112} Duration of effective serum concentrations of methotrexate is important; accumulation of MTXPG\textsubscript{c} was less with high-dose 4-h infusions than with high-dose 24-h infusions.\textsuperscript{113} Folinic acid rescue is necessary after high-dose methotrexate; however, excessive use can counteract antileukaemic effects of methotrexate and increase the risk of relapse.\textsuperscript{114}

Reinduction therapy is a crucial element of protocols for acute lymphoblastic leukaemia. Intensified reinduction therapy with vincristine and asparaginase improved the outcome of patients with high-risk disease.\textsuperscript{115} However, an identical second reinduction cycle did not improve the outcome of patients with high-risk disease and a rapid marrow response to 7 days of induction therapy or those with standard-risk disease, suggesting that residual leukaemic clones after a course of reinduction therapy might represent intrinsic drug resistance.\textsuperscript{115,116} Whether a second reinduction cycle offers benefits to patients with high-risk disease and a slow early response in the context of contemporary therapy is unclear. Osteonecrosis frequently occurs after reinduction therapy, especially in children aged 10 years or older. Alternate-week (10 mg/m\textsuperscript{2} per day on days 0–6 and 14–20) rather than continuous (10 mg/m\textsuperscript{2} per day on days 0–20) administration of dexamethasone significantly reduced osteonecrosis despite delivering a higher cumulative dose.\textsuperscript{117}

**Continuation therapy**

Continuation therapy typically lasts 2 years or longer and comprises mainly daily mercaptopurine and weekly methotrexate with or without pulses of vincristine and dexamethasone. Mercaptopurine and thioguanine are structural analogues of hypoxanthine and guanine, respectively, and inhibit de-novo purine synthesis. Although thioguanine forms the active-metabolite thioguanine nucleotides in fewer steps and is more cytotoxic in vitro to lymphoblasts than is mercaptopurine, randomised studies have not consistently shown improvements in event-free\textsuperscript{118,119} or overall\textsuperscript{120} survival, and protracted doses of 40 mg/m\textsuperscript{2} per day or more were associated with death during remission, veno-occlusive disease, portal hypertension, and thrombocytopenia.\textsuperscript{118–120} Thus, mercaptopurine is preferred for continuation therapy.

TPMT catalyses S-methylation of thiopurines to inactive methylated metabolites. Patients with heterozygous or homozygous TPMT deficiency become moderately to pronouncedly myelosuppressed when given thiopurines.\textsuperscript{121} They can develop secondary malignant disease, especially at high doses (eg, mercaptopurine 75 mg/m\textsuperscript{2} per day).\textsuperscript{122} Adherence of less than 95% to planned mercaptopurine doses is associated with relapse.\textsuperscript{123} Therefore, uninterrupted, pharmacogenetics-based mercaptopurine dosing is important.\textsuperscript{1}

After thioguanine nucleotides are incorporated into DNA, DNA mismatch repair enzymes exert cytotoxic effects. Deficiency of such enzymes (eg, MSH2) renders leukaemic cells thiopurine-resistant.\textsuperscript{124} MSH2 expression was low or undetectable in roughly 11% of children with newly diagnosed acute lymphoblastic leukaemia due to partial or complete somatic deletion of genes that regulate MSH2 degradation (MTOR, HERC1, PRKCZ, and PIK3C2B).\textsuperscript{124} These children had a high incidence of relapse.

**Haemopoietic stem cell transplantation and cellular therapy**

Allogeneic haemopoietic stem cell transplantation is an option for children with very-high-risk or persistent disease.\textsuperscript{125} Contemporary protocols with high-resolution HLA typing, case-based conditioning, and improved supportive care have reduced relapse-related mortality, regimen-related toxic effects, and infection.\textsuperscript{126–127} Furthermore, leukaemia-free survival is not affected by the source of stem cells (matched related, matched unrelated, cord blood, or haploidentical donor).\textsuperscript{127–129}

In view of the development of disease detection and frontline therapies, indications for allogeneic haemopoietic stem cell transplantation should be reassessed continuously. MRD of 1 cell in 10000 or more before transplantation is strongly associated with relapse, and new strategies are needed to reduce the disease burden before and after the procedure.\textsuperscript{128,129} Patients with BCR–ABL1-positive acute lymphoblastic leukaemia who go into remission after multidrug chemotherapy with ABL1 kinase inhibitors and young children (<6 years) with B-cell disease in delayed remission after induction failure can be treated without transplantation.\textsuperscript{130,131,132} Is the procedure beneficial in infants? Its role, if any, is limited to a small high-risk group.\textsuperscript{133} Although haemopoietic stem cell transplantation during first complete remission is a key element of treatment in many adult centres, the greater application of paediatric regimens in adult patients should decrease its use.\textsuperscript{134}

