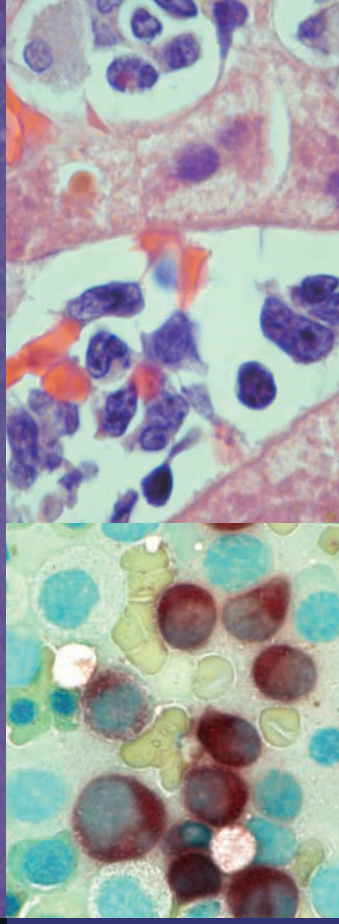
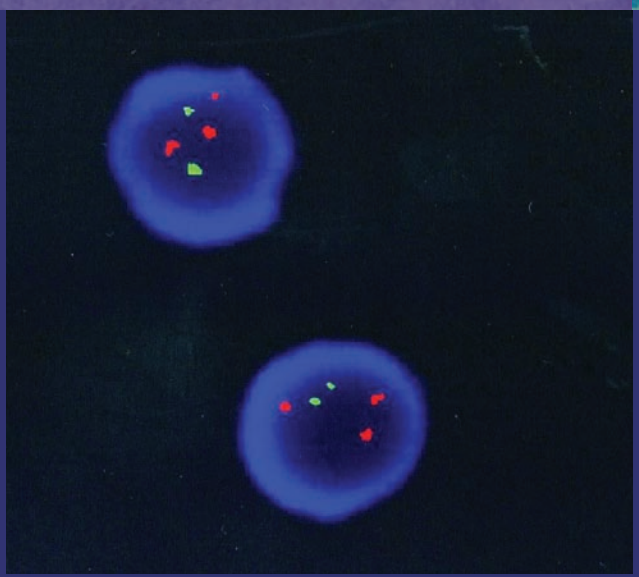
Cytogenetics, FISH and molecular testing in hematologic malignancies





informa
healthcare

Wojciech Gorczyca

Cytogenetics, FISH and molecular testing in hematologic malignancies

Cytogenetics, FISH and molecular testing in hematologic malignancies

Wojciech Gorczyca MD PhD
Genzyme Genetics
New York, NY
USA

informa
healthcare

First published in the United Kingdom in 2008 by Informa Healthcare,
Telephone House, 69-77 Paul Street, London EC2A 4LQ.
Informa Healthcare is a trading division of Informa UK Ltd.
Registered Office: 37/41 Mortimer Street, London W1T 3JH.
Registered in England and Wales number 1072954.

Tel: +44 (0)20 7017 5000
Fax: +44 (0)20 7017 6699
Website: www.informahealthcare.com

A CIP record for this book is available from the British Library.
Library of Congress Cataloging-in-Publication Data

Data available on application

ISBN-10: 0 415 42009 1
ISBN-13: 978 0 415 42009 9

Distributed in North and South America by
Taylor & Francis
6000 Broken Sound Parkway, NW, (Suite 300)
Boca Raton, FL 33487, USA

Within Continental USA
Tel: 1 (800) 272 7737; Fax: 1 (800) 374 3401
Outside Continental USA
Tel: (561) 994 0555; Fax: (561) 361 6018
Email: orders@crcpress.com

Distributed in the rest of the world by
Thomson Publishing Services
Cheriton House
North Way
Andover, Hampshire SP10 5BE, UK
Tel: +44 (0)1264 332424
Email: tps.tandfsalesorder@thomson.com

Composition by Cepha Imaging Pvt. Ltd., Bangalore, India
Printed and bound in India by Replika Press Pvt Ltd

Contents

Preface

Cytogenetics, fluorescence in situ hybridization (FISH) and molecular tests, especially polymerase chain reaction (PCR), play an important role in the management of patients with hematologic malignancies by helping to establish the diagnosis, as well as predict prognosis, response to treatment and disease progression.[1–48] Chromosomal and molecular abnormalities provide the most reliable criteria for classification of hematopoietic tumors and often comprise the basis for targeted therapy. For example, Philadelphia chromosome (Ph), rising from t(9;22),*BCR/ABL* confirms the diagnosis of chronic myeloid leukemia (CML) while the presence of t(15;17)*PML/RARa* confirms the diagnosis of acute promyelocytic leukemia (APL). Others include: t(8;21) or inv(16) in a subset of acute myeloid leukemia (AML), chromosome 14 abnormalities in T-prolymphocytic leukemia (T-PLL), iso(7q) in hepatosplenic T-cell lymphoma, t(11;14)*CCND1/IGH* in mantle cell lymphoma, t(14;18)*IGH/BCL2* in follicular lymphoma, t(2;5)*NPM/ALK* in anaplastic large cell lymphoma (ALCL), t(8;14)*c-MYC/IGH* in Burkitt lymphoma, *JAK2*^{V617F} mutation in classic non-CML chronic myeloproliferative disorders, especially Polycythemia vera, and cryptic deletion on 4q12 associated with the *FIP1L1/PDGFRA* fusion gene in chronic eosinophilic leukemia (CEL) or systemic mastocytosis with eosinophilia. Lymphomas most often display balanced translocations, whereas myelodysplastic syndromes are often marked by deletions or additions of genetic material (e.g. monosomies and trisomies). Nonrandom chromosomal aberrations detected by G-banding, FISH, and/or PCR identify patients at different risk and help to determine the most appropriate management (e.g. aggressive treatment in high-risk groups versus the wait-and-watch approach or less aggressive treatment for low-grade neoplasms). Cytogenetic and molecular analysis helps to identify chromosomal/oncogenic abnormalities that can be targeted by specific therapies exemplifying the ever-expanding personalized approach to management of cancer patients. Imatinib (Gleevec) and novel tyrosine kinase inhibitors (Dasatinib, Nilotinib) successfully target BCR/ABL fusion in CML. Neoplastic cells in CEL or systemic mastocytosis with eosinophilia with *FIP1L1/PDGFRA* also respond to imatinib, albeit with higher sensitivity. All-trans-retinoic acid (ATRA) therapy revolutionized treatment of APL by targeting *PML/RARα* fusion. Myelodysplastic plastic (MDS) patients with del(5q) respond to lenalidomide. A combination of molecular genetic techniques and G-banding allows for early detection of recurrence, identification of low levels of residual disease (minimal residual disease; MRD), and evaluation of engraftment status after allogeneic stem cell transplantation.

This book provides a review of chromosomal and molecular changes in hematologic malignancies and correlates the karyotypic and genetic abnormalities

with morphology, immunophenotype, and clinical data. It is divided into three chapters. The first describes the karyotype and the basics of cytogenetics, FISH, and molecular testing. Chapter 2 lists the most common chromosomal and molecular aberrancies and their utility in diagnosis, prognosis, and monitoring. Chapter 3 covers specific hematologic malignancies and provides a short description of the clinical, pathologic, and cytogenetic/molecular characteristics.

Wojciech Gorczyca

Acknowledgments

I would like to express my great appreciation to Dr Kelly Cornish (Commissioning Editor at Informa Healthcare, London, UK) for her help and support during preparation of this book. I am also grateful to Robert Peden and the whole staff of Informa Healthcare in the UK for years of support, encouragement, expertise and professionalism during work on this book as well as my prior publications. This book would not possible without the help of Michael Lorenzo, Joseph Tripodi, and Charles Barnabe from Genzyme's laboratory in New York, whose contributions I want to acknowledge.

CHAPTER 1

Introduction

Recurring chromosomal abnormalities are involved in the pathogenesis of hematologic malignancies and are important indicators for their diagnosis and prognosis. The methods used to detect the genetic changes in hematologic malignancies include:

(a) conventional cytogenetics (karyotyping on cells derived from direct preparations or short-term cultures using banding analysis; G-banding)
(b) molecular cytogenetics, e.g. fluorescence in situ hybridization (FISH), multicolor FISH, and spectral karyotyping (SKY)
(c) molecular techniques to analyze DNA, RNA, or proteins directly, e.g. the polymerase chain reaction (PCR), reverse transcriptase PCR (RT-PCR), quantitative real-time RT-PCR (RQ-PCR; qRT-PCR), Southern blotting, and microarray analysis.

Conventional cytogenetics using classical karyotyping of chromosomes remains the most comprehensive method for assessing chromosome abnormalities, especially numeric and structural chromosome aberrations. Technical issues associated with cytogenetics (e.g. requirement for fresh sample, difficulties in identification of masked or cryptic aberrations due to limited resolution by classic banding techniques) have resulted in an increased use of molecular cytogenetic techniques, such as FISH, to identify specific abnormalities that are useful in either the diagnosis or management of hematologic disorders. Automation and high sensitivity have led to the recent increase in popularity of PCR technologies in diagnosis and especially in disease monitoring.

Many chromosomal and molecular changes define specific hematologic entities and syndromes, and have important therapeutic and prognostic impact: t(15;17)$^{PML/RAR\alpha}$ is characteristic for acute promyelocytic leukemia (APL), a unique variant of acute myeloid leukemia (AML) treated with ATRA and arsenic dioxide; t(8;21) or inv(16) comprises the favorable risk group of AML, whereas a complex karyotype in AML predicts a poor prognosis; t(2;5)$^{NPM/ALK}$ defines a subset of anaplastic large cell lymphoma associated with a good prognosis and chemosensitivity; t(8;14)$^{MYC/IGH}$ is important in the diagnosis of Burkitt's lymphoma and, when accompanied by *BCL2* or *BCL6* rearrangements, defines the aggressive subset of diffuse large B-cell lymphoma; iso(7) is seen typically in hepatosplenic γδ T-cell lymphoma and inv(14) in T-prolymphocytic leukemia (T-PLL). The t(9;22)$^{BCR/ABL}$ is typical for chronic myeloid leukemia (CML), although it may be seen in a subset of precursor B-lymphoblastic leukemia (B–ALL) or AML, where it is associated with a poor prognosis. Understanding the role of *BCR/ABL* fusion in the pathogenesis of CML led to the development of imatinib (Gleevec), a selective *BCR/ABL* kinase inhibitor that replaced interferon

and/or allogeneic stem cell transplantation as the frontline therapy for CML.[42, 49–54]

A significant subset of hematopoietic malignancies shows lack of chromosomal rearrangements in conventional cytogenetic studies and only molecular tests are able to visualize the underlying genetic defect(s). For example, *FLT3* mutations belong to the most frequent mutations in AML (~23%) with the majority of patients (~70%) displaying a normal karyotype.[55–62] Similarly, *MLL* mutations occur mainly in cytogenetically normal AML.[63–68] Molecular technology, especially real-time quantitative RT-PCR (qRT-PCR), plays an important role in monitoring patients after treatment, best documented in CML patients on imatinib.[41,42]

KARYOTYPE

A karyotype is a set of the chromosomes from one cell. There are 46 chromosomes occurring in 23 pairs (Figure 1.1). Chromosomes are distinct bodies found in the nucleus of cells, best visible in the phase of the cell cycle called metaphase. Chromosomes are composed of protein and DNA and hold the genetic information in the form of linear sequences of four bases (A,T,C,G). The DNA sequence for a single trait is called a gene. Each chromosome contains a few thousand genes, which range in size from a few thousand bases up to 2 million bases. The first 22 pairs are labeled longest to shortest. The last pair are called the sex chromosomes, which are labeled X or Y. Females have two X chromosomes (XX), and males have an X and a Y chromosome (XY). Each chromosome has a short or p (petit) arm and long or q (next letter in the alphabet) arm, which are separated by a region known as the centromere. The centromere is a condensed part of the chromosome which binds together two sister chromatids and constitutes the attachment site for spindle fibers during cell division.

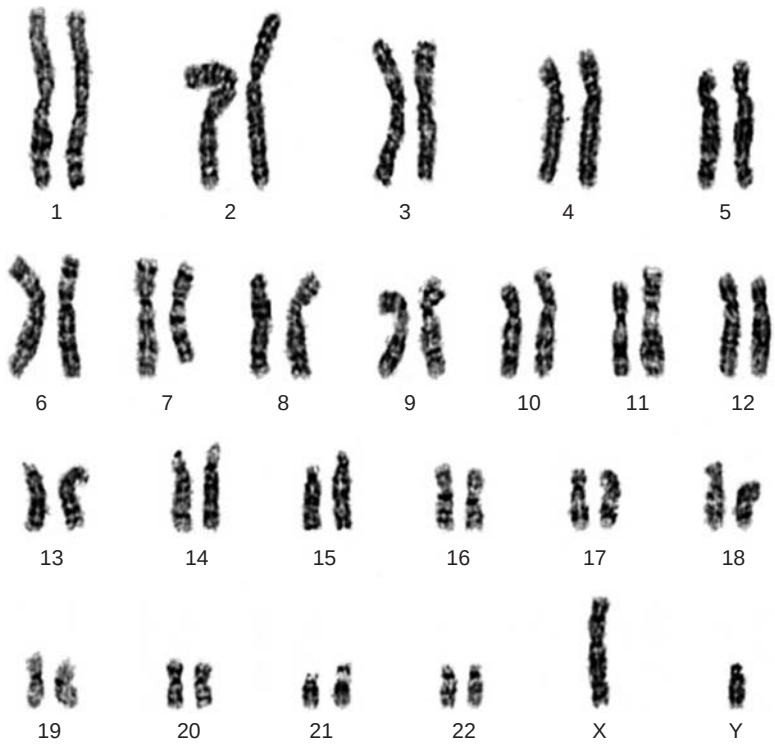


Figure 1.1 Normal karyotype (male)

Group	Chromosomes	Characteristics	Example
A	1-3	Large metacentric chromosomes	
B	4-5	Large submetacentric chromosomes	
C	6-12 and X	Medium-sized submetacentric chromosomes	
G	13-15	Large acrocentric chromosomes	
E	16-18	Medium-sized acrocentric chromosomes	
F	19-20	Short metacentric chromosomes	
G	21-22 and Y	Short acrocentric chromosomes	

Figure 1.2 Types of chromosomes

Types of chromosomes are presented in Figure 1.2. Each chromosome arm is defined further by numbering the bands (light and dark bands are visible under the microscope after staining with various dyes); the higher the number, the further that area is from the centromere. The band width and the order of bands are specific for each chromosome and allow their identification (Figures 1.3–1.25).

NOMENCLATURE

Chromosomal aberrations include numeric and structural abnormalities (Figure 1.26). A cell with 46 chromosomes is called diploid, and a cell with an abnormal number of chromosomes is called aneuploid (<46, hypodiploid; >46, hyperdiploid). An insertion (ins) is a structural rearrangement in which part of a chromosome is inserted to a new location on a chromosome. Monosomy refers to a single chromosome and trisomy to three chromosomes. Loss of a chromosome is designated by a minus sign (–; monosomy) and loss of part of a chromosome by del. Deletions can be either terminal or interstitial. The chromosomes that are most commonly lost include –5, –7, –X, and –Y, and those most commonly duplicated (+; trisomy) include +4, +6, +8, +9, +10, +11, +12, +13, +14, +19, +20, and +21. Common chromosomal deletions include del(5q), del(6q), del(7q), del(13q), and del(20q). Isochromosome (i) is an abnormal chromosome with two chromosome arms positioned as mirror images of each other (duplication of one of the arms, resulting in a metacentric chromosome with identical genes on both arms). The most common isochromosomes include i(1q), i(7q), i(9q), i(11q), i(17q), i(21q), and i(22q). A chromosomal inversion (inv) is a 180° rotation of a chromosome segment (part of a chromosome is reversed in orientation). The common inversions include inv(3) and inv(16). A chromosomal translocation (t) is a relocation of material from one chromosome to a different chromosome. Translocations can be either reciprocal (mutual exchange of segments of chromosomes) or Robertsonian (centric fusion of the long arms of acrocentric chromosomes and loss of their short arms). Most of the translocations are reciprocal and result in either synthesis of novel fusion protein or relocation of an oncogene to a locus that is highly transcribed. The common translocations

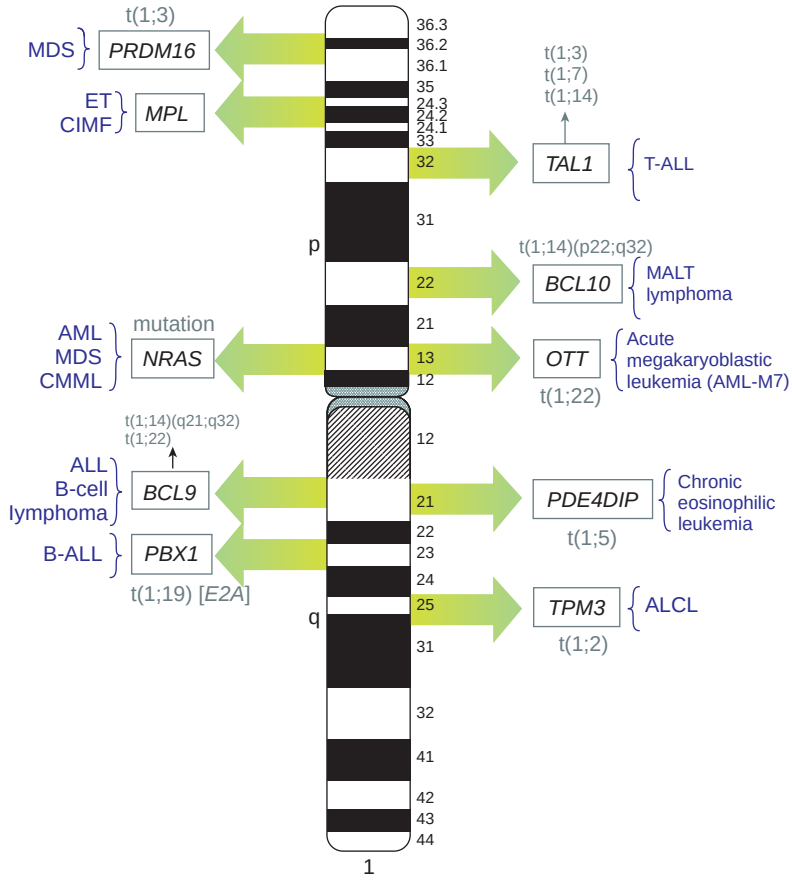


Figure 1.3 Chromosome 1

include t(9;22), t(15;17), t(14;18), t(11;14), t(8;14), and t(8;21). A Robertsonian translocation product is considered a derivative chromosome and is described by der. Marker chromosomes (mar) are abnormal chromosomal structures which cannot be subclassified by cytogenetics. The presence of a marker chromosome is depicted as a +mar. Two cells with identical structural aberrations are defined as a clone. Most common terms and abbreviations used in cytogenetics/FISH studies are presented in Table 1.1.

CONVENTIONAL CYTOGENETICS

Cytogenetics is the study of chromosomes. Somatic cells in humans have 46 chromosomes (diploid number) and gametic cells have 23 chromosomes (haploid number). Women have 22 pairs of autosomal chromosomes and two X chromosomes, while men have 22 pairs of autosomal chromosomes and two sex chromosomes, X and Y (Figures 1.3–1.25). Based on the location of the centromere and chromosome size, they can be divided into metacentric, submetacentric, and acrocentric and subclassified into seven groups (A to G) (Figure 1.2). The structures of all chromosomes and the location of most common genes involved in hematopoietic malignancies are presented in Figures 1.3 through 1.25.

Cytogenetic aberrations, both structural (translocations and inversions) and numeric (deletion or gain of whole or portion of chromosome) occur in the majority of hematopoietic tumors.[5,8,10,13,15,22,28,69–109]

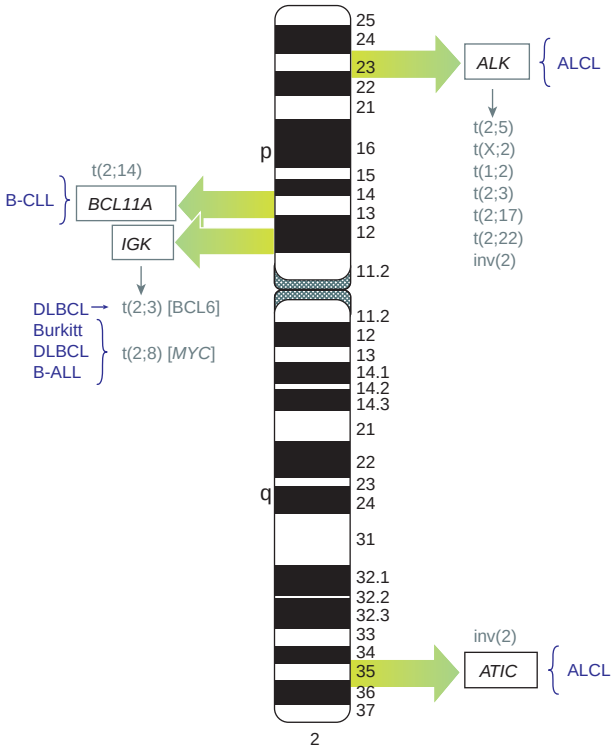


Figure 1.4 Chromosome 2

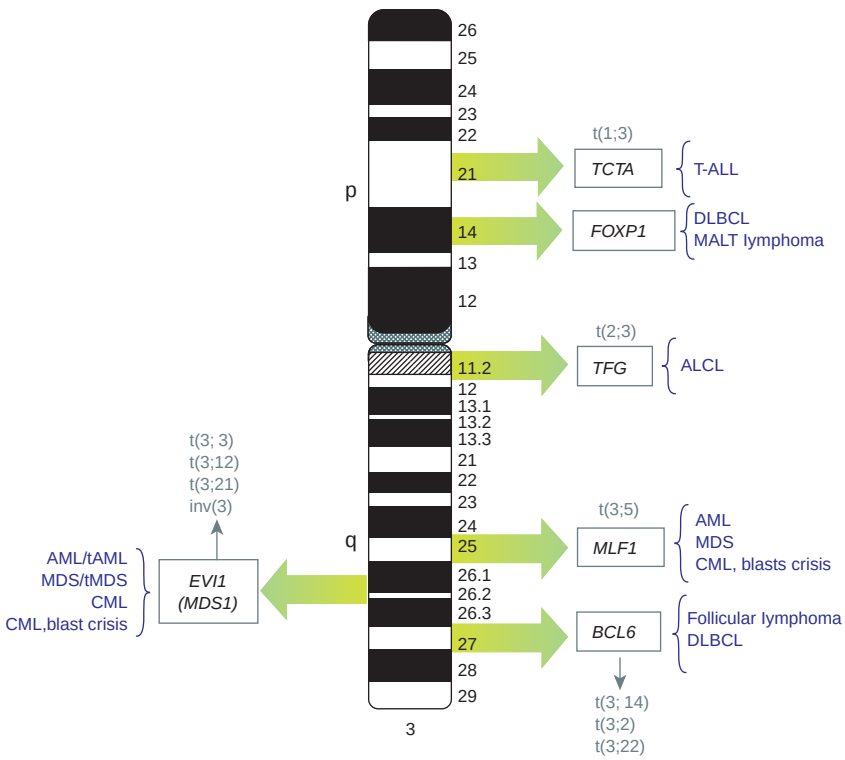


Figure 1.5 Chromosome 3

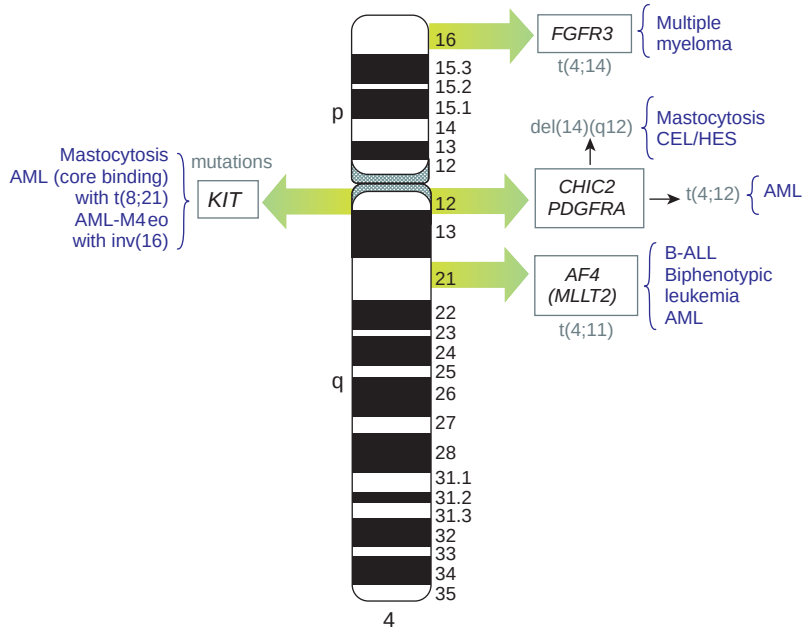


Figure 1.6 Chromosome 4

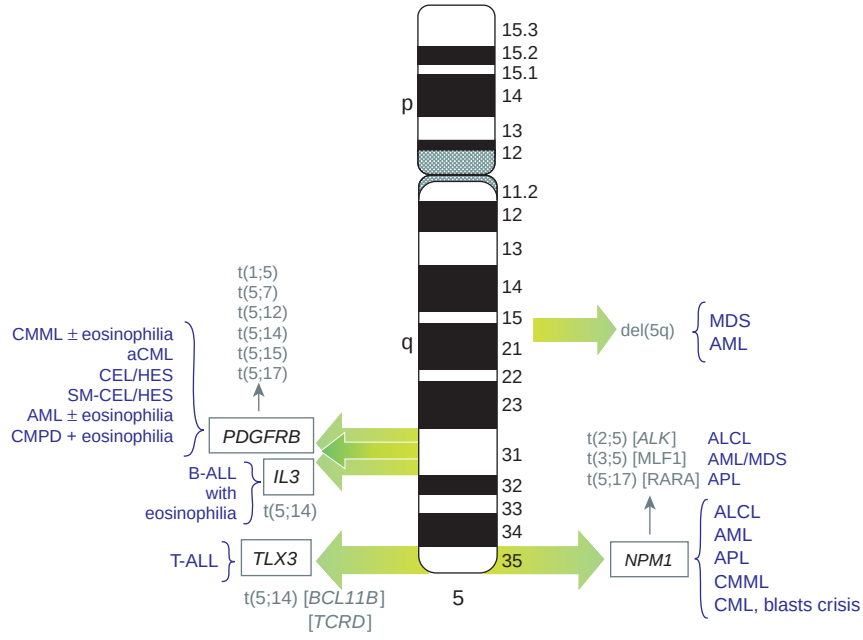


Figure 1.7 Chromosome 5

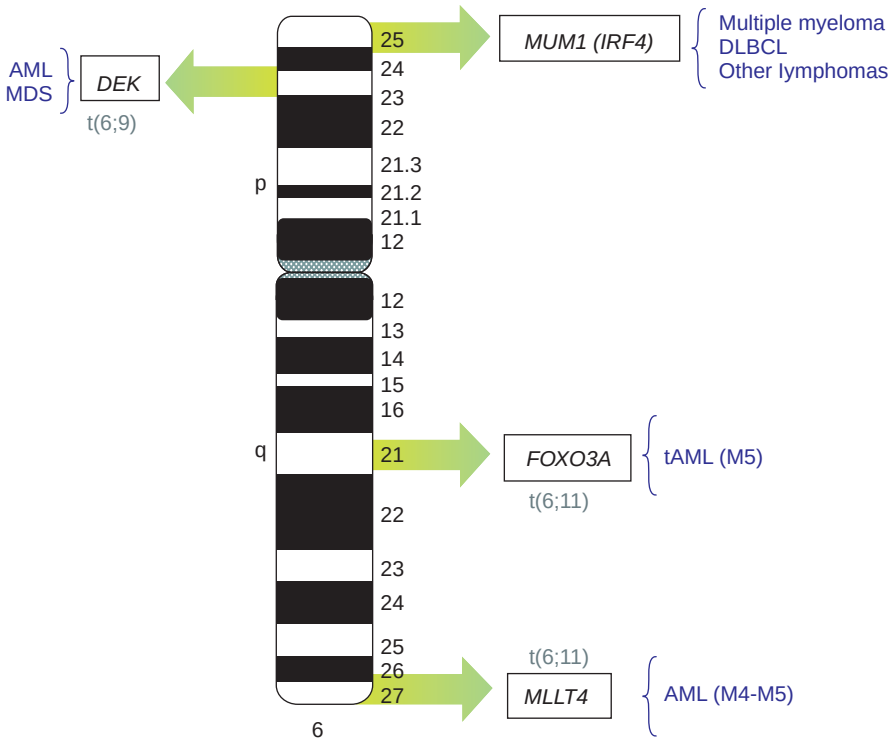


Figure 1.8 Chromosome 6

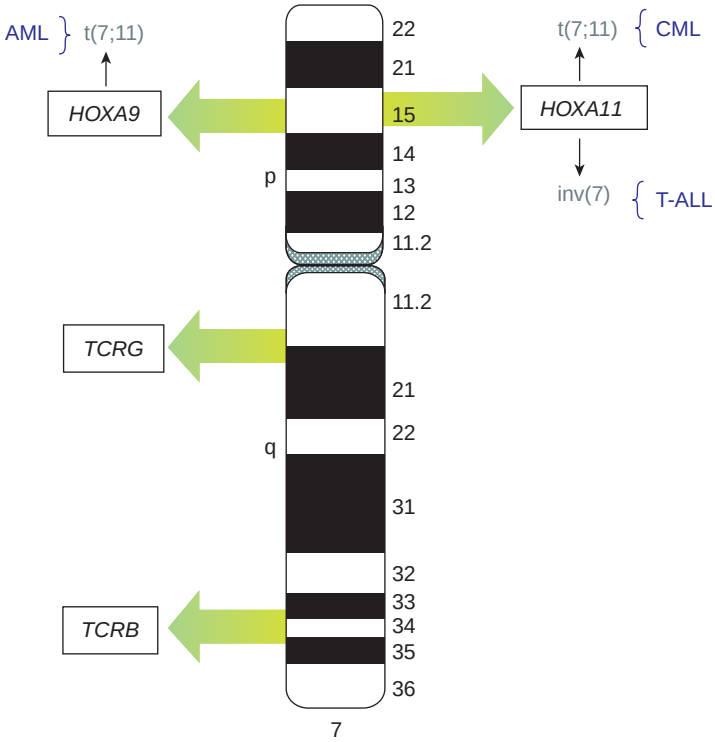


Figure 1.9 Chromosome 7

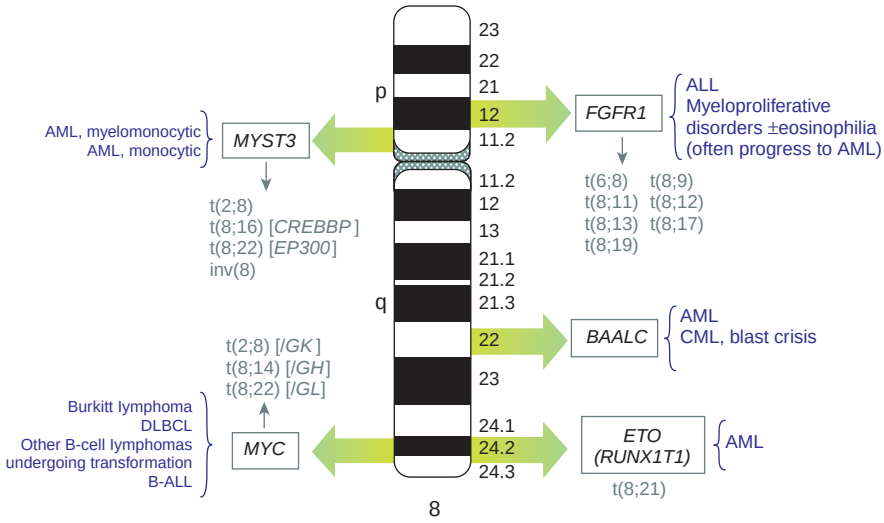


Figure 1.10 Chromosome 8

Chromosomal translocations result in altered gene function, either due to deregulated gene expression or abnormal activity of a novel fusion protein, resulting from the juxtaposition of coding sequences from two different genes, which under normal circumstances are separated. Chromosomal deletions result in the loss of genetic material crucial in maintaining proper functions of the cells, such as proliferation, cell cycle control, and programed cell death (apoptosis).

Methodology

Conventional cytogenetic studies are performed on fresh (unfixed) specimens containing viable cells (Figure 1.27). The specimen is either prepared immediately (direct preparation) or, more often, cultured for 1 to 3 days, with or without mitogenic stimulants (in the cases of acute leukemias, cells are usually cultured without mitogens). For hematologic malignancies, suitable specimens include: bone marrow aspirate (preferable source), fresh tissue (lymph node, extranodal tumorous infiltrate), effusion (pleural, abdominal, and pericardial), cerebrospinal fluid (CSF), and blood. Specimens should be collected under sterile conditions. Sodium heparin (green top tube) is the anticoagulant of choice, since the results with other agents (e.g. EDTA) are less optimal. Blood and bone marrow are transported at room temperature or refrigerated, whereas samples from fresh tissue are best transported on ice, to minimize autolysis and bacterial overgrowth, which may lead to culture failure. Cells from acute leukemias or high-grade lymphomas tend to proliferate spontaneously in culture and do not require mitogenic stimulus, whereas other cell types, e.g. plasma cells, CLL cells, or low-grade lymphomas, often require addition of mitogens. Amphopterin, thymidine, methotrexate, or 5-fluorodeoxyuridine may be used to synchronize the dividing cells in order to obtain a high mitotic index. Synchronization is often combined with mitotic inhibitors such as colchicine, which blocks proliferation by interfering with mitotic spindle formation. Use of hypotonic solutions (e.g. 0.075 M KCl) before fixation improves chromosome morphology. A methanol and acetic acid mixture (e.g. Carnoy's solution) is commonly used as a fixative. The harvested cells can be stained with ethidium bromide, Giemsa, or Wright's methods, but the Giemsa banding method or its variant, a

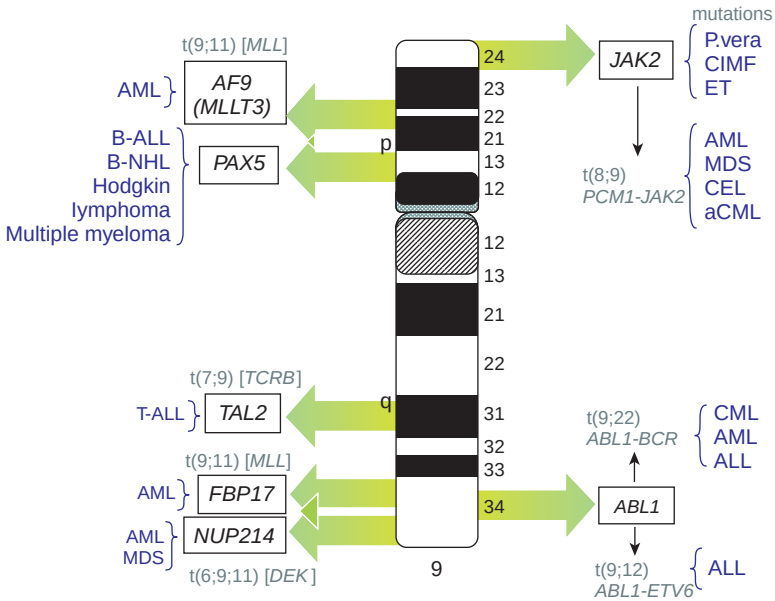


Figure 1.11 Chromosome 9

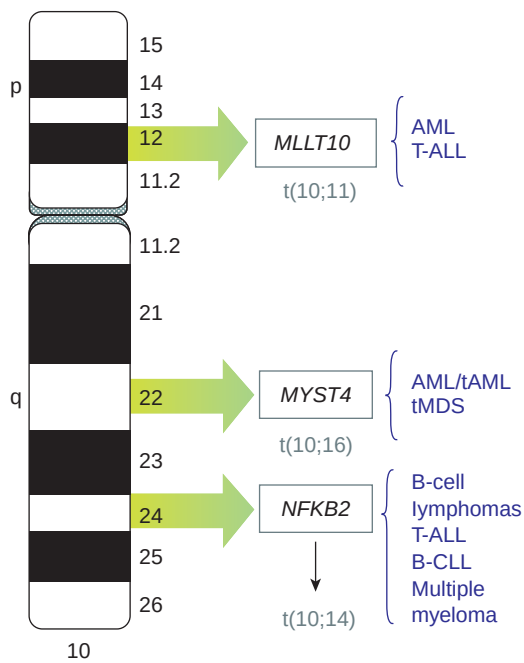


Figure 1.12 Chromosome 10

high-resolution banding, is most often applied (G-banding). Chromosomal abnormalities are described according to the International System for Human Cytogenetic Nomenclature (Table 1.1). The detection of any abnormality in at least two cells is required to confirm clonality.

Table 1.2 presents the advantages and disadvantages of cytogenetic analysis.

FLUORESCENCE IN SITU HYBRIDIZATION

FISH uses test probes against target DNA in the nucleus of interphase cells or metaphase chromosomes.[2,3,27,110–120] FISH allows the analysis of specific DNA changes in tissues (cells) or intact chromosomes and does not require metaphase chromosomes. Many of the clinical applications of FISH include chromosome enumeration using α-satellite probes (e.g. gain or loss of a chromosome), marker identification, gene mapping, deletion, amplification, or translocation as well as whole chromosome 'painting'. In hematologic malignancies, the most common chromosomal abnormalities

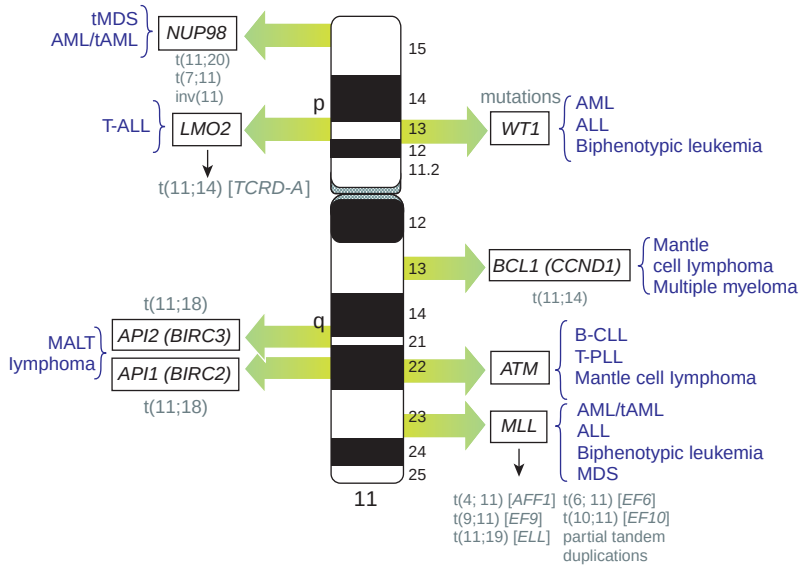


Figure 1.13 Chromosome 11

targeted by FISH include: t(9;22)[BCR/ABL] in CML (and subsets of AML and ALL), t(15;17)[PML/RARα] in acute promyelocytic leukemia (APL), t(14;18)[IGH/BCL2] in follicular lymphoma (FL), t(11;14)[CCND1/IGH] in mantle cell lymphoma (MLC), del(13q), del(11q), and del(17p)[TP53] in B-CLL, del(5q) in MDS, del(13q)[RB1], t(11;14)[CCND1/IGH], and del(17p)[TP53] in multiple myeloma (MM), t(8;14)[MYC/IGH] in Burkitt lymphoma (BL), t(11;18)[API2/MALT1] and t(14;18)[IGH/MALT1] in marginal zone lymphoma (MALT lymphoma), t(8;21)[ETO/RUNX1] in AML, and t(12;21)[ETV6/RUNX1] in ALL.

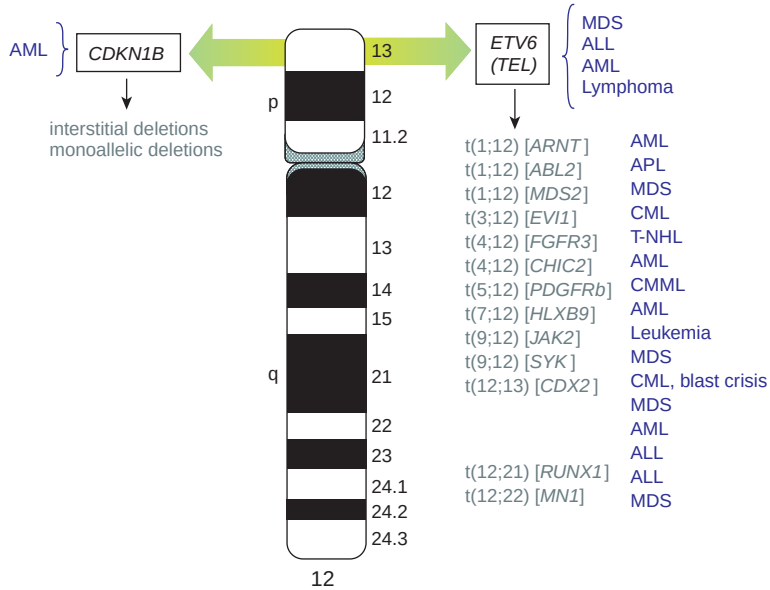


Figure 1.14 Chromosome 12

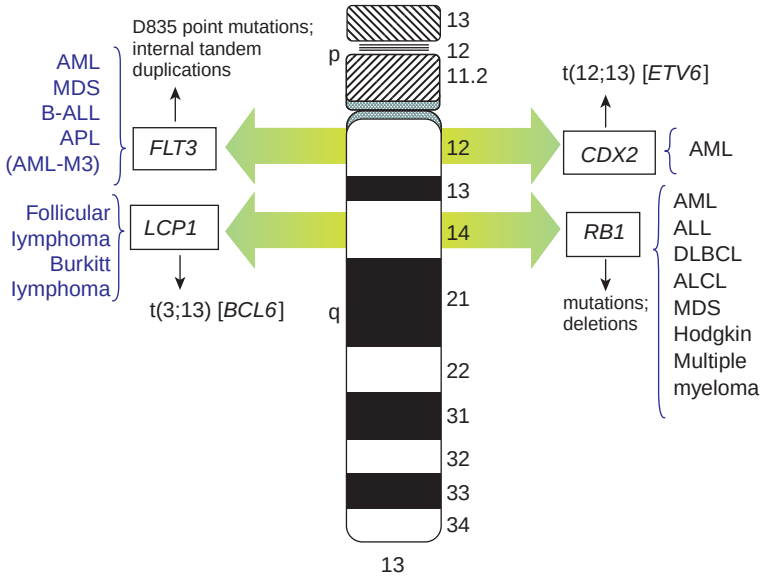


Figure 1.15 Chromosome 13

Methodology

The test can be performed indirectly or directly. The FISH protocol (Figure 1.28) includes the following steps: denaturation, in which the probe and target DNA are denatured by incubation at high temperature; hybridization, in which the probe is hybridized to the chromosomal target; washing to remove the unbound probe; and analysis under the fluorescence microscope. In contrast to Southern blot hybridization, in the FISH protocol DNA is analyzed in the cell or on the chromosome and is not being extracted and run in a gel. In the indirect FISH method, biotin- or digoxigenin-labeled nucleotides are visualized in the second step by fluorescein isothiocyanate (FITC)-conjugated or CY3-conjugated avidin or rhodamine-conjugated antidigoxigenin antibodies, whereas in the direct method the probe(s) are already labeled with fluorochrome(s). With the application of several filters, two or three probes can now be detected

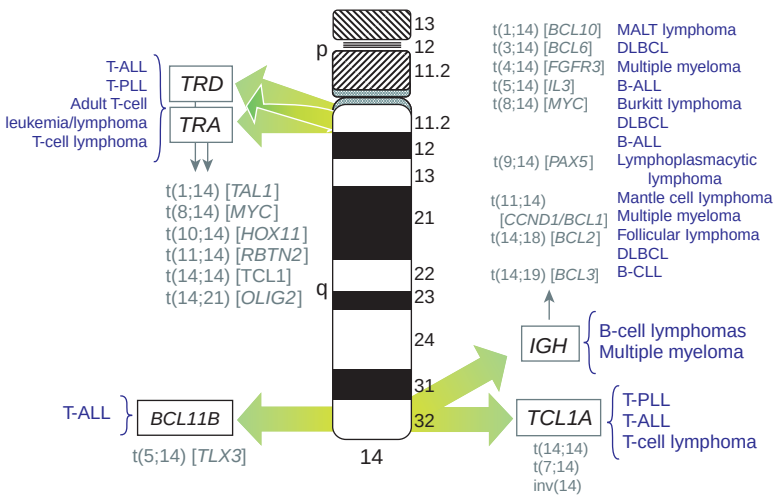


Figure 1.16 Chromosome 14

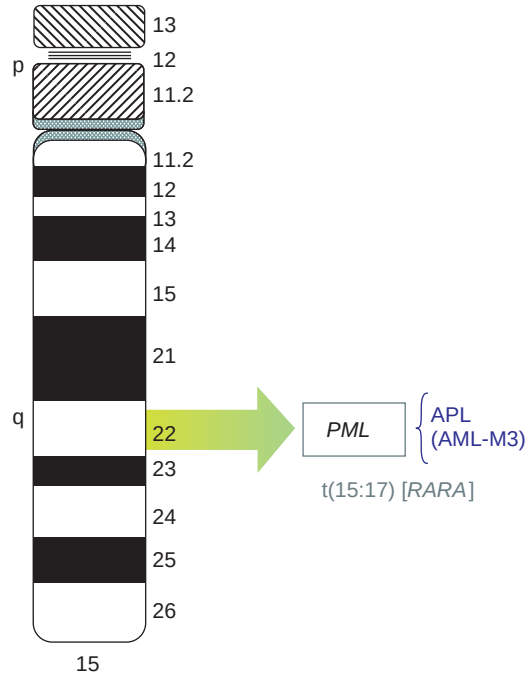


Figure 1.17 Chromosome 15

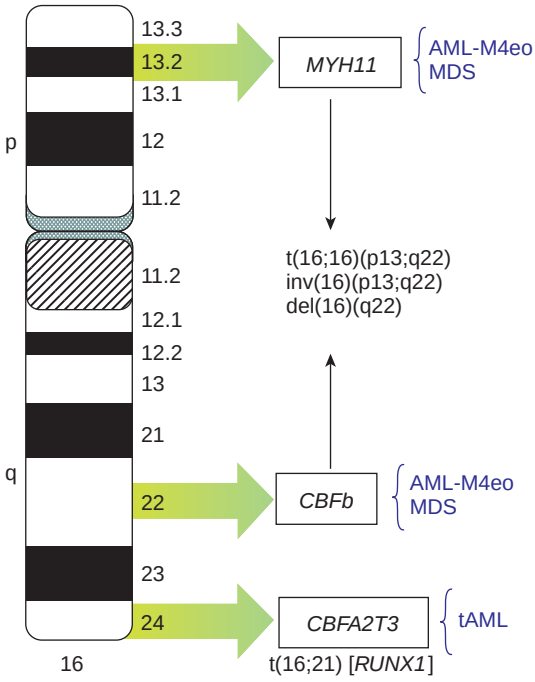


Figure 1.18 Chromosome 16

simultaneously (Figure 1.29). In the detection of translocation, two probes are labeled with different fluorochromes: a normal cell will display four signals (two of each color), while a cell with translocation will show two adjacent signals leading to a different colored fluorescence signal. In the case of a break-apart probe, the target DNA is labeled with two different probes directed at two opposite areas of the gene (3′ and 5′). The fusion of the signal seen as a different color (e.g. yellow) would indicate the normal allele, whereas the two different signals (e.g. red and green) would indicate the presence of translocation. The counterstain using either propidium iodide (PI) or DAPI can be used to identify chromosomes.

FISH can be used in both fresh specimens and in routinely fixed, paraffin-embedded material. Fresh cell suspensions fixed with methanol and glacial acetic acid are commonly used in FISH methodology. Formalin-fixed tissue sections from paraffin blocks offer a big advantage in terms of tissue availability (for new testing as well as retrospective analysis) since this is the most common method of processing surgical specimens in pathology laboratories. However, FISH is difficult to accomplish using thin sections of paraffin-embedded tissue because of the high cellularity and truncated cells that interfere with accurate scoring of the individual nuclei. The isolation of individual nuclei from thick tissue cores of paraffin-embedded material described by Remstein et al,[121] and later modified by Paternoster et al,[122] helped to circumvent some of the problems associated with thin sections. The paraffin sections are treated with xylene (deparaffinization) and proteinase K (protein digestion), and centrifuged to isolate nuclei which then are resuspended onto slides, ready for FISH probes. The major advantage of FISH performed on nuclei isolated from thick sections is the lack of truncation artifacts (nuclei are intact), and the major disadvantage is the lack of correlation with histomorphology. FISH studies performed on whole thin sections allow correlation of the FISH results with tissue architecture (the sections are counterstained with DAPI to visualize nuclei,

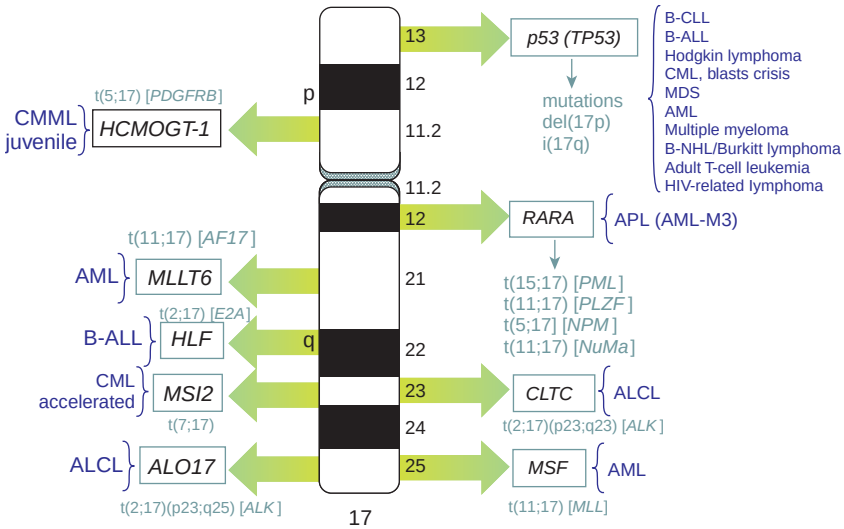


Figure 1.19 Chromosome 17

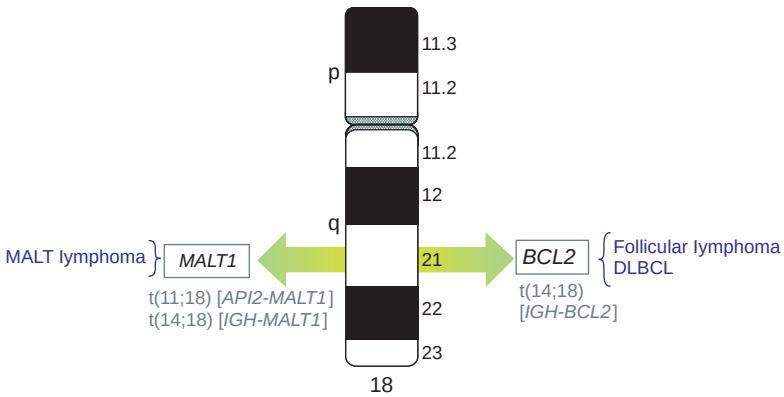


Figure 1.20 Chromosome 18

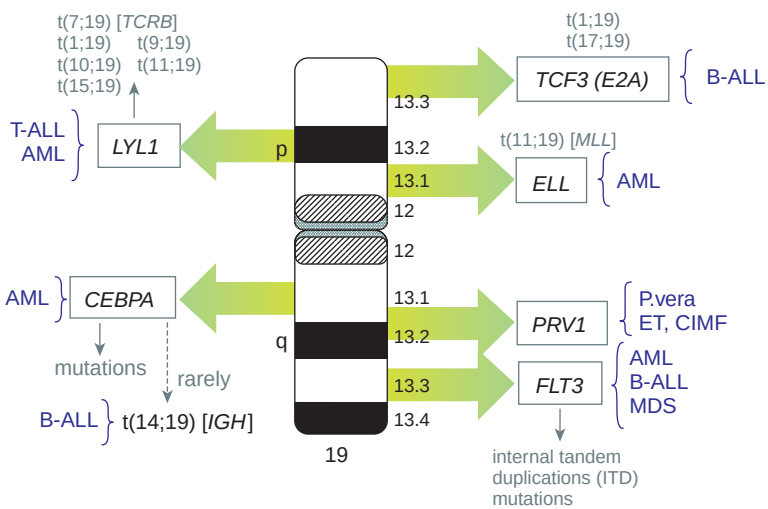


Figure 1.21 Chromosome 19

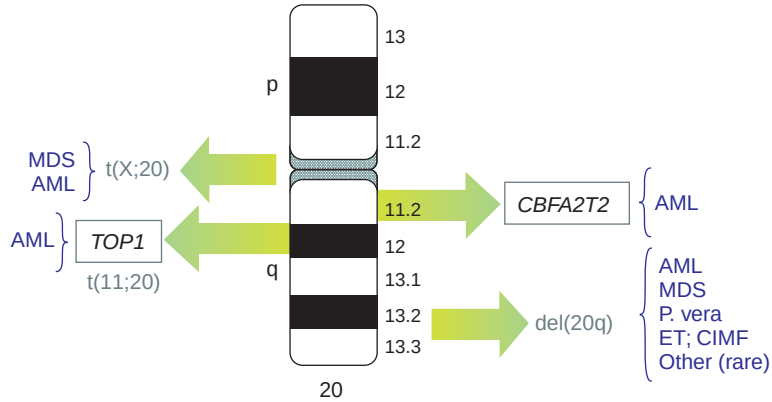


Figure 1.22 Chromosome 20

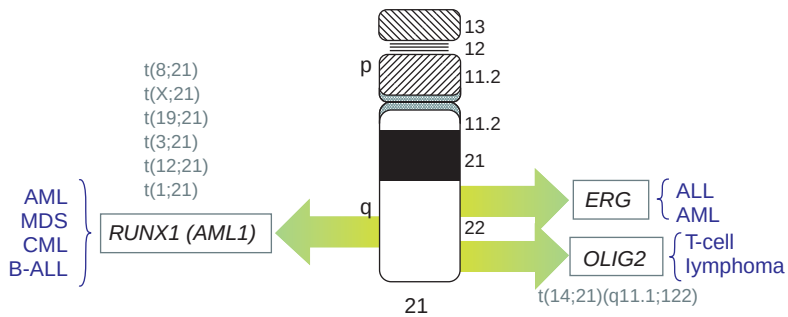


Figure 1.23 Chromosome 21

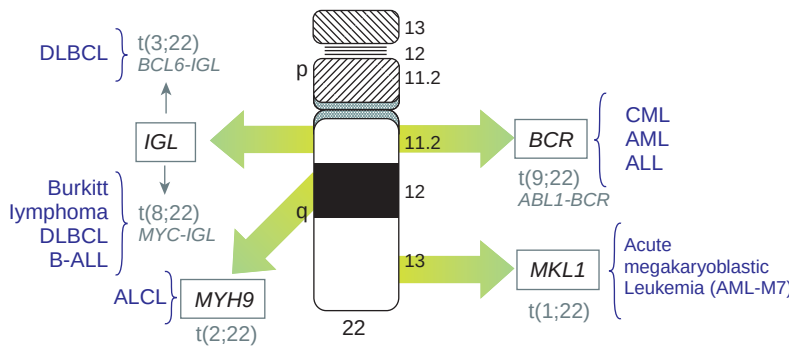


Figure 1.24 Chromosome 22

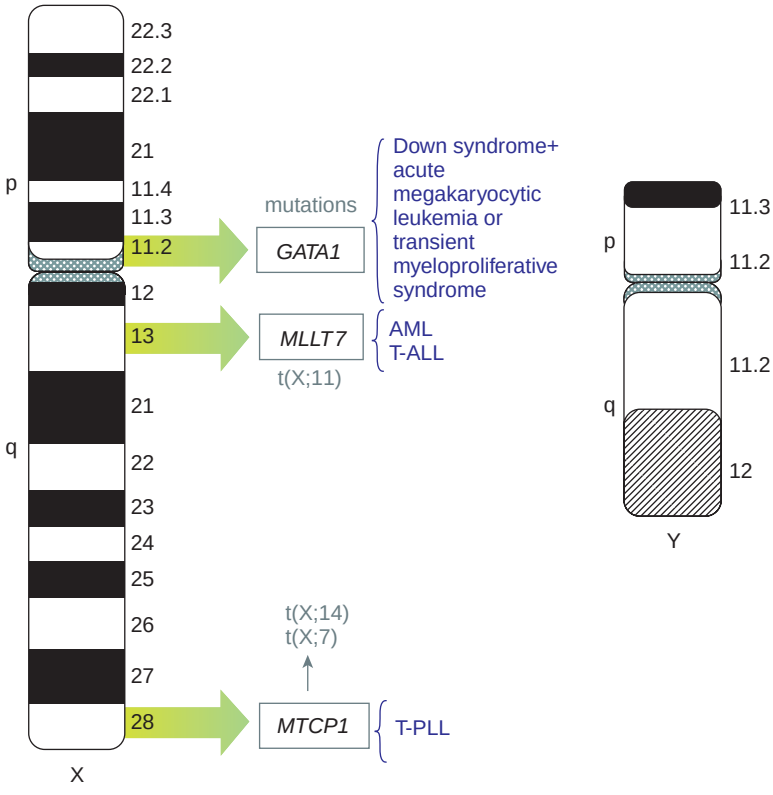


Figure 1.25 Chromosomes X and Y

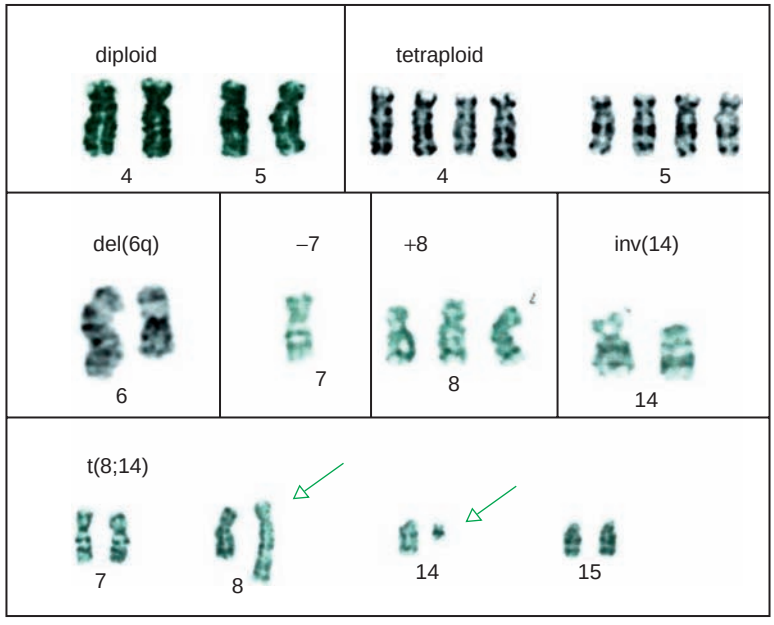


Figure 1.26 Examples of chromosomal aberrations

Table 1.1 Most common abbreviations used in cytogenetics/FISH

Abbreviation	*Definition*
add	additional material of unknown origin
cen	centromere
del	deletion (loss of part of chromosome)
dic	dicentric
der	derivative chromosome
dup	duplication
fra	fragile site
i (iso)	isochromosome
idic	isodicentric chromosome
ins	insertion
inv	inversion
mar	marker chromosome; a structurally abnormal chromosome that cannot be identified with standard cytogenetics
mos	Mosaic; two or more cell lines present in one individual (two or more cell types are present, which differ in number of chromosomes or their structure)
p	short arm of chromosome
ph	Philadelphia chromosome
q	long arm of chromosome
r	ring chromosome
rcp	reciprocal
rea	rearrangement
rec	recombinant chromosome
t	translocation
tel	telomere (end of chromosome arm)
ter	terminal end of chromosome
+	plus sign in front of chromosome number; gain of chromosome
–	minus sign in front of chromosome number; loss of chromosome
[ ]	square brackets; number of cells in each clone

and can be compared to corresponding routine H&E sections). The results of FISH analysis performed on paraffin-embedded tissue are comparable to those obtained in frozen samples.[123]

FISH probes

The FISH probes can be generally subclassified into the following categories:

(a) centromere-specific probes
(b) whole chromosome ('painting') probes
(c) single-copy (locus-specific) genomic probes
(d) spectral karyotyping (SKY; multiplex metaphase FISH; multicolor FISH).

Centromere-specific probes (centromere-enumerating probes, CEPs) are repetitive sequence probes that target highly repetitive 171 kb α-satellite sequences of centromeric DNA. Since the α-satellite sequences of chromosomes differ, those probes can detect specific chromosomes, and therefore are useful to demonstrate numeric chromosomal aberrations in metaphase or interphase nuclei (monosomy, trisomy, other aneuploidies). Due to sequence similarities between chromosomes 13 and 21 and between chromosomes 14 and 22, CEPs cannot be applied to identify those chromosomes.

Whole chromosome probes (WCPs; chromosome 'painting' probes) consist of numerous overlapping probes that recognize and bind to specific nonrepetitive DNA sequences along the entire length of individual (targeted) chromosomes. WCPs can be used to identify marker chromosomes (rearranged chromosomes of unidentified origin) or translocations that are otherwise not evident or difficult to interpret with routine banding cytogenetics.

The single-copy genomic probes (syn: locus-specific; gene-specific, or unique sequence probes) target distinct chromosomal regions (not repetitive sequences). They are used to detect the presence of amplification (increased number of copies of a specific locus), or to detect the presence of rearrangement. FISH with unique sequence probes can detect cryptic rearrangements not visible in standard (banding) cytogenetics analysis. In contrast to centromere-specific probes which yield a strong fluorescence signal, a single-copy genomic probe needs to be at least 20 kb in size to yield a sufficient signal. Assays for gene amplification (e.g. n-*MYC* in neuroblastoma or *HER2* in breast carcinoma) often employ a probe specific for the target gene together with a probe for the corresponding centromere. This approach helps to differentiate true amplification from cases with aneuploidy; in gene amplification

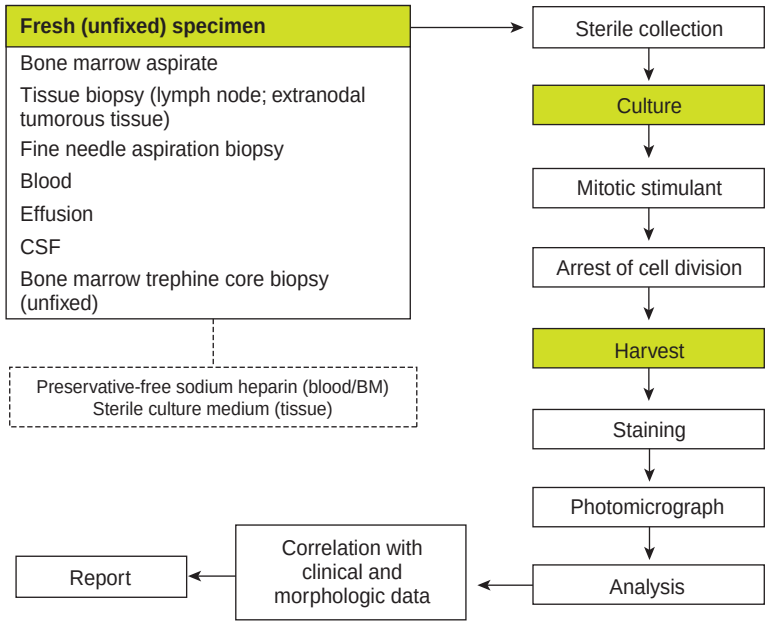


Figure 1.27 Algorithm for cytogenetic analysis

Table 1.2 Advantages and disadvantages of cytogenetics

Advantages	*Disadvantages*
Overview of whole karyotype	Requires fresh tissue
Can detect complex abnormalities	Balanced translocations
Unexpected findings	Labor intensive
Provides an overall evaluation of the whole genome and helps direct further analysis (specific probes for FISH and/or PCR)	Low mitotic index (lack of dividing cells in low-grade tumors or tumors with low proliferation fraction, e.g. CLL or multiple myeloma, etc.)
	Time consuming; long TAT
	Technically difficult: presence of multiple abnormal cell lines, complexity of chromosomal pattern
	Requires highly skilled personnel
	Cannot detect minute deletions
	Culture failure (lack of viable cells, bacterial overgrowth, stimulation of benign, non-neoplastic elements, sampling error)

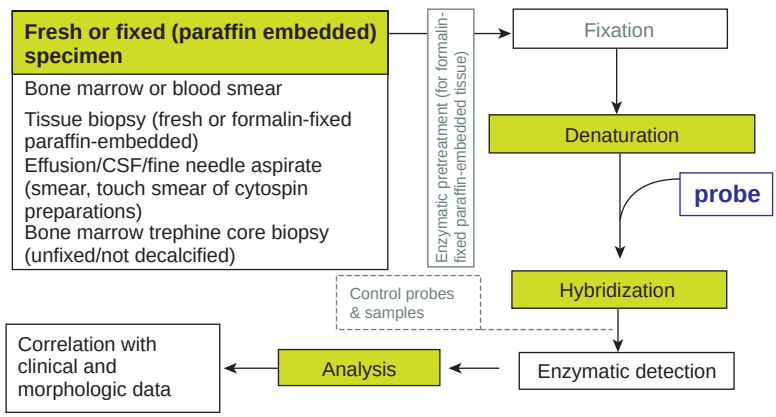


Figure 1.28 Algorithm for FISH analysis

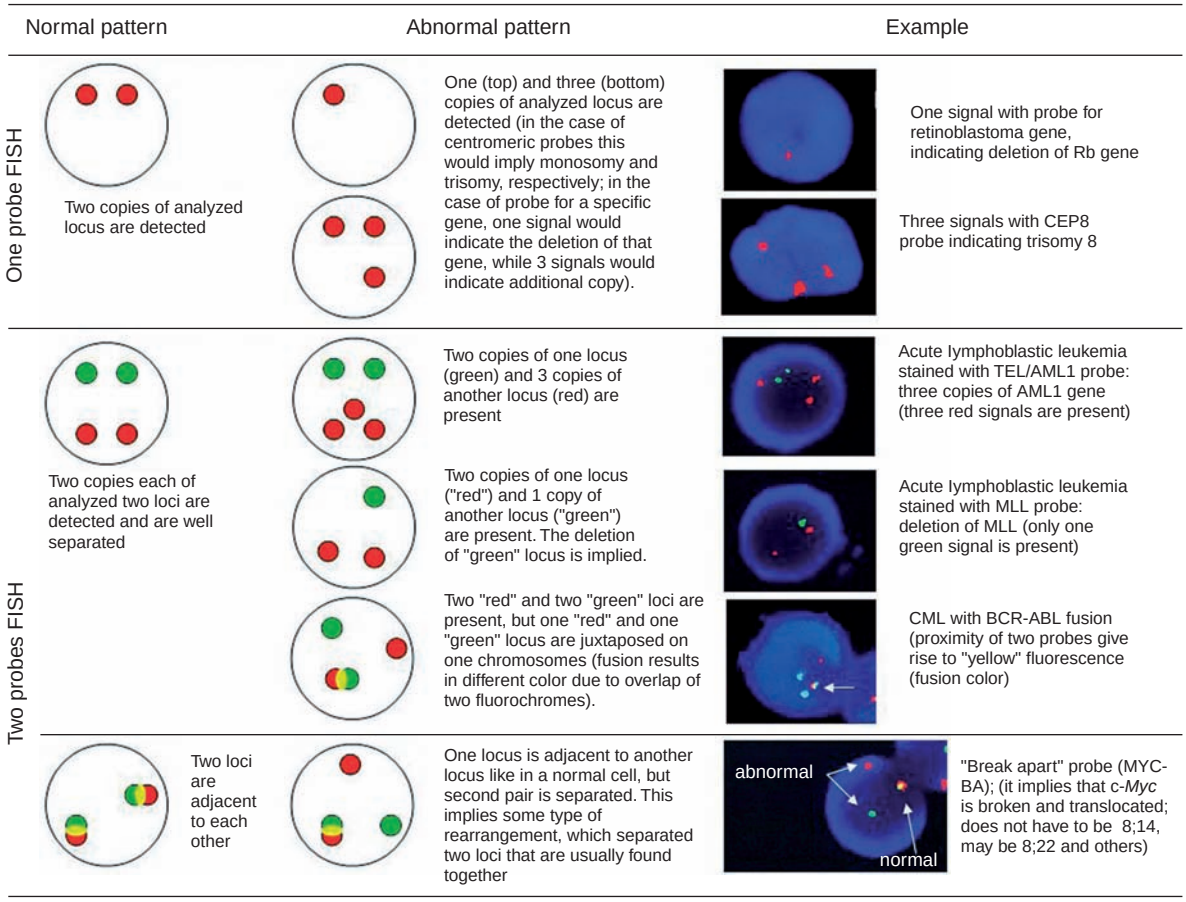


Figure 1.29 Interpretation of FISH results

the number of signals from the target gene is higher than the centromere signals. Locus- or rearrangement-specific probes have an advantage over PCR methodology for the identification of abnormalities involving multiple partners, such as *BCL6* (3q27) or when genomic breakpoints are widely scattered (e.g. *MYC/IGH* or *CCND1/IGH*).[124–127]

FISH probes for chromosomal translocations are most widely used in the evaluation of hematopoietic tumors. Two types of probes are used to detect translocations: (single- or dual-) fusion probes and break-apart probes (Figure 1.29). A dual-fusion probe consists of a pair of probes labeled with two different colors (fluorochromes), green (e.g. FITC) and red (e.g. rhodamine) directed against translocation breakpoint regions in the two different genes involved in a reciprocal translocation. In a normal cell there are two green and two red signals corresponding to two separate loci that are not in close proximity (no translocation). In cells with translocation between the targeted loci, there is one green and one red signal (normal chromosome) and one yellow signal indicating the fusion between two loci (yellow fluorescence being the result of overlap between the green and red signals) (Figure 1.29). Variant and complex patterns may also be identified and provide additional clinical information on the underlying chromosomal changes. The break-apart(BA) the probe

consists of two probes that target opposite regions (translocation breakpoint) of the targeted gene. The interpretation of results is opposite to that with fusion probes: the fusion (yellow) signal is normal, and separate green and red signals indicate translocation (BA). In a benign cell (without translocation), there are two fusion yellow signals indicating an intact gene (the probes are in close proximity, generating two yellow spots due to the overlap between the green and red fluorescence). In a cell with translocation, there will be one fusion yellow signal corresponding to a normal chromosome, but the second set of probes will be split, yielding one green and one red signal (indicating two derivative chromosomes). Commonly used BA probes in hematologic tumors include: *MLL*-BA (AML/ALL), *CBFB*-BA (AML), *RARα*-BA (acute promyelocytic leukemia; APL), *MYC*-BA (Burkitt lymphoma; BL), *MALT1*-BA (MALT lymphoma), *ALK*-BA (anaplastic large cell lymphoma; ALCL), and *IGH*-BA (lymphoma/MM).

