Clinical review

Science, medicine, and the future
Childhood leukaemia

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The risk of any child developing acute leukaemia is about 1 in 2000 with 400-450 new cases a year in the United Kingdom. Cure rates approaching 75% can be achieved with combination chemotherapy, but this figure disguises success rates that vary from 10% to 90% with the different biological subtypes of the disease. In this review I discuss how new insights into the underlying molecular biology of leukaemia have changed our understanding of the disease. Not only is there the prospect of better treatment and the introduction of new biologically based therapies, but, as the causes of disease are being unravelled, the possibility of prevention may not just be wishful thinking.

Methods

This article is based on information and views published from my laboratory plus comprehensive, prospective screening of leading journals for leukaemia and cancer research.

A diverse disease with variable clinical outcome

It has long been recognised that childhood leukaemia is not one homogeneous disease. The major morphological division into acute lymphoblastic leukaemia and acute myeloblastic leukaemia is supplemented by the identification of a range of subsets based on gene expression, antigens that delineate cell type or differentiation status, and chromosomal and molecular abnormalities. These include chromosome translocations— exchanges of large tracks of DNA between chromosomes, resulting (at the point of exchange) in the generation of chimeric or fusion genes[1]—and changes in chromosome number (hyperdiploidy or hypodiploidy). At a more subtle level, there may also be gene deletions or single nucleotide base changes in genes.

This molecular archaeology has uncovered what has long been suspected—that the acute leukaemias are biologically diverse diseases.[2] Moreover, in acute lymphoblastic leukaemia these subgroups segregate with age (fig 1), which may help explain the considerable differences in outcome between infants aged < 1 year, children (2-10 years old), and adults.[3] A similar spectrum of molecular diversity exists for acute myeloblastic leukaemia. Several of these molecular abnormalities have independent prognostic importance in the context of particular treatment regimens (see table).[2]

Summary points

Different chromosomal and gene abnormalities in leukaemia define biological subsets of disease with prognostic importance

Chromosome translocations generate chimeric fusion genes, which provide stable sensitive markers that are unique for each patient's leukaemic clone and can be used to track its origins and response to treatment

The common chromosome translocations in childhood leukaemia seem to initiate disease and often arise prenatally

One or more postnatal genetic alterations are also needed for leukaemia development, and in childhood acute lymphoblastic leukaemia these may be caused by abnormal immune responses to infection

Proteins coded by fusion genes operate principally by blocking cell differentiation in leukaemic cells and provide potential targets for new treatments

What most descriptions of leukaemic cells obscure, however, is the dynamic, evolving nature of the disease, a feature it shares with all other cancers.[4] Leukaemia is a clonal disease (originating in a single cell) and evolves by the accrual of mutations within a clone. This results in progressive genetic diversification followed by a "natural selection" of dominant mutant subclones. Clinical outcome depends on not only the nature of the leukaemic clone but how far it has evolved by the time pathological symptoms are recognised, a correct diagnosis is made, and treatment started. Diagnostic delay increases the probability that the clone will have progressed to the point where additional mutations have been acquired, including those endowing drug resistance, rendering eradication more difficult.

The fetal origins of childhood leukaemia

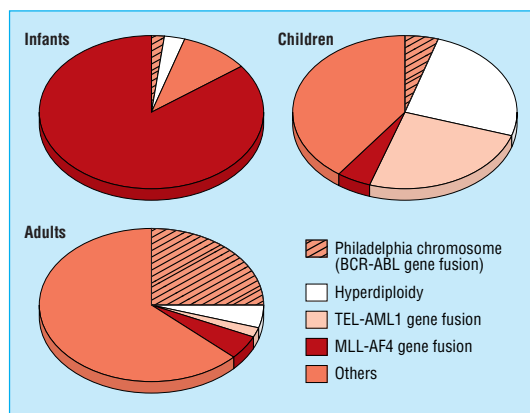There is now compelling evidence that chromosome translocations are often the first or initiating events in

Fig 1 Major molecular subsets of acute lymphoblastic leukaemia in infants (<1 year old), children (2-10 years old), and adults

Prognostic correlates of molecular genetic abnormalities in childhood leukaemia

Molecular marker	Associated prognosis
Infant acute lymphoblastic leukaemia	
MLL-AF4 fusion gene	Very poor
Acute lymphoblastic leukaemia	
Hyperdiploidy*	Very good
TEL-AML1 fusion gene	Good to very good†
Hypodiploidy‡	Very poor
BCR-ABL fusion gene§	Very poor
CDK4I/p16del¶	Very poor
Acute myeloblastic leukaemia	
AML1-ETO fusion gene	Intermediate
MLL-AF10 fusion gene	Intermediate

