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REVIEW

The clinical relevance of chromosomal and genomic abnormalities in B-cell precursor acute lymphoblastic leukaemia

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ABSTRACT

Acute lymphoblastic leukaemia (ALL) occurs at all ages but is the most common cancer of childhood. The current treatment of paediatric ALL is highly successful with up to 90% children being cured. In contrast, survival rates for adult ALL are significantly lower at around 40%. The discovery and characterisation of genetic abnormalities have increased our understanding of the biology of the disease and provided important prognostic and predictive markers which have improved patient outcome. Not only is the spectrum of these aberrations vast but, due to advances in technology, continually expanding. A wide range of chromosomal and genomic abnormalities have been reported as being associated with patient outcome but only a subset are currently used to risk stratify patients. This review highlights the main genetic abnormalities which are used to manage patients with B-cell precursor ALL and discusses the evidence which has been accumulated on several newly described genomic abnormalities.

1. Introduction

The presence of acquired chromosomal and genetic abnormalities in the leukaemic cells of patients with acute lymphoblastic leukaemia (ALL) is one of the principal hallmarks of the disease. Over the past four decades numerous structural and numerical aberrations have been discovered and characterised in ALL. These anomalies are leukaemia-specific and are used to diagnose and classify the disease. The current WHO Classification of B lymphoblastic leukaemia defines seven genetic subtypes: t(9;22)(q34;q11.2)/*BCR-ABL1*, *MLL*/11q23 translocations, t(12;21)(p13;q22)/*ETV6-RUNX1*, t(1;19)(q23;p13.3)/*TCF3-PBX1*, t(5;14)(q31;q32)/*IGH@-IL3*, hyperdiploidy and hypodiploidy.[1] Many of these aberrations are linked to key patient and disease characteristics such as age, white count cell and immunophenotype. Furthermore, several genetic aberrations have been shown to be strongly associated with patient outcome. More recently, the presence of certain genetic alterations has been used to direct therapy. As with all risk factors, the prognostic impact of genetics is treatment dependent. A major challenge facing researchers is the identification of genetic subgroups whose outcome can be improved by modulation of treatment intensity and to distinguish them from subgroups that require the development of novel therapies. In addition to their clinical impact, the characterisation of genetic aberrations, in particular reciprocal chromosomal translocations, has led to the identification of genes and chimeric fusion genes which are key drivers of leukaemogenesis. The spectrum of genetic abnormalities that have been described in ALL is vast and rapidly expanding, and has been comprehensively and expertly reviewed very recently.[2] Therefore the focus of this review will be the principal genetic abnormalities which are currently used to manage the treatment of patients with B-cell precursor ALL; as well as newly described aberrations which are likely to impact on clinical care in the near future.

2. Cytogenetic and molecular genetic techniques

A range of genetic techniques can be employed to detect chromosomal and genetic abnormalities in patients with ALL. Table 1 provides an overview of the principal cytogenetic techniques and contrasts them with the main analogous molecular genetic methods. Although these procedures are principally performed at diagnosis, they can also be used at subsequent time points to confirm remission or relapse. There is a wide diversity of genetic abnormalities in ALL and no single technique is sufficiently informative to detect all types of aberration.

Despite the invention of several new technologies, G-banded cytogenetic analysis remains the foundation genetic test in ALL. The major advantage of cytogenetics is that it represents a whole genome analysis which, in a single step, can identify many of the clinically relevant aberrations in ALL. Its two major limitations are (a) the dependence on metaphases representative of the leukemic clone; (b) inability to detect small or subtle abnormalities. Therefore, G-banded analysis must always be supplemented with additional targeted tests (e.g. FISH, RT-PCR etc.) to detect cytogenetically cryptic abnormalities. There is a wide variety of commercially available FISH probes which

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Table 1
Overview of the principal cytogenetic and molecular genetic techniques used for the diagnosis and classification of acute lymphoblastic leukaemia.

Technique (s)	Scope/target of test	Abnormality resolution	Sensitivity	Detectable types of abnormality
G banded cytogenetics/multiplex FISH	Evaluation of chromosome number and morphology	Low	Low/medium	Translocations (gene fusions), deletions, amplifications, aneuploidy.
Locus specific FISH	Enumeration and localisation of specific DNA target sequences	Medium	Medium/high	Translocations (gene fusions), deletions, amplifications, aneuploidy.
DNA index	Measurement of DNA content	N/A	High	Aneuploidy
Reverse Transcription (RT) Polymerase Chain Reaction (PCR)	Qualitative & quantitative assessment of fusion transcripts. Can be multiplexed	N/A	High	Gene fusions (translocations)
Quantitative PCR	Enumeration of specific DNA target sequences	High	High	Copy number alterations – deletions and amplifications
Multiplex Ligation-Dependent Probe Amplification (MLPA)	Enumeration of multiple specific DNA target sequences	High	Medium	Copy number alterations – deletions and amplifications
Single Nucleotide Polymorphism (SNP) Arrays	Simultaneous evaluation of tens of thousands SNPs across the genome.	Very high (depends on distribution of SNPs)	Medium	Copy number alterations (deletions and amplifications), aneuploidy, and copy number neutral (CNN) loss of heterozygosity (LOH)[a]
Array Comparative Genome Hybridisation (aCGH)	Simultaneous enumeration of tens of thousands DNA probes across the genome.	Very high (depends on distribution of probes)	Medium	Copy number alterations – deletions and amplifications – and aneuploidy

[a] Also referred to as acquired uniparental disomy (aUPD).

will reliably detect virtually all clinically relevant translocations, deletion and amplifications in ALL. One advantage of FISH is that the resulting FISH signal patterns can provide useful secondary information. For instance, probes to *ETV6* (12p13) and *RUNX1* (21q11) can not only detect *ETV6-RUNX1* fusion but can detect (a) an independent but very important abnormality – iAMP21[3]; (b) deletion of the non-rearranged *ETV6* allele – an important secondary abnormality in *ETV6-RUNX1* patients[4]; and (c) gain of chromosomes 12 and 21 – the latter of which is almost uniformly present in high hyperdiploidy (HeH).[4] Thus a single FISH test can provide direct evidence for the presence of two abnormalities and indirect evidence for the presence of a third subgroup. The ability to design probes to a single gene locus and enable detection of all rearrangements involving that gene gives FISH a major advantage over RT-PCR when screening for multiple rearrangements involving a common gene (e.g. *MLL-AFF1, MLL-MLLT1, MLL-MLLT3, MLL-MLLT4, MLL-MLLT10*, etc.).[4] However, RT-PCR has a number of important advantages too: (a) it is highly sensitive and so can detect low level clones [5]; (b) it can be multiplexed,[5] allowing the simultaneous detection of several gene fusions; (c) it can detect rare gene fusions which can arise via cryptic chromosomal rearrangements (e.g. insertions).[6]

DNA indexing is a powerful technique for assessing changes in ploidy level. Such changes are common in ALL with high hyperdiploidy, defined as 51–65 chromosomes, being the most prevalent subgroup.[7] Intriguingly, two rarer ploidy subgroups – near-haploidy (<30 chromosomes) and low hypodiploidy (30–39 chromosomes) – can occasionally masquerade as high hyperdiploidy through the evolution of doubled-up sub-clones (see below). High hyperdiploidy is associated with a very good outcome whereas near-haploidy and low hypodiploidy are both linked with high rates of relapse. DNA indexing is a very sensitive method for distinguishing between true high hyperdiploids and hidden near-haploid/low hypodiploid clones.[8,9]

There are several techniques available for the accurate detection of copy number alterations (Table 1). Q-PCR and MLPA are cheaper and easier to perform and analyse but arrays (SNP and CGH) offer massively greater genome coverage.[10–14] The availability of disease-specific customised MLPA kits which target key genes makes MLPA a highly useful adjunct test to cytogenetics and FISH for the detections of multiple CNAs.[14]

Although most genetic laboratories will have the capability to perform all these tests they will usually devise a screening strategy for groups of patients based on the putative diagnosis, patient age and clinical trial requirements. Typically this strategy will use two or three techniques to detect the clinically relevant abnormalities. In the UK, national "best practice" guidelines are published and regularly updated in order to assist individual laboratories formulate appropriate policies. [15]

3. Primary genetic subtypes of B-cell precursor ALL (BCP-ALL)

Table 2 details the chromosomal abnormalities which describe biologically and clinically distinct subtypes of BCP-ALL. They can be divided into three main groups: (1) chromosomal translocations which result in the creation of novel chimeric fusion genes which in turn express leukaemogenic proteins or the over-expression of oncogenes; (2) established ploidy subgroups characterised by the gain or loss of multiple non-random chromosomes; (3) miscellaneous subgroups. Collectively, the major chromosomal translocations in BCP-ALL account for approximately 40–50% of both paediatric and adult cases. Although all chromosomal translocations have been observed at virtual all ages there is a strong correlation between age and the frequency of each translocation (Fig. 1). Ploidy subgroups are defined according to the number of chromosomes (modal chromosome number) present in the major leukaemia clone but are substantially more prevalent among children (40%) than adults (20%).

4. t(12;21)(p13;q22)/*ETV6-RUNX1*

The chromosomal translocation, t(12;21)(p13;q22), results in the chimeric fusion product *ETV6-RUNX1* (formerly *TEL-AML1*) and is the most prevalent translocation in paediatric ALL (~25% BCP-ALL) but it is rare among adults (Fig. 1).[16–18] Unlike many chromosomal translocations, it is cytogenetically cryptic and was discovered by FISH in the mid-1990s. Initially, *ETV6-RUNX1* patients were thought to have an excellent prognosis and they associated with good risk features such as female gender, young age, low white cell count and CD10+ immunophenotype.[19] However, some trial such as MRC UKALLXI showed no favourable outcome for *ETV6-RUNX1* patients[20] while other studies reported a high incidence of the gene fusion among relapse patients and a tendency towards late relapse.[21,22] Nevertheless, it is now clear that the initial optimism was well founded. Virtually all major clinical trial groups around the world have reported that children with *ETV6-RUNX1* fusion enjoy excellent overall survival with very low rates of relapses (Fig. 2).[23–26] There is some evidence to suggest that *ETV6-RUNX1* patients require high doses of asparaginase to achieve this excellent outcome.[27,28] Given that virtually all