**CNS-directed therapy**

Control of CNS disease is a key component of treatment. Prophylactic cranial irradiation (12–18 Gy) is effective, but its use has been reduced or eliminated to prevent acute neurotoxic effects, neurocognitive deficits, endocrinopathies, secondary malignant disease, and excess late mortality.\textsuperscript{135} In the St Jude Total XV and Dutch Childhood Oncology Group acute lymphoblastic leukaemia-9 protocols, cranial irradiation is replaced...
by triple intrathecal chemotherapy with methotrexate, hydrocortisone, and cytarabine for all newly diagnosed patients. The 5-year cumulative risk of isolated CNS relapse was 2.7% with the St Jude and 2.6% with the Dutch Childhood Oncology Group protocol—ie, within the range achieved by prophylactic cranial irradiation (1.5–4.5%). Patients at high risk of CNS relapse—ie, those with any CNS disease (including leukaemia-cell contamination as a result of traumatic spinal tap) or with T-cell disease—should be given intensified intrathecal therapy during early remission-induction therapy. Cranial irradiation can be reserved only for salvage treatment, because the retrieval rate is high in patients with an isolated CNS relapse who did not receive irradiation with initial treatment.

In a randomised study in standard-risk acute lymphoblastic leukaemia, triple-drug intrathecal treatment reduced the frequency of CNS relapse compared with intrathecal methotrexate only, but was associated with increased risk of bone marrow and testicular relapse, possibly because systemic therapy was less intensive. In the St Jude total XV protocol, not only excellent CNS outcomes but also excellent overall outcomes (5-year event-free survival 85–6%, overall survival 93–5%) were achieved. Because CNS and haematological relapses are competing events, systemic chemotherapy with high-dose methotrexate, intensive asparaginase, and dexamethasone, plus risk-based early intensive intrathecal chemotherapy, have substantial roles in prevention of CNS relapse.

Remaining questions and future directions
Some subsets of acute lymphoblastic leukaemia still have an adverse prognosis. Further intensification of available regimens is unlikely to substantially improve survival but will increase short-term and long-term adverse effects. Reduction of treatment intensity should be sought in patients at low risk. Studies of chronic health complications in long-term adult survivors will help to refine treatments, reducing the toxic effects of treatment. Functional genomics and proteomics will improve understanding of the epidemiology and pathogenesis of individual cases, allowing targeted personalised medicine (appendix). Most epidemiological and pathobiological studies have been developed for paediatric disease—these should be expanded to adult patients.

Pharmacological inhibitors of Janus kinases (eg, ruxolitinib) are being investigated in childhood disease harbouring CRLF2 and JAK alterations. Detailed preclinical studies in BCR–ABL1-like disease showed that the ABL1 and PGDGRB inhibitors imatinib and dasatinib are effective against acute lymphoblastic leukaemia cells with NUP214–ABL1 rearrangements and JAK inhibitors are effective against those with BCR–JAK2 or IL7R mutations; such cases might be candidates for targeted therapy. DNA and histone methyltransferase inhibitors and histone deacetylase inhibitors might reactivate silenced tumour-suppressor genes or increase sensitivity to concomitant chemotherapy. Such drugs could be used in infants with MLL-rearranged disease, in whom aberrant DNA and histone methylation are frequently observed, and in patients with CREBBP mutations that encode histone acetyltransferase CREB-binding protein.

Mutations in early T-cell precursor acute lymphoblastic leukaemia are also noted in acute myeloid leukaemia, suggesting that treatments for acute myeloid leukaemia or drugs that target JAK signalling might be beneficial. Monoclonal antibodies to surface antigens such as CD19, CD20, CD22, and CD52 have been used in unconjugated form (eg, rituximab and epratuzumab), conjugated to immunotoxins or chemotherapeutic drugs (eg, moxetumomab, inotuzumab ozogamicin), or in the form of bispecific antibodies (blinatumomab). The incorporation of rituximab into the hyper-CVAD regimen (fractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone) seems to improve outcome in adults younger than 60 years with CD20-positive, BCR–ABL1-negative B lymphoblastic leukaemia. Blinatumomab, a bispecific, single-chain antibody to CD19 and CD3ε, recruits and activates CD3 effector cytotoxic T cells and is cytotoxic to CD19-expressing target cells bound to the other arm of the antibody; it was active against relapsed and refractory B lymphoblastic leukaemia in an adult phase 2 study.

Emerging evidence shows that newly diagnosed acute lymphoblastic leukaemia comprises several subclones and that chemoresistance is frequently driven by subpopulations harbouring genetic alterations that confer resistance. Thus, efforts should be made to identify patients at high risk of relapse through use of highly sensitive methods to detect these subpopulations at diagnosis. Treatment should target these subpopulations to improve the efficacy of therapeutic regimes while reducing intensity.

Contributors
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Conflicts of interest
HI receives unrelated research support from Bayer and Onyx. MG and CGM declare that they have no conflicts of interest.

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www.thelancet.com Vol 381 June 1, 2013


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