All fusion genes are generated by chromosome exchanges (translocations).
*Extra copies (such as 3 instead of 2) of certain chromosomes.
†Patients with TEL-AML1 fusion gene seem less likely to relapse if treated with regimens that include high dose L-asparaginase.
‡Loss of some whole chromosomes.
§This alteration (the Philadelphia chromosome) is much more common in adult acute lymphoblastic leukaemia.
¶CDK4I codes for cyclin D kinase inhibitor, which restrains the cell cycle.

leukaemia, occurring prenatally during fetal development. This evidence comes from two sources—identical twin infants or children with concordant acute lymphoblastic leukaemia[5 6] and retrospective scrutiny of neonatal blood spots or Guthrie cards.[7 8]

The most common structural genetic abnormality in childhood leukaemia is a fusion of two genes, TEL and AML1. This is generated by a chromosome translocation between chromosomes 12 and 21. Simultaneous breaks in the TEL gene (chromosome 12) and AML1 gene (chromosome 21) are followed by error-prone repair that stitches up the DNA across chromosomes 12 and 21, joining the normally separate TEL and AML1 genes together to form a chimeric or fusion gene (fig 2). As in other chromosomal translocations, the DNA breaks always occur in non-coding regions (introns) of genes. The precise breakpoints in the TEL and AML1 genes can be identified or mapped by the "long distance" polymerase chain reaction (PCR). Breaks always occur, more or less randomly, within a limited region of these genes, but each patient's leukaemic cells have a unique (or clone specific) breakpoint in the DNA sequence.

Analysis of pairs of identical twins with concordant acute lymphoblastic leukaemia shows that leukaemic cells from both twins in a pair share the identical breakpoints in TEL and AML1 genes or, in the case of

infant twins with acute lymphoblastic leukaemia, the same breakpoints in the MLL gene.[5 6] Monozygotic twins are, of course, themselves monoclonal and genetically identical, but gene breakpoints in leukaemic cells are not inherited—they disappear in remission.

The only plausible explanation for twin leukaemias sharing the same gene breakpoints is that the chromosomal breaks generating the fusion gene must have occurred just once, in one blood stem cell, in one twin in utero. Subsequently, but still in utero, descendent progeny of this transformed cell spread to the other twin, presumably via the anastomoses that exist within shared, single (monochorionic) placentas.[5] We assume that at this early stage a clinically silent or covert preleukaemic clone is generated which, after birth, may evolve to full blown leukaemia anything from two months to 14 years later.[3 6]

Further evidence that childhood leukaemia can originate before birth comes from scrutiny of neonatal blood spots or Guthrie cards (fig 3). PCR tests for specific fusion genes, designed for each patient, can detect as few as 1-20 leukaemic cells in a blood spot. The presence of the same fusion gene sequence in a neonatal blood spot as is in the patient's leukaemic cells at diagnosis[7 8] provides unequivocal evidence that
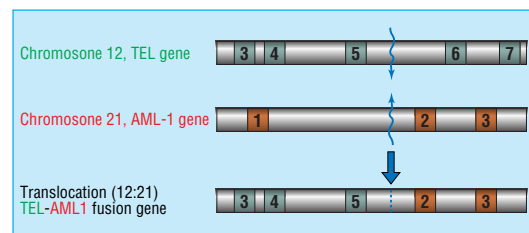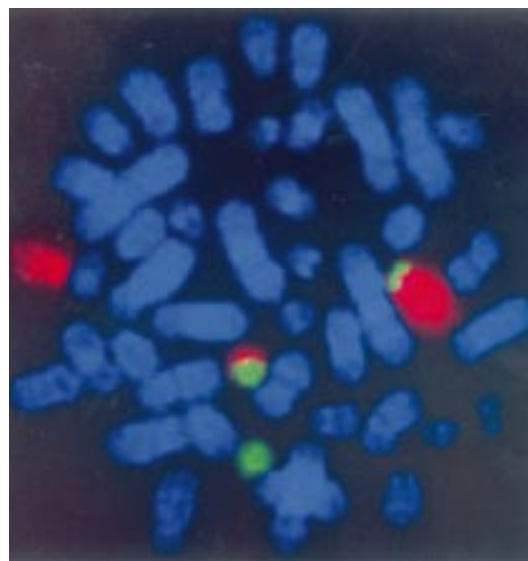

Fig 2 Chromosomal translocation to form the TEL-AML1 fusion gene in childhood acute lymphoblastic leukaemia. Top: Fluorescence in situ hybridisation labelling of dividing leukaemic cell chromosomes with specific probes for chromosome 12 (red) and chromosome 21 (green) reveal two red and green chromosomes (one large, one small). These are copies of chromosomes 12 and 21 between which there has been a reciprocal exchange of DNA. Bottom: The TEL and AML1 genes lie at the breaks and are brought together by the exchange. The genes break in non-coding (grey) regions between the coding regions (numbered, green or red), and re-joining of the two broken genes forms a novel fusion gene
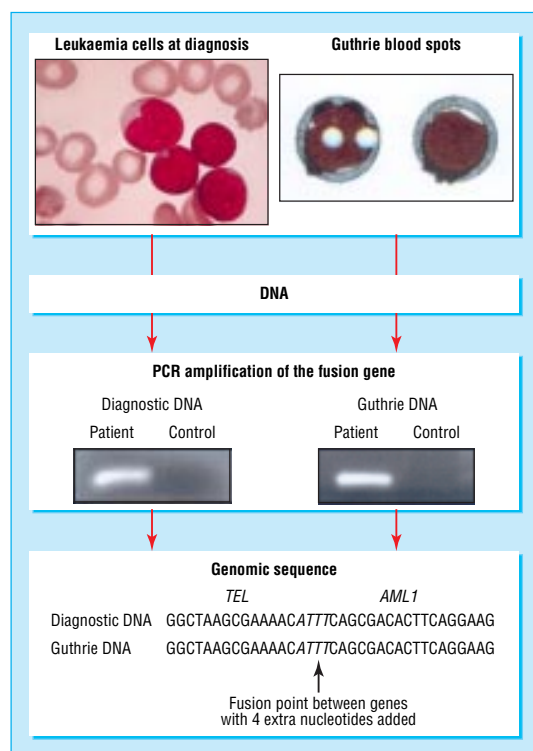
Fig 3 Identification of fusion genes in neonatal blood spots of patients with leukaemia. At diagnosis of childhood acute lymphoblastic leukaemia, a TEL-AML1 fusion gene can be identified in the leukaemic cells. The TEL-AML1 sequence is first determined by long range PCR, then oligonucleotide primers are designed for that unique sequence and for use in short range (conventional) PCR. DNA is extracted from a diagnostic sample for PCR and, in parallel, a segment from a neonatal blood spot is subjected to PCR. If successful, both samples from the patient amplify to produce a nucleotide sequence visualised as a band in a gel. Sequencing of these bands shows them to be identical

leukaemia has been initiated prenatally, probably by formation of the fusion gene itself. The conclusions from Guthrie card studies is that leukaemia is fetal in origin in all cases of infant leukaemia (with fusions of the MLL gene), in most cases of the common form of childhood acute lymphoblastic leukaemia (with TEL-AML1), and in about half of cases of childhood acute myeloblastic leukaemia (with translocation of chromosomes 8 and 21).

The "two hit" model for childhood leukaemia

Although there are no accurate data for concordance rates of leukaemia in infant twins, anecdotally it seems to be exceptionally high, perhaps approaching 100% (that is, if one twin has it, so will the other). If correct, this suggests that MLL gene fusion in utero has a dramatic impact, ensuring subsequent leukaemia. But for children aged 2-6 years (with acute lymphoblastic leukaemia) the concordance rate is considerably lower, around 5%. This still represents a 100-fold extra risk of leukaemia for the twin of a patient with acute lymphoblastic leukaemia but also indicates the need for some additional postnatal event(s) for which there is a 1 in 20 chance, or 95% discordance. This suggests, at a minimum, a "two hit" model for the natural course of childhood leukaemia (fig 4).[3]

If this model of leukaemia development is correct, then, for every child with acute lymphoblastic leukaemia diagnosed, there should be at least 20 healthy children who have had a chromosome translocation, a functional leukaemia fusion gene, and a covert preleukaemic clone generated in utero. This possibility has been investigated by screening unselected samples of newborn cord blood for fusion genes. About 600 samples have been screened, and around 1% have a leukaemic TEL-AML1 fusion gene (H Mori, et al, personal communication). This 1% represents 100 times the cumulative rate or risk of acute lymphoblastic leukaemia (with a TEL-AML1 gene), indicating that the frequency of conversion of the preleukaemic clone to overt disease is low. The real bottleneck in development of acute lymphoblastic leukaemia therefore seems to be a stringent requirement for a second "hit" after birth—that is, exposure and additional chromosomal or molecular abnormality.

Causal mechanisms

A key issue to resolve is what exposures or events might precipitate the chromosome breaks whose improper repair initiates or promotes childhood leukaemia. Given the biological diversity of leukaemia, it is highly unlikely that there is a single cause. Even for a defined biological subtype of the disease, there probably isn't one cause as such but a causal mechanism. As with other cancers, this is likely to involve an interaction of exposure (exogenous or endogenous) with inherent genetic susceptibility, and chance.[4]

Epidemiological evidence suggests that ionising radiation, certain chemicals (such as benzene), viruses (human T cell leukaemia/lymphoma virus type I, Epstein-Barr virus), and bacteria (*Helicobacter pylori*) may play a part in the development of some subtypes of leukaemia and lymphoma in adults and children. Whether any of these exposures have a major role in childhood leukaemia is uncertain, but large scale case-control molecular epidemiological studies in Britain and the United States may provide answers. The UK children's cancer study (UKCCS) seeks to address several hypotheses on different exposures, combined with definition of biological subtypes of disease and genetic studies.[9] It and a parallel US study have already ruled out electromagnetic fields as a major factor in leukaemia aetiology.[10]

A critical role for infection?

Two hypotheses have suggested that an abnormal response to common infections plays a decisive role in the development of childhood acute lymphoblastic
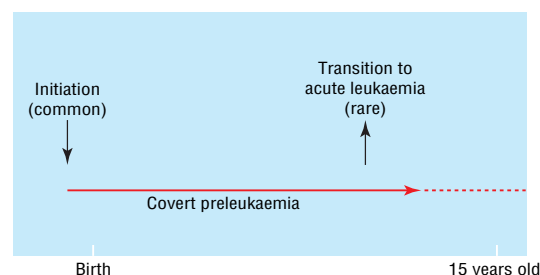

Fig 4 Natural course of childhood leukaemia

leukaemia. One proposes that transiently increased rates of leukaemia (of any subtype), sometimes in clear geographical clusters, are due to population mobility and mixing resulting in infection occurring in previously unexposed or susceptible individuals.[11] The "delayed infection" hypothesis suggests that acute lymphoblastic leukaemia in children is caused by a lack of exposure to infection and a failure of immune system modulation in infancy.[12] Later, an abnormal immune response occurs to one or more common bacterial or viral infections incurred after (delayed) mixing with infectious carriers, such as other children in playgroups or schools.

This second hypothesis is similar to the "hygiene hypothesis" put forward to explain allergies and asthma and type 1 diabetes.[13] It suggests that it is the aberrant response to infection that promotes the crucial second, postnatal event.[12] Epidemiological support for the delayed infection hypothesis come from studies of children with acute lymphoblastic leukaemia. These show that such children are less likely to have had some common infections in infancy, have had fewer social contacts in infancy, are more likely to have been first born, and are less likely to have received certain vaccinations, particularly for *Haemophilus influenzae*.[14 15]

Over the next year or two, it should become clear whether childhood leukaemia involves infectious exposures. If it does, this raises the possibility of prevention, but it cannot apply to all types of childhood leukaemia. For acute lymphoblastic leukaemia in infants (with MLL gene fusions), aetiological hypotheses and the available epidemiological data are distinct. A recent international epidemiological study of infant leukaemia has implicated transplacental chemical exposures to pesticides (Baygon) and a drug (dipyrone) during pregnancy.[16]

Inherited susceptibility

Inherited genetic variation is likely to be important in determining differential susceptibility to leukaemia, as in other cancers and diseases. Risk of infant leukaemia has been associated with polymorphic variants of the NQ01 gene, which codes for an enzyme that detoxifies benzene metabolites and quinone-containing flavonoids and other substances.[17] For typical childhood acute lymphoblastic leukaemia, there is preliminary evidence that HLA class II alleles influence risk,[18] and inherited variations in other immune genes that influence responses to infection probably play a role in susceptibility. Finally, risk of acute lymphoblastic leukaemia in infants, older children, and adults has been linked to inheritance of alleles of MTHFR, a key gene in the folate metabolism pathway.[19] The reason for this association may lie in the way that folate metabolism affects the fidelity of DNA replication and, possibly, vulnerability to chromosomal breaks. Recent data suggest that folate intake in pregnant women may be an important dietary modifier of risk for paediatric acute lymphoblastic leukaemia.[20]

New therapeutic targets and better indicators of prognosis

Genetic alterations in leukaemia (and other cancers) affect the complex signalling networks that control cell

proliferation, cell differentiation, and cell death by apoptosis.[21] These effects help to explain why certain molecular abnormalities result in adverse clinical outcomes. They also present new opportunities for targeting treatments. For example, the BCR-ABL fusion gene (found in the Philadelphia chromosome associated with acute lymphoblastic leukaemia and chronic myeloid leukaemia) results in the production of an active kinase enzyme (from the ABL part of the gene) that drives cell proliferation independently of normal requirements for growth factor and blocks apoptosis and therefore drug responsiveness pathways. Normal p53 protein in cells is required to induce cell death after anoxia or DNA damage from exposure to drugs or irradiation. Mutations or deletions in the p53 gene are rare at presentation of leukaemia but are more common at relapse, helping to explain the therapeutic "resistance" of more advanced disease.

The fusion genes generated by chromosome translocation (TEL-AML1 in acute lymphoblastic leukaemia, AML1-ETO in acute myeloblastic leukaemia, and PML-RARA in acute promyelocytic leukaemia) primarily block cell differentiation. The aberrant proteins produced by these genes inhibit gene activity and differentiation by recruiting repressor molecules.[22] These repressors include histone deacetylase enzymes. These enzymes can, however, be counteracted by selective inhibitors, and one promising line of treatment—at present targeted at acute promyeloblastic leukaemia with PML-RARA fusions—is to use such drugs to reverse the block to normal cell development.[23] The success in using a derivative of retinoic acid to induce remission in acute promyeloblastic leukaemia[24] is also encouraging in this respect.

Another promising approach is the use of STI-571, a selective inhibitor of Abl protein and related kinases, which is has been shown to have a major impact on chronic myeloid leukaemia.[25 26] Hopefully, other small compounds can be designed that will block the signalling pathways that are overactive in paediatric and adult leukaemic cells, preferably in a non-toxic fashion as with STI-571.

Another hope is that the ability to obtain more comprehensive or complete genetic profiles of leukaemic cells may allow prognosis to be defined extremely accurately.[27] Prognosis in paediatric acute lymphoblastic leukaemia can already be assessed with some accuracy using highly sensitive polymerase chain reaction methods to detect molecular markers of leukaemic cells, such as clonal rearrangements of immunoglobulin genes or T cell receptor genes or the

unique leukaemia fusion genes. With such methods, quantitative assessment of levels of residual leukaemic cells during treatment is predictive of later outcome[28] and may therefore provide a good guide to individual patient management.

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A memorable lecture
Lessons of yore

Recently, while working as a senior house officer in a busy emergency department and only weeks away from giving birth to my first child, I came across some of my great grandfather's university lecture notes. He studied medicine at Queen's College in Belfast and later became a general practitioner in the north of England.

The notes I discovered were beautifully handwritten in black ink with neat headings and subheadings, all bound together in a black A5 notebook. In short, the type of lecture notes my peers and I could only have dreamt of. The subject was "Midwifery," and the first session in the lecture series on pregnancy was taken on 17 October 1901. As I browsed through the pages, I mused on what antenatal care I would have received if I had been born a century earlier.

Lecture 15, for instance, informed me of the relevance of food, clothing, air, stimulants, household conditions, and exercise. The influence of diet was noted as "very real." Prochownik of Hamburg was quoted as advising special diets in the following cases:

"In fat women whose previous labours have been difficult owing to defective muscular action, by special dieting easier confinements are procured and the patients are enabled to suckle their children.

"In cases of contracted pelvises (i.e. those with a conjugate of 31/4 to 4 inches) special dieting will so influence the size, weight and osseous development of the fetus that normal labour is obtained."

(Why did we never hear about this Prochownik chap when we were learning about cephalopelvic disproportion in obstetrics and gynaecology?)

My desire to have lived at the turn of the 19th century culminated when I read that "the house should be perfect both in ventilation and sanitation" (I was definitely going to have to have words with my husband) and that regarding sleep "eight to ten hours are necessary. Patients should avoid late hours" (I was also going to have to speak to my consultant about this). When I discovered that "breasts should be free from all compression and should be bathed daily with the following lotion: Boracic acid 3i, Whiskey 3i, Water 3iip," I began to wonder how many women may just have left out the boracic acid and used the potion for more pleasurable purposes.

In a later lecture, however, my great grandfather had made slightly more concerning notes on the aetiology of birthmarks. They can, he wrote, be explained by "maternal impressions … The impression must be a very strong one—fright etc." This was followed by an example: "Lady driving saw child run over—over neck. She was three months pregnant. At labour child had scar across neck." Having already worked in an inner city emergency department for several months, where trauma victims were the largest patient group, I began to wonder whether my unborn child would have any normal anatomy left. I closed the lecture notes and decided it was time to start my maternity leave.

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