Leukaemias and lymphomas are clonal disorders of the haemato-
opietic cell characterized by somatically acquired genetic
alterations. The discovery that molecular abnormalities are involved
in their pathogenesis has greatly improved our understanding of
these diseases. In leukaemias, more than 300 chromosomal
rearrangements have been detected and more than 100 of these
have been cloned and characterized until now. Molecular abnormalities in Haematologic malignancies are diverse
but can be grouped in two categories:
- abnormal rearrangements caused by chromosome
translocations, inversions and duplications which usually result in
oncogene activation; the result of these rearrangements can be
either fusion proteins or deregulated expression of genes;
- mutations and deletions of tumour suppressor genes (e.g. p53, atm).

Additionally, normal rearrangements in lymphocyte antigen
receptor genes can serve in detection of clonality that highly
correlates with (but is not equal to) malignancy. Perhaps the best studied is t(9;22) translocation, giving rise to the
bcr/abl fusion protein. Fusion of BCR to ABL results in constitutive activation of ABL that is necessary and sufficient for induction of
chronic myelogenous leukaemia (CML). At least 95% of CML cases
carry t(9;22) which is not pathognomonic for CML, as it is also
present in 15% of adult and 5% of pediatric ALL and in 2% of
AML. The t(9;22) is a reciprocal translocation in which a large
segment of the Abelson proto-oncogene (abl) at 9q34 is juxtaposed
within the breakpoint cluster region gene (bcr) at 22q11, resulting
in bcr-abl fusion gene (Figure 1).

Transcription from this gene yields chimeric mRNA molecules. The
final product is a protein with elevated tyrosine kinase activity that
seems to exert its effects by interfering with cellular signal
transduction pathways normally involved in the control of cell
death and proliferation and cell-cell adhesion. The BCR-ABL fusion
protein can vary from 190 kDa to 230 kDa, depending on the site of the
breakpoint in the BCR gene. In almost all CML the breakpoints in the BCR gene are found within the M-bcr region with hybrid BCR-
ABL transcript of 8.5 kb containing either BCR exon b2 or b3 and
ABL exon 2. This mRNA encodes the 210 kDa BCR-ABL protein
(p210). The majority of CML patients have transcripts with b3-a2 or
b3-a2 junctions. In 5% of cases, both b3-a2 and b2-a2 transcripts
can be formed as a result of alternative splicing. In very rare cases
of Ph+ CML, the breakpoint in the BCR gene involves the ALL-
associated m-bcr region, which results in production of smaller
p190 fusion protein. A very small proportion of Ph+ CML patients
display a larger BCR-ABL fusion transcript that is caused by
breakpoints in the micro breakpoint cluster region (7-bcr) and
results in a larger fusion protein p230.

Known genetic aberrations have been included in World Health
Organization (WHO) classification of acute leukaemias and Revised
European-American Lymphoma (REAL) classification of
lymphomas. Genetic characterization became an essential part of
the diagnostic work-up in order to define the risk of relapse and
assign patients to distinct treatment options. The WHO classification recognizes in acute myeloid leukaemia (AML) four subgroups with distinct prognostic outcomes (Table 1):
- AML with recurrent genetic abnormalities including t(8;21),
t(15;17), inv (16) or t(16;16) and 11q23 abnormalities;
- AML with multilineage dysplasia;
- therapy related AML and MDS and
- AML classified as FAB subtype M0-M7

Table 1. The most common chromosomal translocations in acute myeloid leukaemia

<table>
<thead>
<tr>
<th>FAB</th>
<th>Abnormality</th>
<th>Fusion gene(s)</th>
<th>Frequency (%)</th>
<th>Detection system</th>
<th>Prognostic outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>M4</td>
<td>q21(q22)q23</td>
<td>aMLL-AF8</td>
<td>3</td>
<td>FISH, RT-PCR</td>
<td>Favourable</td>
</tr>
<tr>
<td>M4</td>
<td>q21(q22)q23</td>
<td>aMLL-ETV6</td>
<td>1</td>
<td>FISH, RT-PCR</td>
<td>Unfavourable</td>
</tr>
<tr>
<td>M4</td>
<td>q21(q22)q23</td>
<td>aMLL-RUNX1</td>
<td>1</td>
<td>RT-PCR</td>
<td></td>
</tr>
<tr>
<td>M3</td>
<td>q21(q22)q23</td>
<td>aMLL-RUNX1</td>
<td>1</td>
<td>RT-PCR</td>
<td></td>
</tr>
<tr>
<td>M0-M7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M0-M7</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

The revised WHO classification of ALL relies upon initial immunophenotypic characterization that defines pre B, preT and Burkitt cell leukaemia. Each entity is then characterized with distinct cytogenetic subgroups (Table 2). The result of translocations in pre B cell forms is the generation of fusion genes (proteins). In pre T-cell ALL most translocations result in deregulated expression of genes juxtaposed to heterologous promoters, usually of a T-cell receptor gene.

Table 2. The most common chromosomal translocations in acute lymphoblastic leukaemia

<table>
<thead>
<tr>
<th>Disease</th>
<th>Abnormality</th>
<th>Fusion gene(s)</th>
<th>Frequency (%)</th>
<th>Detection system</th>
<th>Prognostic outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>pre B</td>
<td>(5;12)(p21;q13)</td>
<td>BCR-ABL</td>
<td>50</td>
<td>FISH, RT-PCR</td>
<td>Unfavourable</td>
</tr>
<tr>
<td>pre T</td>
<td>(9;14)(q34;q11)</td>
<td>ETV6-AK1</td>
<td>10</td>
<td>FISH, RT-PCR</td>
<td>Unfavourable</td>
</tr>
<tr>
<td>Burkitt</td>
<td>(2;22)(q13;q12)</td>
<td>MFC16</td>
<td>1</td>
<td>FISH</td>
<td>Intermediate</td>
</tr>
</tbody>
</table>

The majority of B cell lymphomas and a minority of T cell lymphomas are characterized by recurring chromosome translocations (Table 3). Many involve immunoglobulin or T cell receptor loci with various partner chromosomes and lead to abnormal proto-oncogene expression. A few result in the production of a novel fusion protein. Aneuploidy and deletion of specific chromosome regions are common secondary chromosomal events which are rarely specific to a particular type of lymphoma but provide valuable prognostic information.

Table 3. Chromosomal rearrangements in non-Hodgkin lymphomas

<table>
<thead>
<tr>
<th>Lymphoma type</th>
<th>Translocation</th>
<th>Genes involved</th>
<th>Molecular analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCL</td>
<td>t(11;14)(q13;q32)</td>
<td>BCL1, IgH</td>
<td>PCR</td>
</tr>
<tr>
<td>FL-DCL</td>
<td>t(14;18)(q32;q21)</td>
<td>BCL2, IgH</td>
<td>PCR, FISH</td>
</tr>
<tr>
<td>CLL-SLL</td>
<td>t(11;14)(q13;q32)</td>
<td></td>
<td>PCR, FISH</td>
</tr>
<tr>
<td>DLCL-PLL</td>
<td>t(13;19)(q14;q13)</td>
<td>BCL2, IgH</td>
<td>Southern, FISH</td>
</tr>
<tr>
<td>ALCL</td>
<td>t(8;14)(p13;q32)</td>
<td>NPM, ALK</td>
<td>RT-PCR</td>
</tr>
<tr>
<td>LPL</td>
<td>t(14;16)(q32;q21)</td>
<td></td>
<td>PCR, FISH</td>
</tr>
</tbody>
</table>

MCL - mantle cell lymphoma; FL- follicular lymphoma; DCL-Diffuse large cell lymphoma; CLL-SLL- chronic lymphocytic leukemia/ small cell lymphoma; ALCL-anaplastic large cell lymphoma; LPL-lymphoplasmacytoid lymphoma.

8.1 Methodological approaches for detecting leukaemia/lymphoma-associated abnormal rearrangements

Haematologic malignancies are analyzed and classified on the basis of properties including morphology, cell surface markers, immunohistochemistry, and cytogenetic abnormalities. Additionally, the knowledge about haematopoietic differentiation and genetic abnormalities in haematologic malignancies have aided in establishment and interpretation of gene expression data.

Structural changes affecting chromosomes can be analyzed by using a variety of techniques including comparative genomic hybridization (CGH) and fluorescence in situ hybridization (FISH). Finer resolution of these alterations can be obtained by using the polymerase chain reaction (PCR), DNA sequencing, and genomic and cDNA array analysis.

Table 4 provides a listing of some currently used techniques in molecular diagnostics today.

Table 4. Summary of techniques for molecular analysis of hematopoietic disorders

<table>
<thead>
<tr>
<th>Test for Genome-Wide Screening of Chromosomal Abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spectral karyotyping (multicolor fluorescence in situ hybridization)</td>
</tr>
<tr>
<td>Comparative genomic hybridization</td>
</tr>
<tr>
<td>Test for Targeting Specific Chromosomal Abnormalities</td>
</tr>
<tr>
<td>FISH (fluorescence in situ hybridization)</td>
</tr>
<tr>
<td>PCR (Polymerase chain reaction analysis of DNA)</td>
</tr>
<tr>
<td>RT-PCR (reverse transcriptase PCR analysis of RNA)</td>
</tr>
<tr>
<td>Real-time PCR</td>
</tr>
<tr>
<td>Genotyping for allelic polymorphisms (PCR-SSP)</td>
</tr>
<tr>
<td>Fluorescence in situ hybridization (FISH)</td>
</tr>
<tr>
<td>Tests for Gene Expression Profiling</td>
</tr>
<tr>
<td>Global microarrays</td>
</tr>
<tr>
<td>Focused microarrays</td>
</tr>
<tr>
<td>Microarray of amplified RNA from microdissection</td>
</tr>
<tr>
<td>Molecular Tests for Minimal Residual Disease Detection</td>
</tr>
<tr>
<td>Nested PCR</td>
</tr>
<tr>
<td>Quantitative real-time PCR</td>
</tr>
</tbody>
</table>

Conventional karyotyping still has a paramount role in diagnosis and classification of human leukaemias and lymphomas. Besides chromosome translocations, other clinically important aberrations are detectable using this technique, allowing implementation of patient stratification in prognostic groups - particularly in AML (Table 1). The study of the full karyotype on metaphases provides important information on distinct lesions that characterize particular leukaemia/lymphoma while additional numerical and structural aberrations carry biological and clinical relevance.

FISH is a very useful technique for detection of targeted chromosomal abnormalities around the time of initial diagnosis or at relapse, when there is a relatively high level of abnormal cells. It can be performed on blood, bone marrow, tissue touch preparations, body fluids, and paraffin-embedded fixed tissue. FISH can be done with metaphase or interphase preparations so it overcomes the need for metaphases that is the biggest problem with lymphoma and chronic leukaemia samples. FISH assays are particularly useful in detection of chromosomal translocations that are not amenable to PCR due to widely distributed breakpoints. FISH is not useful for detection of low-level minimal residual disease (MRD) as the sensitivity is only 10-2.

Reverse transcription (RT)-PCR is a well-established technique for identifying very small amounts of specific mRNA transcripts. The
application of this technique has permitted researchers to detect
cancerous cells at levels well below the threshold of the light
microscope.

Table 5. Sensitivity of various detection methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Sensitivity (1 cell in n cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytogenetics</td>
<td>1/25</td>
</tr>
<tr>
<td>Interphase FISH</td>
<td>1/500</td>
</tr>
<tr>
<td>Immunohistochemical</td>
<td>1/10^9 – 10^10</td>
</tr>
<tr>
<td>Nested PCR</td>
<td>1/10^8 – 10^9</td>
</tr>
<tr>
<td>Real time PCR</td>
<td>1/10^9 – 10^10</td>
</tr>
<tr>
<td>Microsatellite PCR</td>
<td>1/10^7 – 10^8</td>
</tr>
</tbody>
</table>

Additionally, the accurate quantification of target sequences is
possible using automated systems. RT-PCR is widely used in routine
genetic diagnosis and in assessment of the response to treatment.
Quantitative real-time PCR measures the number of target DNA
copies in automated manner using a fluorescence analyzer and is
particularly useful for MRD studies.

8.2 Minimal residual disease in
Haematological malignancies

Although many patients with Haematologic malignancies achieve a
complete clinical remission and remission by morphologic and
immunologic criteria, a relatively high proportion of them will
ultimately relapse. A persistent malignant cellular population
present at low level, below the limit of detection of standard
techniques, is the cause of this relapse and is called minimal residual
(disease (MRD)). Several studies have shown that detection and
quantification of residual tumour cells significantly correlate with
clinical outcome. The quantitative measurement of the decrease in
the leukemic cell load during the initial phases of treatment has a
high prognostic value.

Methods to detect MRD include technologies designed to detect
residual malignant cells beyond the sensitivity of conventional
approaches (Table 5). Ideally, techniques used for MRD detection
should have a sensitivity level in the 10^-9 to 10^-10 range. Only a few
commonly used techniques are sensitive enough for detection of
MRD in leukaemias and lymphomas. Currently, PCR based methods
represent the most widely accepted technologies for MRD detection.
Over the past 15 years, quantitative PCR assays were developed.
Competitive RT-PCR employed to monitor patients after
transplantation or treatment with specific agents are time-
consuming and cumbersome. Quantification of residual disease has
been simplified with the introduction of real-time PCR
methodologies and machines. Nested PCR and quantitative real-time
PCR can be used for disease-associated translocations. If there is not
a good translocation target for PCR analysis, patient-specific gene
rearrangements may be targeted.

8.3 DNA microarrays

In the past several years, experiments using DNA microarrays have
contributed to an increasingly refined molecular classification of
Haematologic malignancies. Quantitative information about the
expression of thousand genes can be generated with rapidity and
reproducibility.

In DNA microarray experiments, DNA probes (cDNA fragments,
generated by PCR of cDNA clone inserts) are arrayed on a platform
(glass slide, nylon membrane, silicone wafer). After target cDNA or
cRNA generated from sample RNA and labeled with fluorescent dye
or biotin is hybridized to the microarray, a scanner measures
fluorescence at the site of each unique probe.

Gene microarrays have been used to profile acute leukaemias and
have identified expression signatures characteristic of AML and ALL.
The same approach has been used to profile subsets of both these
leukaemias. It was demonstrated that expression patterns are
strongly linked to karyotypic status for t(8;21), t(15;17), inv(16),
11q23 and normal controls. Profiles for ALL with rearrangement of
the MLL gene clearly distinguished this category from ALLs and
AMLs without MLL. In ALL, expression profiles subdivided ALL into
T-ALL, hyperploid, BCR-ABL, E2A-PBX1, TEL-AML, MLL and one
previously unrecognized subset of ALL (Figure 2).

Diffuse large B-cell lymphoma (DLBCL) is one disease in which
attempts to define subgroups on the basis of morphology have
largely failed. Using DNA microarrays two molecularly distinct
forms of DLBCL were identified which had gene expression patterns
indicative of different stages of B-cell differentiation: one type
expressed genes characteristic of germinal centre B cells (GC-like
DLBCL) while the second type expressed genes normally induced
during in vitro activation of peripheral blood B cells (PB-like
DLBCL). It was found that GC-like DLBCLs have a more favorable
outcome compared with the PB-like DLBCLs, suggesting that
putative cell of origin might be predictive of response to treatment
in this disease.

Gene microarray helps to progress the study of Haematological
malignancy in the area of classification and outcome prediction, which
then enables tailoring and earlier application of treatment for the
benefit of patient.

Figure 2. Expression profiling-based classification of AML and ALL subtypes.
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References