Spectral karyotyping

Spectral karyotyping (SKY), multiplex metaphase FISH (M-FISH), or multicolor FISH, is a novel 24-color, multichromosomal painting technique, that allows for the simultaneous visualization of all chromosomes of a metaphase in a single hybridization step.[87,113,120,128–131] It allows simultaneous identification of the 22 autosomes and the 2 sex chromosomes. Those new methods are especially useful in evaluation of cases with complex chromosomal abnormalities, which often preclude an accurate interpretation by conventional cytogenetics. SKY in combination with the standard banding method and FISH is useful for exploring undefined or complex chromosomal abnormalities as well as identifying the chromosomal origins of marker chromosomes and other extrachromosomal structures without prior knowledge of them. It is a sensitive and valuable method that enables screening for the recurrent chromosomal aberrations in hematologic disorders.

Comparative genomic hybridization

Comparative genomic hybridization (CGH) is a technique developed by Kallioniemi et al based on the comparison of two genomic DNA populations, an analyzed sample (tumor) and a karyotypically normal reference.[132] Differentially labeled test DNA from a tumor and control (normal) reference DNA (metaphase chromosomes from blood lymphocytes) are hybridized simultaneously onto normal human metaphase chromosomes, competing for the same targets. The hybridization products are detected with two different fluorochromes (usually red and green). The fluorescence signal (ratio of the green and red fluorescence) of the tumor sample is compared to that of a control cell. Changes in the ratio of the intensities of the two fluorochromes as compared to the expected one-to-one ratio seen in the control sample indicates regions of gain or loss of DNA sequences, such as deletions, duplications, or amplifications (areas with a green to red ratio >1 indicate amplifications, a value <1 indicates deletions and ~1 (yellow/orange) indicates regions that are normally present). The presence and localization of chromosomal imbalances can be detected and quantitated by analyzing the ratio of the test fluorescence to reference fluorescence along the target chromosome using the digital image analysis. CGH is complementary to conventional cytogenetics (CC) and FISH, and provides additional information for the correct localization of the aberrations found in CC; it is most helpful in samples not processed successfully in CC.[114]

Table 1.3 presents the advantages and disadvantages of FISH analysis.

MOLECULAR PATHOLOGY

The polymerase chain reaction (PCR), developed by Mullis and Faloona, is a core technique for most tests used in molecular diagnostics.[133–135] It targets a segment of DNA (or RNA in reverse-transcriptase PCR) and produces multiple copies (usually between

Table 1.3 Advantages and disadvantages of FISH analysis

Advantages	*Disadvantages*
Does not require mitotically active cells; genomic aberrations can be measured in dividing and nondividing cells	It is not a screening test (it requires a targeted approach for suspected abnormality, based on clinical data, morphologic features, or findings in classic cytogenetics)
Does not require culturing	Requires specific probes
Allows for disease monitoring	Low resolution of some probes
Can be performed on fixed or unfixed tissue and on paraffin-embedded material	Cannot detect minute aberrations (small intragenic mutations, deletions, or insertions); targeted abnormalities need to be large (between 20 kb and 200 kb in size)
Fast, short TAT	Less sensitive than PCR-based essays
High efficiency and specificity	Tumor aneuploidy may result in inappropriate signal counts
More sensitive than cytogenetic karyotyping or CGH	Susceptible to artifacts in sections from paraffin-embedded tissue (e.g. underestimation of copy number due to transsection of nuclei)
High detection rate	Cross-hybridization artifacts (e.g. centromeric probes for chromosomes 13 and 21, or 14 and 22)
Large number of cells can be analyzed	Truncation artifacts in tissue sections
Allows for correlation with morphology	
Allows choice of a specific area for evaluation	
Can detect minute deletions	
Multifluorescent SKY (M-FISH) techniques provide an overall evaluation of the whole genome and help to direct further analysis	

10^7 and 10^{11}) of a DNA region of interest. PCR enables the detection of malignant cells below the threshold of karyotyping or morphology, even when combined with immunophenotyping. Basics steps in PCR technology are provided in Figure 1.30. Fresh tissue is the best sample source for PCR analysis, but fixed tissue (formalin, ethanol) may also be used. Carnoy's, Zanker's, B5, and Bouin's fixatives are suboptimal for PCR. Tissue exposed to decalcifying solutions (e.g. bone marrow trephine core biopsy) is not suitable for PCR analysis. Three major steps in PCR include denaturation of double-stranded DNA into single-stranded DNA (ssDNA), hybridization (annealing) of oligonucleotide primers to both ends of a target sequence, and a synthesis achieved by addition of four nucleotide bases and a *Taq* polymerase. A PCR reaction usually involves 30–40 cycles. The denaturation is achieved by high temperature (95°C), which, by breaking the hydrogen bonds between complementary bases, creates single-stranded DNA. In the next step the two primers join (hybridize) the single-stranded template DNA. In the final step (synthesis), *Taq* polymerase synthesizes new DNA strands, using the oligonucleotide primers as starting points.

Nested PCR

Nested PCR involves two PCR reactions for a single locus. It uses two sets of primers, the second of which lies within the first set of primers (the first target is longer and the second is shorter), which increases both specificity and sensitivity. Because of the high sensitivity of this methodology, it is used for a target expressed at a very low level (or by a small subset of the cells in the analyzed sample).

Reverse-transcriptase PCR

RT-PCR methodology amplifies RNA and consists of two major steps: first it employs an enzyme, reverse transcriptase, which converts RNA into the single-stranded complementary DNA (reverse transcription),

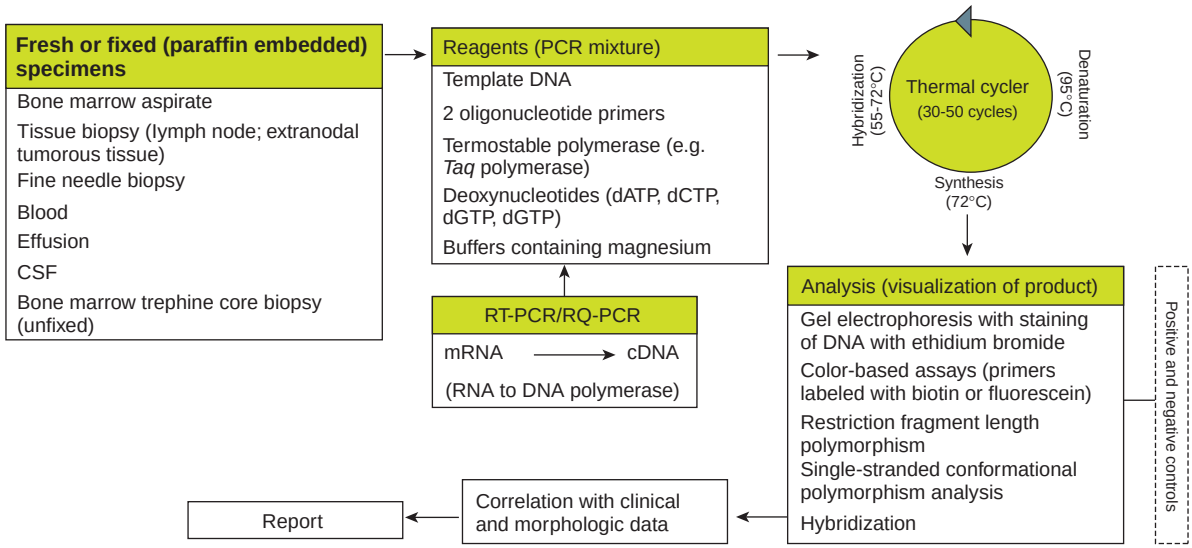


Figure 1.30 Algorithm for PCR analysis

which in the second step serves as a template for conventional PCR (second strand reaction). The original RNA is degraded by RNase. It be performed on fresh or fixed tissue. It is widely used in the diagnosis of genetic diseases, since it makes it easier to detect the presence of specific aberrations by directly detecting the product (mRNA), e.g. fusion transcripts encoded by the translocations. Additionally, it is very useful in monitoring some hematologic malignancies during treatment, since it allows for quantification of the analyzed products (see below).

Real-time quantitative PCR

Qualitative PCR (e.g. RT-PCR) fails to detect disease-specific changes in certain subsets of patients, especially in patients monitored for MRD. This is often associated with a poor quality of RNA. Quantitative real-time PCR (RQ-PCR; qRT-PCR) became a more powerful tool for monitoring patients, giving the clinicians the best chance of detecting the earliest stages of molecular relapse. This technique allows simultaneous amplification and detection of PCR products in a single reaction tube. The reaction is monitored in real time after each cycle of amplification by computer software permitting both detection and quantification of PCR products. After each amplification cycle, a dye is released and its amount is measured in real time to ensure that quantitation of DNA occurs in the exponential phase. The results are presented in the form of a graph which plots the dye (corresponding to the amount of the starting template) versus the number of PCR cycles (Figure 1.31). The quantitation is based on the number of cycles required to reach a designated threshold (the lower the number of cycles to reach the threshold the higher the expression of the mRNA or DNA copy number), using the fluorescence-based quenching methods (fluorescence resonance energy transfer; FRET). The most commonly used systems for qRT-PCR include the *Taq*Man system, Molecular beacons, Scorpions, and SYBR green. *Taq*Man probes depend on the 5′-nuclease activity of the DNA polymerase used for PCR to hydrolyze an oligonucleotide that is hybridized to the target amplicon. The fluorochromes used in *Taq*Man (reporters) include FAM,

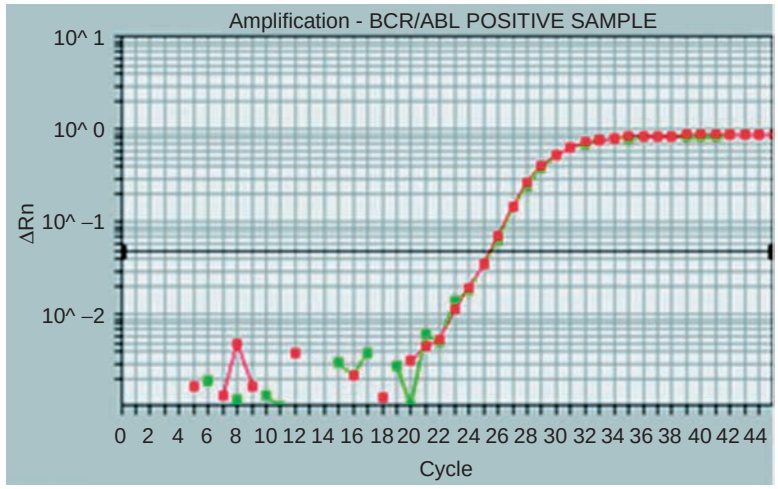


Figure 1.31 PCR (*BCR/ABL* positive sample)

HEX/JOE/VIC, and TET. The fluorescence signal is quenched by either TAMRA or DABCYL (quenchers). Figure 1.32 illustrates the chemistry of *Taq*Man methodology. In the unhybridized state, the fluorochrome (reporter) and the quencher are close to each other, allowing for FRET (there is no fluorescent signal from the probe because the fluorescence of the reporter dye is being quenched). During PCR, when the *Taq* polymerase replicates a template and the 5′-exonuclease activity of the polymerase degrades the probe, the reporter and the quencher become unlinked and FRET no longer occurs. Thus, fluorescence increases in each cycle, proportional to the amount of probe cleavage.

Table 1.4 presents the advantages and disadvantages of PCR methodology.

Gel electrophoresis

Gel electrophoresis (most often using polyacrylamide gels; PAGE) is used to separate DNA products based on their size. DNA is then visualized by chemical stains (unlabeled probes) or fluorescence (fluorochrome-labeled probes). Capillary electrophoresis employs thin glass tubes (capillaries) in which DNA products are separated and then visualized by either emitted fluorescence or under UV light absorbance.

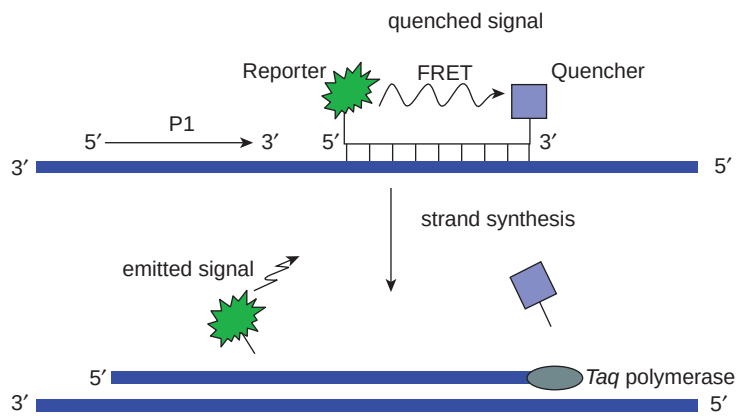


Figure 1.32 The chemistry of *Taq*Man methodology in qRT-PCR

Table 1.4 Advantages and disadvantages of PCR analysis

Advantages	*Disadvantages*
High specificity	DNA sequence has to be known, to design specific probe
High sensitivity	Very high sensitivity leading to possible contamination
Fast TAT	Potential for carry over
Allows use of very small amount of nucleic acid	Nonspecific PCR products
Allows use of many types of specimens (blood, bone marrow, buccal cells, CSF, paraffin-embedded tissue sections)	Limited detection of chromosomal breakpoints spread over a large genomic region [e.g. t(11;14) involving *CCND1* gene]
Highly automated	Cost
PCR products can be directly labeled by fluorochrome(s)	Targeted nature of the test (PCR analyzes only the target region); morphologic (phenotypic)–genotypic correlation can be achieved with microdissection, laser capture microdissection, or flow cytometry sorting
	False positive or false negative results DNA polymorphism in the primer binding site and some mutations may alter the structure of the target region, so that it cannot be amplified
	Positive results in healthy individuals [e.g. t(14;18) and t(2;5)]

Single nucleotide polymorphism

Single nucleotide polymorphism (SNP) arrays offer the ability to define simultaneously the copy number changes and LOH (loss of heterozygosity) events.[136–138] Two major technologies which allow simultaneous genotyping of over half a million SNPs across the genome include Affymetrix SNP arrays with oligonucleotide probes spotted on gene chips (adopted from the microarray hybridization chip technology) and Illumina arrays, in which allele-specific oligonucleotide probes are adsorbed to microbeads arranged on a microarray.[138]

DNA microarrays

DNA (genomic) microarray analysis is a highly promising new technique with broad applications, including the prediction of cancer outcomes by simultaneous analysis of the expression of thousands of genes.[139–141] A DNA chip or microarray consists of tens of thousands of fragments of DNA bound to a silicon chip. The methodology involves the extraction of RNA from a sample, its conversion to cDNA by labeled probes, hybridization of these labeled cDNAs, and laser scanning of the hybridized array. A reference RNA is labeled with a different fluorochrome. Spots appear either yellow (if the subject and reference cDNA hybridize equally), or red or green, if either the sample cDNA or control probe predominate (Figure 1.33). The automated examination of a chip delivers the information about thousands of probe sites. The genomic microarray technique is similar to comparative genomic hybridization (CGH) except that the labeled test and reference DNAs are hybridized to a microarray rather than to a normal metaphase spread.

Microarray technology will most likely lead to identification of new tumors and change the criteria by which current hematopoietic cancers are subclassified. In addition, it will provide new targets for treatment, and identify new prognostic and predictive parameters and markers for monitoring minimal residual disease (MRD). Microarray analysis has been used to screen for chromosomal aberrations (genomic imbalances, mutations, and structural changes) and large-scale gene expression analysis.

IMMUNOPHENOTYPING BY FLOW CYTOMETRY

Flow cytometry (FC) plays an important role in a multimethodology approach to the diagnosis of

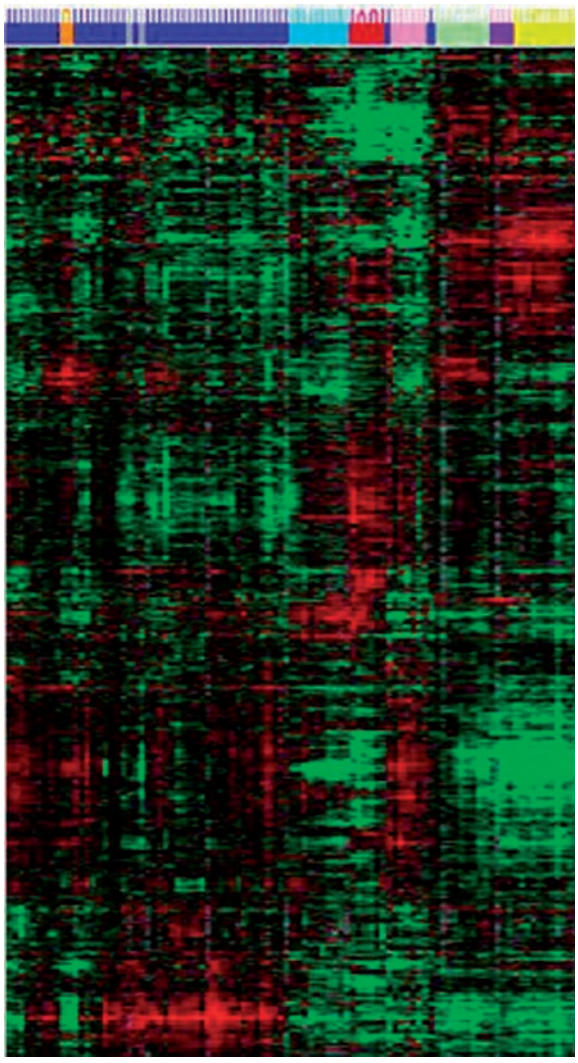


Figure 1.33 DNA microarray

hematologic tumors, by providing data on the extent of involvement, prognosis, and post-treatment monitoring.[23,38,142–151] FC analysis requires fresh (unfixed) material. Types of specimens suitable for FC include blood, bone marrow aspirates (and fresh core biopsy), fresh tissue samples (excisional or core biopsies), fine needle aspirates, and body fluids. FC allows distinction between benign and neoplastic processes. While no single marker permits accurate lineage assignment, analysis with panels of antibodies allows for separation of hematologic tumors into various, very precise subtypes, each of which has different prognosis and treatment requirements. FC can precisely differentiate between B- and T-cell malignancies, and between mature (peripheral) and precursor tumors, and among the latter, it can determine the myeloid or lymphoid lineage. Multiparameter (4- or 6-color) FC analysis enables simultaneous evaluation of several markers on a single cell, allowing for an accurate characterization of analyzed populations. FC can be used to analyze DNA ploidy, proliferation (S-phase), and programmed cell death (apoptosis). Determination of the expression of ZAP70 and/or CD38 by FC can serve as a prognostic marker in patients with B-chronic lymphocytic leukemia (B-CLL). The recognition of an aberrant pattern of antigen expression can be used to monitor patients following chemotherapy or bone marrow transplant and to help distinguish recovering bone marrow from residual disease. The sensitivity of flow cytometry in evaluating MRD is approaching that of molecular tests (PCR). Identification of individual malignant cells in patients during and/or after treatment is a strong parameter in evaluating patients' response to treatment and also a prognostic indicator. FC analysis enables the physicians to monitor treatment and modify it based on FC findings. In acute lymphoblastic leukemia (ALL), the MRD-based evaluation of the initial response to front-line therapy emerged as a highly relevant diagnostic tool, particularly in childhood ALL, where MRD has been shown to be an independent prognostic factor allowing a precise risk group classification. The presence of MRD in patients with CLL in complete remission after treatment with alemtuzumab (Campath) or autologous stem cell transplantation correlates with a shorter duration of the response and/or survival compared to patients achieving MRD-negative complete remission. The MRD status at the end of therapy is more predictive of the duration of remission than conventional response criteria and identifies the patients at risk of early disease progression.

Methodology

In the FC protocol, the sample is incubated with antibodies, followed by red blood cell lysis, washing, fixation in paraformaldehyde, and FC analysis. Whole blood lysis represents the most commonly used technique for sample preparation. Routinely 5–10 000 cells are collected. Monoclonal antibodies used in FC are conjugated with fluorochromes, which are excited or stimulated by laser(s) in flow cytometry. The commonest fluorochromes excited at 488 nm (argon laser) include: fluorescein isothiocyanate (FITC), phycoerythrin (PE), propidium iodide (PI), 7-amino-actinomcyin D (7AAD), peridinchlorophyl-A-protein (PerCP), and dimers of thiazole orange (TOT-1). FC is much faster than immunohistochemistry and can analyze thousands of cells within seconds. Another advantage of FC immunophenotyping is that it allows the correlation of several markers on a single cell, and detects the intensity of staining and aberrant expression of antigens. FC has a high sensitivity for B-cell lymphoproliferative disorders and acute leukemia, and a high specificity for several categories of those neoplasms. All these properties are used in diagnostic hematopathology for the subclassification of neoplasms. The major disadvantage of FC is a need for liquid cell suspensions and therefore a lack of correlation with histomorphologic features (tissue architecture). FC requires viable fresh (unfixed) material. In a subset of neoplasms, especially high-grade lymphomas, decreased viability precludes FC analysis. Flow cytometry analysis requires at least 10–20 000 cells/events acquired by tube, which often limits its use in specimens from CSF, fine needle aspirates, and paucicellular (or fibrotic) lesions. Dropout of neoplastic cells due to low viability or sample bias due to focal (partial) tissue involvement may lead to false negative flow results. Figure 1.34 illustrates the gating strategy applied in the FC analysis of peripheral blood and bone marrow. Based on the intensity of CD45 staining (x-axis) and side scatter (SSC, y-axis) one can distinguish several major populations in normal

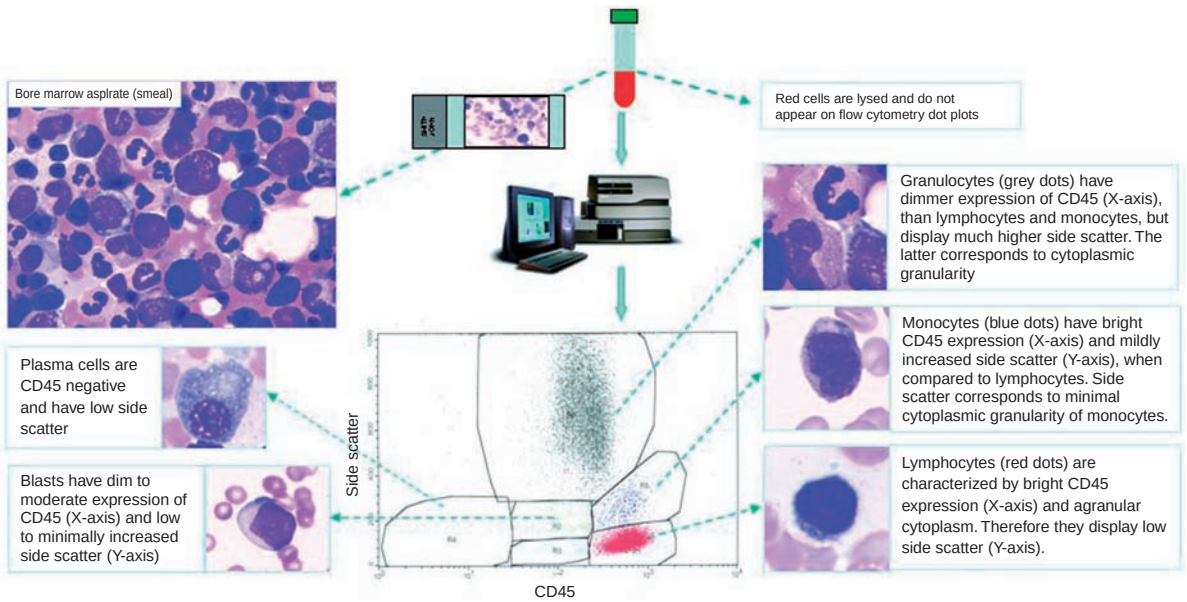


Figure 1.34 Flow cytometry (gating strategy). Part of the sample from the bone marrow aspirate or blood (tube) is smeared on the microscope glass slide for morphologic correlation, while the rest is incubated with antibodies, lysed, fixed and submitted for flow cytometry analysis; *see text for details*

Table 1.5 Phenotypic markers in major types of hematopoietic cells

Target	*Marker/antibody*
Activated cells	CD30, MUM1, CD138
Anaplastic large cell lymphoma	ALK-1, CD30, pan-T antigens, CD43, UCHL1, EMA
B-cell markers	CD19, CD20, CD79a, CD22, PAX-5, Oct-2, Bob-1
Blastic markers	CD34, CD117, CD1a, TdT
Germinal center cells	CD10, bcl-6
Granulocytes	CD10, CD11b, CD15, CD16, MPO
Hodgkin lymphoma	CD30, CD15, PAX5
Immature T-cells/thymocytes	CD1a, CD4, CD8, TdT, pan-T antigens, CD10
Mast cells	Mast cell tryptase, CD117, CD43, CD2
Megakaryocytes	CD41, CD61, (CD79a)
Monocytic markers	CD11b, CD11c, CD14, CD64, CD68, CD163, HLA-DR
Myeloid markers	CD13, CD15, CD33, CD68, MPO
NK cell markers	CD16, CD56, CD57
Plasma cell markers	CD38, CD138, cytoplasmic light and heavy chains, CD56, CD117, MUM1
Red cell markers	GPHA, hemoglobin A
T-cell markers	CD2, CD3, CD5, CD7, CD43, UCHL1

bone marrow: lymphocytes (bright CD45 and low SCC; red), monocytes (bright CD45 and increased SSC; blue), and granulocytes (moderate CD45 and high SSC; gray). Red blood cells and their precursors are eliminated from FC analysis by lysis.

IMMUNOHISTOCHEMISTRY

Immunohistochemistry plays an important role in diagnostic hematopathology. It is helpful in the determination of (a) cell origin, (b) degree of differentiation (maturation), and (c) prognosis. The availability of monoclonal or polyclonal antibodies (Tables 1.5 and 1.6), automation, and protocols for antigen retrieval designed for formalin-fixed, paraffin-embedded tissues makes immunohistochemical techniques a common practice in everyday diagnostic surgical pathology. Immunohistochemistry permits the differentiation of epithelial tumors (cytokeratin positive) from hematolymphoid tumors

Table 1.6 CD antigens and other commonly used immunophenotypic markers – expression in hematopoietic tumors

Antibody	*Reactivity*
AE1/AE3	epithelial cells, mesothelial cells
ALK1	anaplastic large cell lymphoma
Annexin	hairy cell leukemia
Bcl-2	mature B-cells (except benign germinal center cells), T-cells, follicular lymphoma, epithelial tumors
Bcl-6	germinal center B-cells, follicular lymphoma, subset of DLBCL
CD1a	thymocytes, immature T-cells
CD2	T-cells, large granular lymphocytes, NK-cells, some acute promyelocytic leukemias, mast cells (neoplastic)
CD3	T-cells, primary effusion lymphoma (PEL; subset)
CD4	T-cells (helper/inducer), monocytes, myeloblasts, blastic NK-cell lymphoma (DC2 leukemia)
CD5	T-cells, B-CLL/SLL, mantle cell lymphoma
CD7	T-cells, some myeloblasts
CD8	T-cells (cytotoxic), large granular lymphocytes

Table 1.6 CD antigens and other commonly used immunophenotypic markers – expression in hematopoietic tumors—cont'd

Antibody	*Reactivity*
CD10	follicle center cells, follicular lymphoma, some diffuse large B-cell lymphomas, precursor B-ALL, precursor T-ALL, thymocytes, Burkitt's lymphoma
CD11b	granulocytes, monocytes
CD11c	monocytes, hairy cell leukemia, large granular lymphocytes, activated T-cells, marginal zone B-cell lymphoma
CD13	myeloid cells, rare precursor B-ALL
CD14	Monocytes
CD15	granulocytes, Hodgkin lymphoma (classical)
CD16	granulocytes, NK-cells, large granular lymphocytes
CD19	B-cells, precursor B-ALL, subset of AML [*AML1/ETO* with t(8;21)]
CD20	B-cells, rare plasma cell myelomas
CD22	B-cells
CD23	B-SLL/CLL, plasma cells, follicular dendritic cells
CD25	hairy cell leukemia, subset of B- and T-cell lymphomas
CD30	Hodgkin lymphoma, anaplastic large cell lymphoma, subset of diffuse large B-cell lymphoma, subset of T-cell lymphomas (NOS), primary mediastinal B-cell lymphoma, lymphomatoid papulosis
CD33	myeloid cells, rare precursor B-ALL, rare blastic NK-cell lymphomas
CD34	myeloblasts, lymphoblasts, endothelial cells
CD38	plasma cells, activated B- and T-cells, subset B-CLL/SLL, epithelial cells
CD41	Megakaryocytes
CD43	myeloid cells, T-cell lymphomas, precursor B- and T-cell leukemias, B-cell lymphoma (subset), plasma cells
CD56	NK-cells, large granular lymphocytes, blastic NK-cell lymphoma/leukemia, monoblasts, subset plasma cell myelomas, rare B- and T-cell lymphomas
CD57	NK-cells, large granular lymphocytes
CD61	Megakaryocytes
CD79a	B-cells, plasma cells, megakaryocytes
CD103	hairy cell leukemia, rare T-cell lymphomas
CD117	AML, mast cells, stromal tumors (GIST), plasma cells
DBA-44	hairy cell leukemia, subset of B-cell lymphomas
Heavy chains	B-cells, plasma cells, DLBCL with ALK expression (IgA)
HLA-DR	AML (except acute promyelocytic leukemia), B-cells, monocytes
Light	B-cells (surface), plasma cells (cytoplasmic) chains
MUM1	activated cells, plasma cells, subset of DLBCL, Hodgkin lymphoma
PAX5	B-cells, Hodgkin lymphoma, subset plasma cell myelomas
TdT	precursor B-ALL, precursor T-ALL, some AML, hematogones
ZAP-70	T-cells, subset of B-CLL, NK-cells

(CD45 positive), but also allows for specific and detailed subclassification of tumors (melanoma versus sarcoma, T- versus B-cell, small lymphocytic versus mantle cell, myeloid versus lymphoblastic). It is also used to determine the prognosis and to optimize treatment strategies (estrogen/progesterone receptors in breast cancer, ZAP70 in B-CLL/SLL, Ki-67 proliferation fraction). Figure 1.35 presents the principles of immunohistochemical staining. In the indirect (two-step) method, antigen is detected by a primary antibody, which in turn is detected by a secondary antibody conjugated with an enzymatic complex. A colorimetric reaction of the substrate (e.g. DAB, alkaline phosphatase) identifies the presence of any given antigen under the microscope with its correlative morphologic parameters. In a direct (one-step) method, the primary antibody is conjugated with an enzymatic complex. The two-step protocol is more sensitive. Depending on the location and distribution of the antigen(s), the color reaction may be

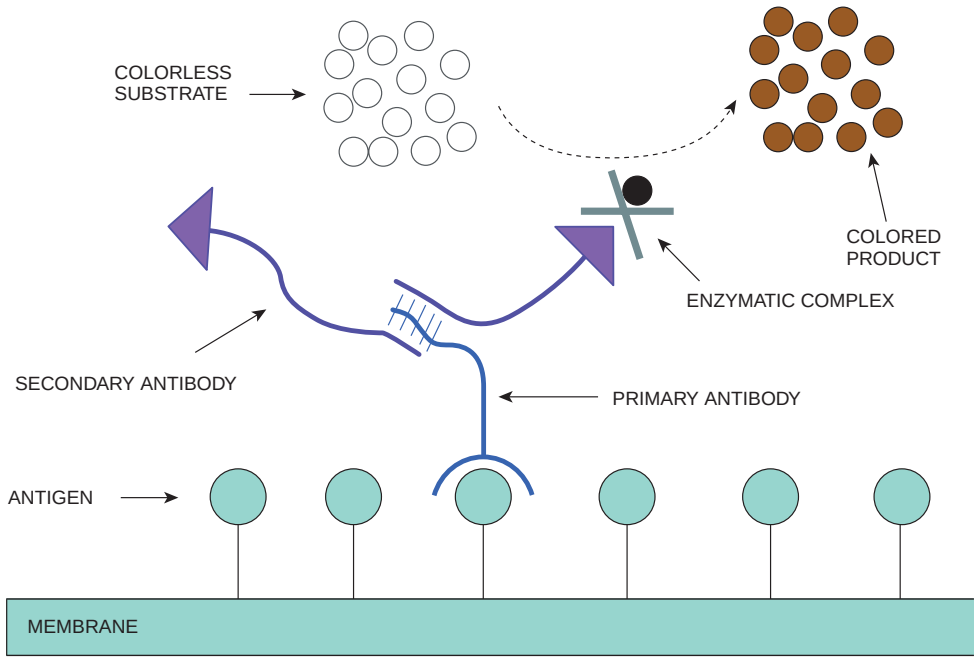


Figure 1.35 Immunohistochemical detection of antigen by indirect method (scheme), *see text for details*

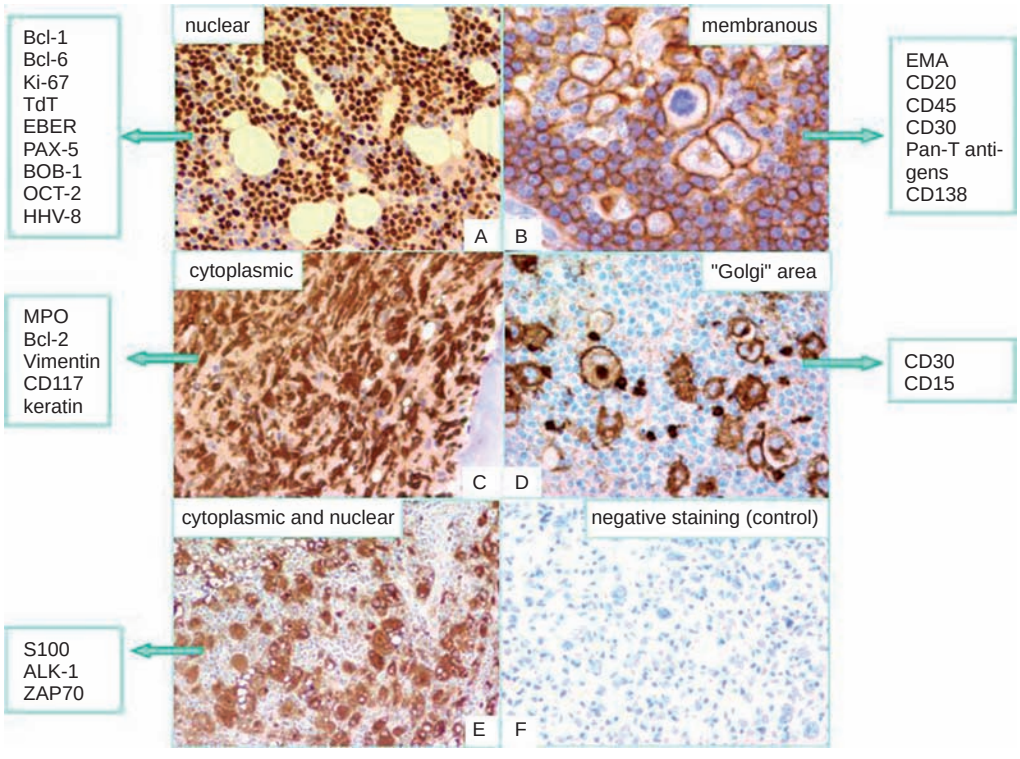


Figure 1.36 Major patterns of the immunohistochemical (IHC) staining in formalin-fixed and paraffin-embedded tissue sections. The pattern of IHC staining depends on the localization of the antigen: nuclear, cytoplasmic, cytoplasmic and nuclear or membranous

visible in the nucleus, cytoplasm, cytoplasmic membrane, and/or nucleolus (Figure 1.36). Nuclear staining is characteristic for bcl-1, bcl-6, BOB-1, EBER, HHV-8, Ki-67 (MIB-1), OCT-2, Pax-5, and TdT; membrane staining occurs with CD20, CD30, CD45, CD138, EMA, and pan-T antigens; cytoplasmic staining is seen with bcl-2, CD117, cytokeratins, myeloperoxidase, and vimentin; staining of the Golgi area as well as the membrane (dot-membrane pattern) is seen with CD15 and CD30, and cytoplasmic and nuclear staining is typical for ALK-1, ZAP70, and S100.

CHAPTER 2

Applications of cytogenetics, FISH, and PCR in diagnosis, prognosis, and disease monitoring

MOST COMMON CHROMOSOMAL/MOLECULAR ABNORMALITIES

Tables 2.1 and 2.2 present common chromosomal abnormalities in hematopoietic tumors.

Deletions and monosomies

Deletions of parts of chromosome(s) or monosomies belong to frequent abnormalities in hematopoietic neoplasms. A deletion can occur on any chromosome, at any band, and can be any size (large or small).

del(5q)

- MDS/tMDS
- AML/tAML
- Plasmacytoid dendritic cell leukemia/lymphoma (blastic NK-cell lymphoma/leukemia; DC2 acute leukemia)
- ALL (rare cases)

Deletion of 5q (Figure 2.1) occurs in MDS and AML.[21,152–159] The presence of del(5q), either as the sole karyotypic abnormality or as part of a complex karyotype, has distinct clinical implications for MDS and AML. Patients with MDS and del(5q) can be grouped into those with isolated del(5q) (good prognosis), del(5q) with additional abnormality (prognosis worse than for isolated 5q deletion), and 5q deletion with complex karyotype (poor prognosis). The 5q– syndrome is a distinct category of MDS defined by less than 5% blasts, isolated deletion of the long arm of chromosome 5, and a low probability of transformation to AML.[1,153,154,156–160] The molecular mechanisms associated with del(5q) are still unknown. The 5q– syndrome occurs in elderly patients (median age 67 years), more often in women than men, and is associated with macrocytic anemia with low reticulocyte counts and high erythropoietin levels.[156] The projected median survival of patients with isolated del(5q) is 107 to 146 months.[155,156] Recent clinical results with lenalidomide, an oral immunomodulatory drug, have shown durable erythroid responses, including transfusion independence and complete cytogenetic remissions in patients with del(5q) MDS with or without additional chromosomal abnormalities, suggesting that

Table 2.1 Most common chromosomal and molecular markers in the diagnosis of hematopoietic malignancies

Marker	*Neoplasm*	*Comments*
BCR-ABL [t(9;22)]	CML, CML in blast crisis	confirms the diagnosis of CML
	AML ALL	poor prognosis
PML-RARα [t(15;17)]	APL (AML-M3)	good response to ATRA
t(8;14)	Burkitt lymphoma Subset of DLBCL	BCL2–/BCL6– BCL2+ and/or BCL6+
JAK2	Polycythemia vera Essential thrombocythemia Chronic idiopathic myelofibrosis	
inv(16)/t(16;16)	AML	good prognosis, response to AraC
ETO [t(8;21)]	AML	good prognosis, response to AraC
BCL1 (*CCND1*) [t(11;14)]	Mantle cell lymphoma Subset of plasma cell myeloma	confirms the diagnosis of MCL
Clonal *IG* heavy chain rearrangements (14q32)	B-cell lymphomas (confirms clonality)	exceptions: see Table 2.4
Clonal *TCRB* or *TCRG* gene rearrangements r(7q34 or 7q15)	T-cell lymphomas (confirms clonality)	exceptions: see Table 2.4
ALK1/NPM [t(2;5)]	ALCL	
del(5q)	5(q)– syndrome (MDS) MDS or AML	sole abnormality del(5q) as part of complex aberrations
inv(14)	T-PLL	
i(7q)	Hepatosplenic γδ T-cell lymphoma	
BCL2 rearrangement [t(14;18)]	Follicular lymphoma Some DLBCL DLBCL	does not occur in Burkitt lymphoma
BCL6 rearrangements [t(3;n)]	Some DLBCL	does not occur in Burkitt lymphoma
c-*MYC* [t(8;14)]	Burkitt lymphoma Some DLBCL	c-*MYC* may be rearranged to *IGK* (chromosome 2) and *IGK* (chromosome 22) loci, as well
PAX5/IGH [t(9;14)]	Lymphoplasmacytic lymphoma	
API2/MALT1 [t(11;18)]	Extranodal marginal zone B-cell lymphoma (MALT type)	

lenalidomide can overcome the pathogenic effect of 5q deletion in MDS and restore bone marrow balance.[153]

Deletions of a 5q are not limited to the 5q– syndrome. They are present in other types of MDS (both *de novo* and therapy-related), usually in high-grade MDS (such as RAEB) as well as AML.[152,161] MDS cases with 5q– plus additional abnormalities, including del(12p), del(7q), del(14q), i(11q), and i(17q), show a neoplastic evolution in a short period of time.[162] Monosomy 5/del(5q) may be seen in all types of AML, but is more common in acute erythroid leukemia (AML-M6). Monosomy 5/del(5q) and monosomy 7/del(7q) represent the most common cytogenetic abnormalities in therapy-related MDS and AML (t-MDS/t-AML) and are strongly associated with prior exposure to alkylating agents.[163] AML with deletion of (5q) or monosomy 5 (–5) is associated with a very poor prognosis, similar to AML with complex chromosomal abnormalities, monosomy 7 (–7), and t(9;22).[15,17,18,161,164] Mutations of the *NRAS* and *TP53* genes and internal

Table 2.2 Most common chromosomal and molecular markers in the diagnosis of hematopoietic malignancies

Tumor type	*Chromosomal/ genetic alteration(s)*
Acute lymphoblastic leukemia	*TEL-AML1* t(9;22) [*BCR-ABL*] MLL-BA
Anaplastic large cell lymphoma	t(2;5) [*NPM/ALK*]; rearrangements of 2p23/*ALK*-BA
Acute myeloid leukemia	+8; +12; –5; –7 t(8;21) [*AML1/ETO*] t(16;16)/inv(16) MLL-BA t(9;22) [*BCR-ABL*]
Acute promyelocytic leukemia	t(15;17) [*PML-RARA*]
B-CLL	13q–; 11q–; 17p–
Burkitt lymphoma	t(8;14) [c-*MYC/IGH*, c-*MYC*-BA] t(2;8) [*IGK*/c-*MYC*] t(8;22) [c-*MYC*/*IGL*]
Chronic idiopathic myelofibrosis	*JAK2* mutations (~40%)
Chronic myeloid leukemia	t(9;22) [*BCR/ABL*]
DLBCL	t(3q27) [*BCL6*] t(14;18) [*IGH/BCL2*] t(8;14) [*MYC/IGH*] (5–15%)
Essential thrombocythemia	*JAK2* mutations (~50%)
Follicular lymphoma	t(14;18) [*IGH/BCL2*]
MDS	+8; +12; –5; –7
MALT lymphoma	t(11;18) [*API2/MALT1*] t(14;18) [*IGH/MALT1*] t(11q21) [*MALT1*-BA] t(1;14) [*BCL10/IGH*] +3/+(3q)
Mantle cell lymphoma	t(11;14) [*CCND1/IGH*]
Multiple myeloma	13q– (RB1) 17p– (TP53) t(11;14) [*IGH/CCND1*] *IGH*-BA IGH-FGFR3
Hepatosplenic γδ T-cell lymphoma	isochromosome 7
Lymphoplasmacytic lymphoma	t(9;14) [*PAX5/IGH*]
P. vera	*JAK2* mutations (>90%)
T-PLL	t(14;14)/inv(14)

tandem duplication (ITD) of the *FLT3* gene are among the most frequently observed molecular abnormalities in MDS and AML, but are uncommon in MDS patients with a 5q deletion (the *TP53* mutation is associated with the more advanced MDS subtypes).[165]

Del(5q) is observed in other hematolymphoid malignancies, including plasmacytoid dendritic cell leukemia/lymphoma (blastic NK-cell lymphoma/leukemia; DC2 acute leukemia),[166–168] adult T-cell leukemia/lymphoma,[169,170] and rare cases of ALL.[171-173]

del(6q)

- FL
- B-CLL
- Diffuse large B-cell lymphoma (DLBCL)
- Marginal zone B-cell lymphoma (MZL; MALT lymphoma)
- T-cell lymphomas
- Waldenström's macroglobulinemia
- T/NK-cell leukemia/lymphoma
- ALL

Aberrations of the long arm of chromosome 6 (Figure 2.2) are among the most common chromosomal abnormalities in lymphoid neoplasms.[174–179] The del(6q) has been reported in follicular lymphoma,[174,175,180,181] Waldenström macroglobulinemia,[182–184] B-CLL,[174,185–187] MZL,[174] ALL,[174,176,188] multiple myeloma,[185,189] diffuse large B-cell lymphoma (DLBCL),[104,174,180,190,191] and T-cell lymphoproliferations.[192,193] In a series reported by Taborelli et al, conventional cytogenetics revealed a 6q deletion in 46% of lymphomas, including two cases that showed 6q deletion as the sole chromosome anomaly.[174] The 6q deletion is usually associated with other chromosomal/genetic abnormalities and likely plays an important role in disease progression.

In patients with FL deletion 6q has been identified as the negative prognostic factor.[175] In a series by Viardot et al, the loss of material on chromosomal bands 6q25q27 was the strongest predictor of a shorter survival in FL patients, followed by elevated

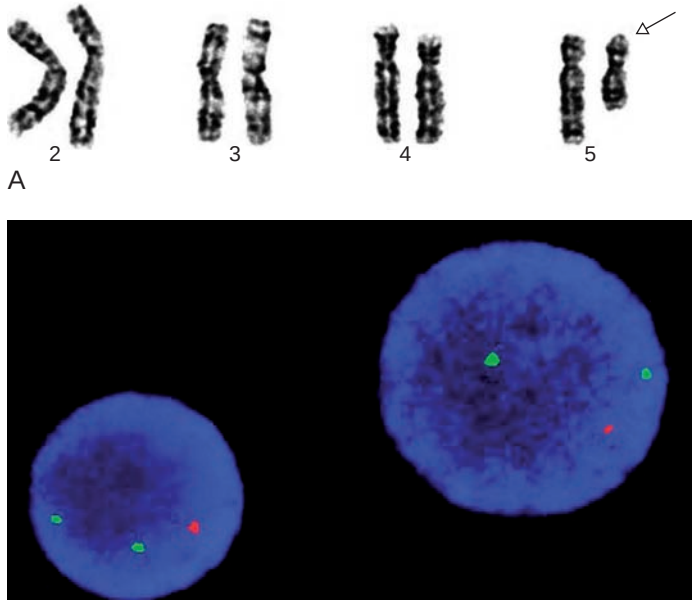


Figure 2.1 Del(5q): A, cytogenetics (partial karyotype); B, FISH (EGR1)

LDH, the presence of more than one extranodal manifestation, and age greater than 60 years.[181] Stilgenbauer et al, did not observe an adverse prognostic impact of 6q deletion in B-CLL.[187] In a more recent report by Cuneo et al, B-CLL with del(6q) was characterized by a high incidence of atypical morphology, a classic immunophenotype with CD38 positivity, and intermediate incidence of *IGVH* somatic hypermutation.[186] Clinicobiologic features and outcome showed that CLL with del(6q) should be allocated in an intermediate-risk category.[186]

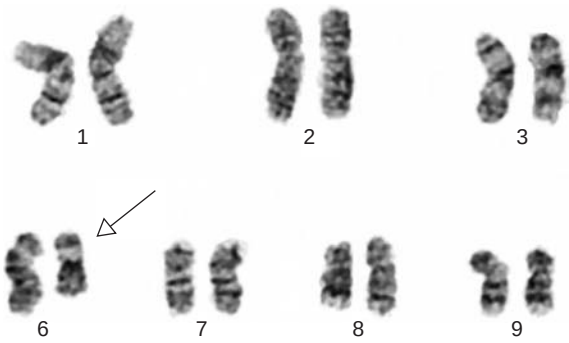


Figure 2.2 Del(6q), cytogenetics (partial karyotype)

Deletion of the long arm of chromosome 6 is found in about half of patients with Waldenström macroglobulinemia.[182–184] The area of minimal deletion falls between 6q23 and 6q24.3, but the deletion usually encompasses a large fragment of the 6q arm. The presence of del(6q) can help to distinguish Waldenström macroglobulinemia from IgM^+ MGUS (monoclonal gammopathy of undetermined significance), since the latter is negative for del(6q).[184] The del(6q) (in conjunction with other abnormalities, such as +3p and +1p) plays a role in MGUS progression to multiple myeloma.[194]

In cutaneous T-cell lymphomas, loss of 6q is associated with a significantly shorter survival.[192] The del(6)(q21q25) is a recurrent chromosomal abnormality in NK-cell lymphoma/leukemia, a highly aggressive malignancy.[193,195]

In pediatric ALL, cytogenetically detectable del(6q) is not associated with adverse risk.[196] In adult ALL 6% of cases have del(6q).[197] A T-cell phenotype

Figure 2.3 Monosomy 6; cytogenetics (partial karyotype)

is more frequently associated with del(6q) in general, and particularly with cases with del(6q) as the isolated abnormality. Patients with ALL and del(6q) have a high complete remission rate (83%); however, they have a lower 18-month event-free survival (31% vs 41%) and a higher relapse rate (70% vs 37%) compared with patients without del(6q). Overall, del(6q) as an isolated change identifies a subset of cases with hyperleukocytosis, a T-cell phenotype, and seems to be associated with an unfavorable clinical outcome.[197]

Monosomy 6 has been reported in MDS, AML, chronic myeloproliferative disorders, and B-cell lymphoproliferations (e.g. follicular lymphoma), usually accompanied by complex abnormalities. Figure 2.3 presents with monosomy 6 in follicular lymphoma.

del(7q)/monosomy 7 (–7)

- AML/tAML
- MDS/tMDS
- ALL
- CML (disease progression)
- Chronic myeloproliferative disorders (P. vera, ET, and CIMF)
- Splenic marginal zone B-cell lymphoma
- Low grade B-cell lymphomas including lymphoplasmacytic lymphoma and B-CLL/SLL

Loss of chromosomal material due to deletion of the long arm of chromosome 7 [del(7q)] (Figure 2.4), or loss of one homolog (– 7) (Figure 2.5), is a common finding in all types of myeloid disorders, especially in patients with AML and MDS, and is invariably

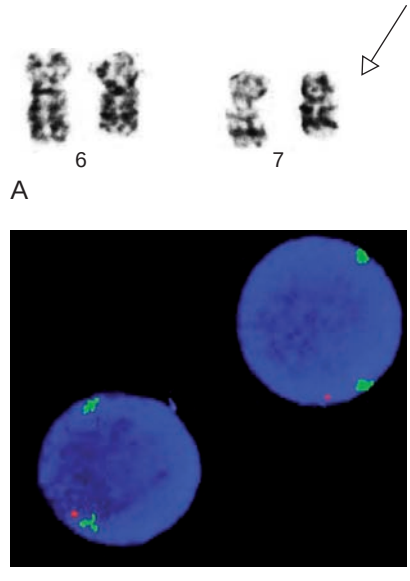


Figure 2.4 Del(7)(q32): A, cytogenetics (partial karyotype); B, FISH (CEP7/D7S522)

associated with a worse response to treatment and a poor prognosis.[198–201] Primary MDS occuring in adults under 50 years often display abnormalities involving chromosome 7 (31%), and almost half of these patients progress to AML with an overall median time to progression of 2 months (range 3 weeks to 3 years).[202] Monosomy 7/del(7q) and monosomy 5/del(5q) represent the most common cytogenetic abnormalities in therapy-related MDS and AML and are strongly associated with exposure to alkylating agents. Monosomy 7 is also reported in myeloid proliferations in children,[203] monosomy 7 being the most common cytogenetic abnormality in pediatric MDS.[204] Monosomy 7 and deletion 7q are rare in childhood AML and represents a heterogeneous group of disorders with additional cytogenetic aberrations having a major prognostic impact.[205] Pediatric AML, MDS, and other myeloproliferations associated with monosomy 7 have a poor prognosis.[206] Children with AML and monosomy 7 have poor disease-free survival when treated by conventional chemotherapy, immunosuppression, or supportive

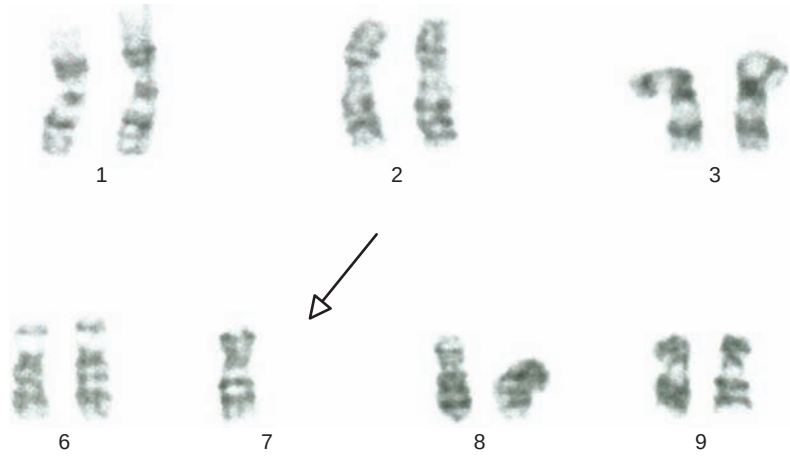


Figure 2.5 Monosomy 7; cytogenetics (partial karyotype)

measures.[207,208] In a series of pediatric AML reported by Hasle et al, cytogenetic aberrations considered favorable in AML [t(8;21)(q22;q22), inv(16)(p13q22), t(15;17)(q22;q21), t(9;11)(p22;q23)] were strongly associated with del(7q) and had a higher 5-year survival rate compared with del(7q) without favorable cytogenetics (75% vs 46%).[205] Patients with −7 and inv(3), −5/del(5q), or +21 had a 5-year survival rate of 5%.[205]

In a series reported by Heerema et al among 1880 children with ALL, 75 (4%) had losses involving chromosome 7, 16 (21%) with monosomy 7, 41 (55%) with losses of 7p (del(7p)), 16 (21%) with losses of 7q (del(7q)), and two (3%) with losses involving both arms.[209] Patients with losses involving chromosome 7 were more likely to be ≥10 years old, National Cancer Institute (NCI) poor risk, and hypodiploid than patients lacking this abnormality.[209] Event-free survival (EFS) and survival of patients with monosomy 7 or del(7p), but not of patients with del(7q), were significantly worse than those of patients lacking these abnormalities (the poorer EFS was maintained after adjustment for a Philadelphia chromosome, NCI risk status, ploidy, or an abnormal 9p).[209]

Deletion of chromosome 7 or del(7q) occurs frequently in both CML and non-CML chronic myeloproliferative disorders (P. vera, essential thrombocythemia, and chronic idiopathic myelofibrosis).[203,210,211] Tripputi et al, analyzed 150 patients with chronic myeloproliferative disorders and found 8 patients with monosomy 7 (cytogenetics) and 4 patients with deletions of the long arm of chromosome 7 (molecular methods).[210] Monosomy 7 and trisomy 8 were the most common clonal chromosomal abnormalities in Ph^- cells in CML patients treated with imatinib.[212,213] In pediatric chronic myeloproliferative disorders, monosomy 7 is associated with a poor prognosis.[203,211]

Rearrangements of 7q (especially 7q deletion) are seen in splenic marginal zone lymphomas, where they are associated with a poor prognosis and risk of large cell transformation.[214–217] Lymphomas with del(7q) often show plasmacytoid features (plasmacytic differentiation).[218,219] Splenic MZL patients with genetic losses [del(7q) or del(17p)] were reported to have a shorter survival than the remaining patients, including those with chromosomal gains.[220] Imbalances on 7q are associated with a shorter survival in patients with Burkitt lymphoma.[221] Among 12 patients with lymphoma and del(7q) reported by Hernandez et al, 11 patients showed small cell morphology (low-grade category), including

B-CLL/SLL, mantle cell lymphoma, and splenic marginal zone B-cell lymphoma, and one patient had diffuse large cell lymphoma.[222] Lymphoplasmacytic features (differentiation) were observed in six out of eleven low-grade lymphomas.[222]

The unbalanced translocation, der(1;7)(q10;p10), is one of the characteristic cytogenetic abnormalities found in MDS and other myeloid neoplasms. In contrast with other –7/7q– cases, where the abnormality tends to be found in one or more partial karyotypes, der(1;7)(q10;p10) represents the abnormality common to all the abnormal clones and usually appears as a sole chromosomal abnormality during the entire clinical course, or if not, is accompanied only by a limited number and variety of additional abnormalities, mostly trisomy 8 and/or loss of 20q.[223] The der(1;7)(q10;p10)-positive MDS cases showed lower blast counts and higher hemoglobin concentrations at diagnosis, slower progression to AML, and significantly better clinical outcome than other –7/7q– cases.[223]

del(9p)

- ALL
- CML, myeloid blast crisis
- Mantle cell lymphoma (MCL)
- DLBCL/post-transplant DLBCL
- T-cell lymphoma (rare cases)

In pediatric ALL, del(9p) belongs to the most common structural rearrangements (17%), followed by t(12;21) (15%), del(6q) (8%), and *MLL* rearrangements (4%).[7] Adult ALL patients with del(9p) have a significantly improved outcome, in contrast to those with Ph chromosome, t(4;11)(q21;q23), t(8;14)(q24.1;q32), or complex karyotype (≥5 chromosomal abnormalities).[224] Although 9p deletions in ALL show considerable variability in both the extent and location, all include the *CDKN2A* locus.[225]

Del(9p) and other structural changes [+3q, +12q, del(6q), del(1p), del(13q), del(10q), del(11q), and del(17p)] may be observed in MCL, including blastoid variant.[226–228] Post-transplant DLBCL shows frequent losses of 6q, 17p, 1p, and 9p.[229] Both *de novo* $CD5^+$ and typical ($CD5^-$) DLBCL may have del(9p).[230,231] Only rare cases of T-cell lymphomas (including anaplastic large cell lymphoma) with del(9p) have been reported.[232,233]

del(9q)

- AML

Del (9q) (Figure 2.6) is a recurrent cytogenetic abnormality in AML.[234] AML with isolated del(9q) is associated with the presence of a single Auer rod, erythroid dysplasia, and granulocytic lineage vacuolation.[234] In a series reported by Peniket et al, the 5-year overall survival for patients with del(9q) was 45% (compared with 35% for the control group), 75% for patients with del(9q) in association with t(8;21), 40% with sole del(9q) (40%), and 26% with del(9q) with other abnormalities.[234] Among AML cases positive for core binding factor (CBF) translocations, loss of sex chromosome, del(9q), and complex abnormalities were more common among patients with t(8;21), while +22 and +21 were more common with inv(16).[235] The overall prevalence of *CEBPA* loss-of-function mutations in AML cases with del(9q), in a noncomplex karyotype was 41%, whereas none of the patients who had a del(9q) in a complex karyotype or together with a t(8;21)

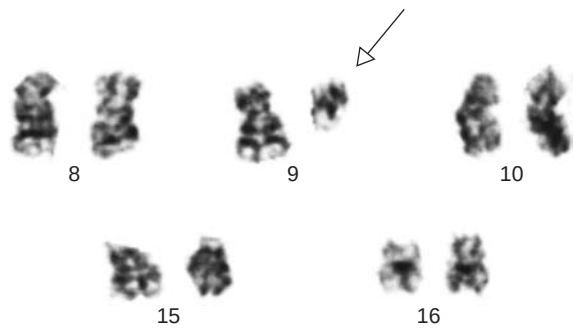


Figure 2.6 Del(9)(q13); cytogenetics (partial karyotype)

demonstrated mutant *CEBPA*.[236] *CEBPA* mutations were reported exclusively in AML and mutated patients preferentially belonged to M1, M2, and M4 FAB subtypes.[237] In the absence of poor prognostic factors, patients with *CEBPA* mutation had a favorable outcome, very similar to that of the t(8;21), inv(16), and t (15; 17) subgroup.[237]

del(11q)

- B-CLL
- MCL
- DLBCL
- AML
- MDS
- ALL
- CMPDs (P. vera, ET, CIMF)
- T-prolymphocytic leukemia (T-PLL)

Structural aberrations involving 11q are among the most common aberrations in a number of hematologic malignancies.[238,239] The 11q contains three genes: *BCL1* (11q13), *ATM* (11q22~23; Figure 2.7), and *MLL* (11q23). Translocations or deletions involving the 11q23 region have been observed in ALL, AML, MDS, T-PLL, and B-cell lymphoproliferations including B-CLL and MCL.

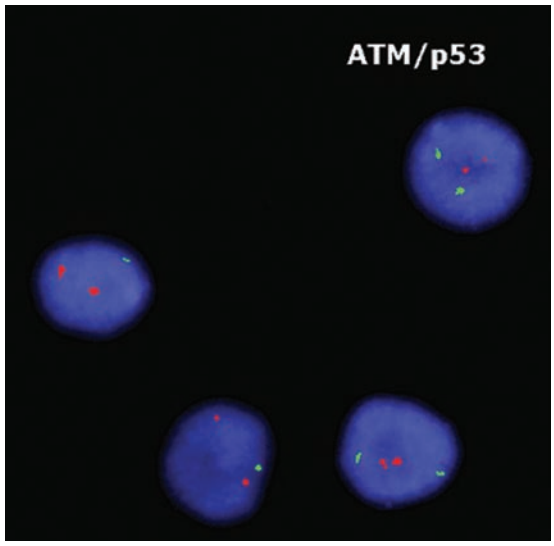


Figure 2.7 Del(11q); FISH

In B-CLL, deletions have been detected in 20–30% of the cases and 11q deletions identify a new clinical subset characterized by younger age, extensive peripheral, abdominal, and mediastinal lymphadenopathy, more advanced clinical stage, more rapid disease progression, and a shorter treatment-free interval.[240] The prognostic effect of 11q deletion on survival strongly depends on the age: in patients less than 55 years old, the median survival time was significantly shorter in the deletion group (64 months vs 209 months), whereas in patients ≥55 years old there was no significant difference (94 months vs 111 months).[240] The high-risk CLL patients characterized by unmutated *IGVH* or high-risk interphase cytogenetics, including either del(17p) or del(11q), appear to have a shorter progression-free survival and overall survival with chemoimmunotherapy.[241] The cDNA array confirmed the prognostic impact of 11q23 deletion in B-CLL.[242]

Apart from B-CLL/SLL, del(11q) can be observed in other non-Hodgkin's lymphomas, mostly mantle cell lymphoma,[226,243] and DLBCL,[239] but also occasionally in marginal zone B-cell lymphoma.[244] Although there are no specific secondary chromosomal changes in MCL, 11q22–23 anomalies as well as 1p21–31 deletions and 8p translocations are often observed.[245] In the case of DLBCL with del(11q), the possibility of Richter's transformation of B-CLL should be excluded.

Del(11q), +8, −7/del(7q), and del(20q) are seen often in non-CML chronic myeloproliferative disorders (P. vera, ET, CIMF).

Chromosomal abnormalities in MDS are predominantly characterized by partial/total chromosomal losses or chromosomal gains, including mainly del(5q)/−5, del(7q)/−7, del(11q), del(12p), del(20q), −Y, and +8.[21,26,246] Del(11q) in MDS is often associated with sideroblastosis (ringed sideroblasts are also seen in Xq13 abnormalities). In the series of 968 MDS patients reported by Sole et al, complex

karyotype, −7/del(7q), and i(17q) were associated with poor prognosis; normal karyotype, loss of Y chromosome, del(11q), del(12p), and del(20q) as single alterations had a good prognosis; and rearrangements of 3q21q26, trisomy 8, trisomy 9, translocations of 11q, and del(17p) were associated with an intermediate prognosis.[247]

Although gains or rearrangements of 11q are often seen in multiple myeloma, no losses of 11q are observed.[248] Campbell et al, described two acute promyelocytic leukemia patients, treated with all-*trans* retinoic acid and combination chemotherapy, who acquired a deletion of 11q within 12 months of diagnosis.[249] Del(11q) has been reported in T-PLL.[238]

del(12p)

- CMPDs (P. vera, ET, CIMF)
- ALL
- MDS/tMDS
- AML/tAML
- DLBCL, post-transplant
- Non-Hodgkin lymphomas

Unbalanced translocations as well as interstitial deletions of the short arm of chromosome 12 [del(12p)] are found as recuring chromosomal changes in a broad spectrum of hematopoietic malignancies including ALL, MDS, AML, chronic myeloproliferative disorders (CMPD), therapy-related MDS or AML (tMDS/tAML), non-Hodgkin's lymphoma, and occasionally other hematopoietic malignancies.[26,115,247,250–253] The cytogenetic abnormalities of 12p in hematologic malignancies result in different molecular changes: deletions of *KIP1*, amplifications of *CCND2*, and structural rearrangements or LOH of *ETV6* (*TEL*).[115,225,254,255] Rearrangement and/or deletion of *ETV6* may occur in up to 70% of patients with abnormalities of 12p.[251]

Heerema et al, showed cytogenetically detectable 12p abnormalities among 1880 children with newly diagnosed ALL in 174 cases (9%): the majority of cases had a balanced translocation, a del(12p), or an add(12p).[252] Del(12p) present as a sole abnormality confers a good prognosis in ALL.[256] The 12p aberrations and near tetraploidy are more common in *ETV6*/*RUNX1*$^{+}$ pediatric ALL.[257] Some rearrangements of 12p which have been described previously as deletions or unbalanced translocations may, in fact, represent 12p13 translocations accompanied by an interstitial deletion,[258] e.g. translocation to 21q [t(12;21)(p13;q22)][259] or 17p [t(12;17)(p13;p12-p13)][260] by FISH analysis.

Abnormalities of the short arm of chromosome 12 (12p) are found in about 5% of AMLs and are often reported in secondary leukemias, especially after prior mutagenic exposure. Deletion 12p occurs frequently in MDS.[21,26,247,261] Del(12p) without other abnormalities is associated with a favorable prognosis in MDS.[247]

Chromosome 12p is the most frequent target of deletions among posttransplant DLBCL.[229]

del(13q)/monosomy 13 (–13)

- B-CLL
- MCL
- B-cell lymphomas, high grade
- Plasma cell myeloma/MGUS
- T-cell lymphomas
- CMPDs (P. vera, ET, CIMF)

Deletions of the long arm of chromosome 13 occur in myeloid and lymphoid tumors, with myeloid breakpoints more often involving the *RB1* gene locus, while more lymphoid breakpoints fall telomeric of *RB1*. The *RB1* locus at 13q14 can be detected by FISH using an RB probe [other FISH probes used to identify del(13q) include D13S25 and D13S319]. The 13q deletion occurs in different types of non-Hodgkin's lymphomas, e.g. B-CLL (Figure 2.8), MCL, a subset of high-grade lymphomas, and in plasma cell neoplasms.[262] Cuneo et al, reported del(13q) in a low-grade lymphoma, MCL, and high-grade lymphoma and found a statistically significant association between 13q−, the presence of splenomegaly and blood involvement, a lower probability of attaining complete remission,

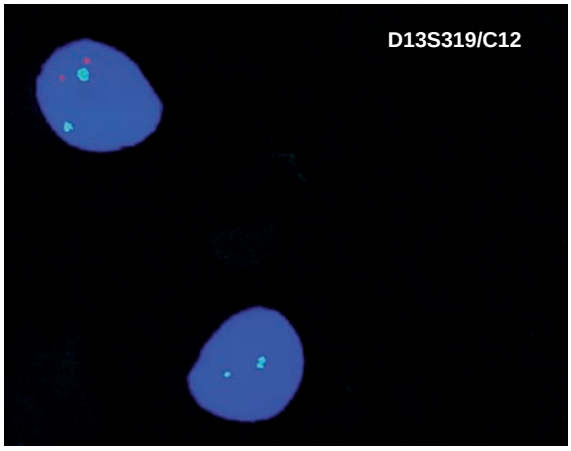


Figure 2.8 Del(13q); FISH

and shorter survival.[262] Loss of 13q in cutaneous T-cell lymphomas is associated with a significantly shorter survival.[192]

In B-CLL deletion of 13q is the most common abnormality (FISH studies) followed by deletions of 11q, trisomy 12, and deletions of 17p.[263] The 13q deletions in B-CLL are associated with the longest (92 months) survival.[264] Coexistence of trisomy 12 and 13q14 deletion is present in 17.5% of patients.[265] In this group, deletion 13q14 was the prevalent clone, with percentages 25–35% higher than those observed for trisomy 12, suggesting clonal evolution.[265]

Monosomy 13 is one of the most frequent chromosomal abnormalities in multiple myeloma (MM; Figure 2.9).[266,267] Zojer et al analyzed del(13q) in newly diagnosed MM and reported monoallelic deletions of the retinoblastoma-1 (*RB1*) gene and the D13S319 locus in 48 of 104 patients (46.2%) and in 28 of 72 (38.9%) patients, respectively.[268] