# Molecular Diagnostics for Haematological Cancer

*a report by*
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## Cytogenetics in Leukaemia

Chromosomal changes play an important role in the diagnosis of leukaemia. The first acquired abnormality to be described was the Philadelphia chromosome (Ph) in chronic myeloid leukaemia (CML) by Nowell and Hungerford in 1960.[1] In 1973, it was identified to arise from a translocation between chromosomes 9 and 22, t (9; 22) (q34; q11).[2] Of greater consequence than the diagnostic association is the strong link between certain abnormalities and prognosis. The improved outcome of children with acute lymphoblastic leukaemia (ALL) and a high hyperdiploid karyotype (51–65 chromosomes instead of the normal 46) was first reported in 1978[3] (see *Figure 1*). Today, karyotype remains the gold standard for classification of patients with acute leukaemia into risk groups for treatment. In ALL, the high-risk group includes patients with Ph, near-haploidy (23–29 chromosomes) and rearrangements involving the myeloid/lymphoid or mixed lineage leukaemia (MLL) gene on chromosome 11. In acute myeloid leukaemia (AML), patients with the translocations t (8; 21) (q22; q22) and t (15; 17) (q22; q21) and the inversion, inv (16) (p13; q22) are classified as favourable risk, whilst those with monosomy of chromosome 7, deletion of the long arm of chromosome 5 and/or a complex karyotype are classified as adverse risk.[4] The changes towards risk-adapted therapy implied that accurate detection of these abnormalities was vital and, in routine screening, detection methods in addition to cytogenetic analysis had to be considered.

## Molecular Technologies

Throughout the 1980s, advances in molecular technologies led to the discovery of the fusion genes resulting from these and other significant chromosomal changes in leukaemia. For example, that the translocation, t (9; 22) (q34; q11), gave rise to a fusion of the breakpoint cluster region (BCR) gene on chromosome 22 and the abl gene on chromosome 9 (BCR-abl fusion) became known, as well as the fusion genes of the favourable risk AML translocations and the multiple partners of the MLL gene. The reverse transcriptase polymerase chain reaction (RT-PCR) was developed as a diagnostic screening method, with the facility to screen for as many as 29 rearrangements simultaneously.[5] This approach has become integrated into clinical practice for disease classification and risk stratification. Recently, gene expression profiling, which measures the relative gene expression of thousands of genes simultaneously, has identified characteristic gene expression signatures for each of the major prognostic groups in acute leukaemia.[6-8] At present, this method will not replace cytogenetics in risk stratification, but may lead to refinements in the future, in particular by the identification of novel significant genes as therapeutic targets.

## Gene Mutations

Gene mutations are emerging as important diagnostic and prognostic markers, the testing for which are becoming integrated into diagnostic procedures. In AML, mutations of the fms-like tyrosine kinase 3 (FLT3) gene show a strong association with karyotype and confer a worse prognosis, particularly when in a homozygous form.[9] Recently, the widespread occurrence of JAK2 V617F mutations was discovered in myeloproliferative disorders (MPD). Detection of this acquired mutation has revolutionised the way in which MPD are diagnosed, as well as serving as an obvious target for signal transduction therapy that will revolutionise the treatment of these patients.[10]

## Minimal Residual Disease

Quantitative molecular methods are now routinely used for the detection of minimal residual disease (MRD),[11] which serve as strong predictors of relapse, particularly in childhood ALL. The proportion of remaining leukaemic cells during and following treatment are measured by standardised analysis of either the level of residual fusion transcript[12] or patient specific immunoglobulin/T cell receptor rearrangements[13] relative to the diagnostic sample.

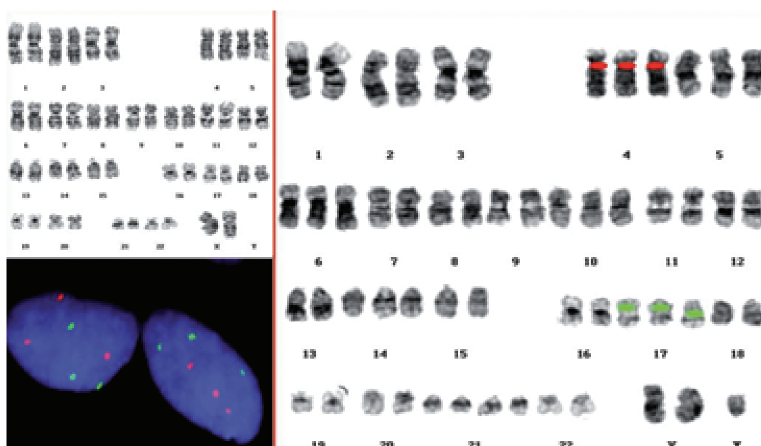
## Fluorescence *In Situ* Hybridisation

In the 1990s there were considerable developments in fluorescence *in situ* hybridisation (FISH) for the detection of significant chromosomal abnormalities in leukaemia.[14] These techniques were evaluated and adopted by a large number of cytogenetics laboratories. Thus, FISH of interphase cells has become established as another important diagnostic molecular method, in particular for the detection of the high-risk chromosomal changes in ALL. The translocation, t (12;21) (p13;q22), giving rise to fusion of the ETV6 gene on chromosome 12 and RUNX1 on chromosome 21 (ETV6-RUNX1 fusion), is found in ~25% of childhood B-lineage ALL.[15] As this translocation is invisible by cytogenetic analysis, FISH also provides an excellent method for detection of this significant fusion gene (see *Figure 2*).

Split-signal FISH, the separation of two normally adjacent signals flanking the gene of interest,[16] or the MLL FusionChip device,[17] have become the methods of choice for the detection of MLL rearrangements. The advantage of these methods over reverse transcriptase-polymerase chain reaction (RT-PCR) is that they ensure detection of translocations involving all partner genes in a single assay. A FISH approach using centromeric probes applied to interphase cells[18] (see *Figure 1*) or flow cytometric measurement of DNA index[19] are the only effective detection methods for high hyperdiploidy and near-haploidy/30–39 chromosomes (abnormalities of established good and poor-risk, respectively) among ALL patients for whom cytogenetic analysis was unsuccessful. As approximately 60% of childhood ALL patients with a failed cytogenetic result harbour a hidden high hyperdiploid clone,[18] routine screening of these cases is advisable to ensure the most appropriate patient management.

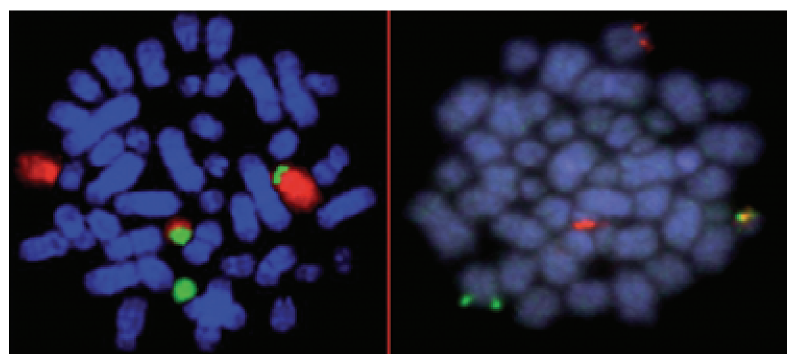
One significant outcome of FISH screening is the additional information provided. FISH is highly effective for the detection of the poor-risk BCR-ABL rearrangements in ALL, as well as revealing the presence of deletions involving the reciprocal translocation product, ABL-BC.[20] Such deletions confer a worse prognosis in CML,[21] which remains to be established in ALL. FISH screening for BCR-ABL also revealed amplification of the ABL gene as a rare cytogenetic subgroup in ALL.[22] It has since been demonstrated to be episomal in origin, involving fusion of the ABL and NUP214 genes. Thus, molecular detection of the NUP214-ABL fusion is now possible by both FISH and RT-PCR. Cells from these patients have been shown to respond to the drug imatinib.[23] These important treatment implications reinforce the need for a routine screening method for reliable detection of this abnormality in T cell ALL (T ALL) patients.

*Figure 1*




*Top Left: karyogram of a normal female karyotype with 46 chromosomes arranged in pairs Right: karyogram from the leukaemic cells of a boy with acute lymphoblastic leukaemia and a high hyperdiploid karyotype. Instead of two copies of each chromosome, as seen in the normal cell, there are three copies of specific chromosomes. The three copies of chromosomes 4 and 17 show the centromeres labelled with probes in red and green, respectively. Bottom left: the three copies of thee chromosomes can be detected in the interphase cells (blue circles) as three red and green signals.*

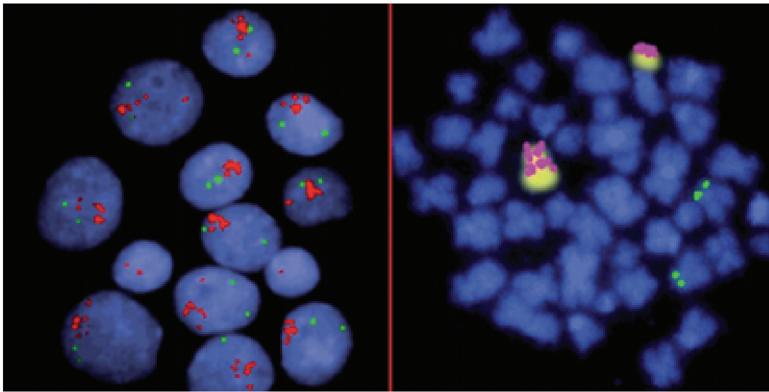
***Figure 2: Two Metaphase Cells (Chromosomes are Stained Blue) from a Patient with the Translocation t (12; 21) (p13; q22) and the ETV6-RUNX1 Fusion***



*Left: chromosome 12 is 'painted' red and chromosome 21 is green. The larger red and green chromosome is the abnormal 12 with chromosome 21 material, whilst the smaller one is the abnormal chromosome 21 with chromosome 12 material, indicating the presence of the translocation.*
*Right: the green signal represents the normal ETV6 gene on the normal chromosome 12. The green signal represents the RUNX1 gene on the normal chromosome 21 and the yellow signal represents fusion of ETV6 and RUNX1 on the abnormal chromosome 21. The small red signal at the top of the image represents the reciprocal RUNX1 signal on the abnormal chromosome 12. An accompanying loss of the normal ETV6 signal is a common secondary genetic event, which would be visualised as a loss of the normal green signal.*

Among ETV6-RUNX1-positive patients, FISH provided simultaneous detection of accompanying chromosomal abnormalities involving RUNX1 and ETV6 (see *Figure 2*), which have been linked to outcome in this patient group.[24,25] Another remarkable discovery was the fortuitous finding of amplification of the RUNX1 gene among ETV6-RUNX1-negative patients, whilst screening for the presence of the fusion (see *Figure 3*). It has been defined as a new recurrent abnormality in approximately 2% of childhood ALL patients,[26,27] which has been renamed as intrachromosomal

*Figure 3: Leukaemic Cells from a Patient with iAMP21*



*Left: interphase cells showing two green signals, which represent the two normal copies of the ETV6 gene, whilst multiple copies of the RUNX1 gene are seen in clusters (red signals). This pattern of signals is characteristic of iAMP21. Right: a metaphase cell showing that the multiple copies of RUNX1 are located on a large abnormal chromosome 21 (yellow). The smaller yellow chromosome represents the normal chromosome 21.*

amplification of chromosome 21 (iAMP21).[28] It is associated with a poor outcome[29,30] and in the UK these patients are now treated with intensive therapy. Thus, accurate identification has become vital; currently, FISH with probes directed to the RUNX1 gene provides the only reliable detection method.

### Array-based Technologies

Array-based comparative genomic hybridisation (aCGH) is a new tool to search for recurrent regions of chromosomal gain or loss throughout the genome from high resolution detection of copy number changes at the DNA level. It has been successfully utilised in both ALL[31] and AML[32] and revealed a large spectrum of genomic imbalances, including novel recurrent changes. A related technique, based on high resolution single nucleotide polymorphisms arrays (SNPA), has also been developed. This approach has successfully demonstrated copy number changes in AML, whilst at the same time defining regions of loss of heterozygosity (LOH). Although LOH usually corresponds to regions of chromosomal deletion, in some cases it has been seen, by SNPA, without a corresponding change in copy number. This situation may arise from acquired uniparental disomy (duplication of one parental chromosome with associated loss of the other). Raghavan et al.[33] have recently shown the importance of these events in giving rise to homozygous mutations of significant genes in AML. Future SNPA screening has the potential to reveal a host of novel genetic mutations, while combined analysis of aCGH/SNPA data against gene expression profiles provides the potential for the detection of novel candidate genes involved in leukaemia. The future development of specific probes and primers directed towards such new abnormalities will allow large-scale screening for the presence of these changes among other patients, to begin to understand their significance in the pathogenesis leukaemia.

### Conclusions

There is no doubt that cytogenetic analysis was instrumental in the realisation of the significance of genetics in leukaemogenesis, as illustrated by the way in which it has become integrated into routine clinical diagnosis for the direction of therapy. Current knowledge of the genetics of leukaemia has emerged from understanding the molecular events within the leukaemic process, facilitated by the development of sophisticated state-of-the-art technologies. The relative merits of these individual methodologies are becoming evident, enabling their integration into routine clinical practice in a complementary fashion. The genetic information potentially available to the clinician at the time of diagnosis is now enormous. Continued collection of data generated by each approach and further streamlining of methodologies will lead to the provision of precise information on which the most appropriate management decisions can be made. The future for molecular diagnosis in haematological cancer is exciting and will be rewarding by ultimately leading to the most appropriate therapy being given to the greatest number of patients. ■

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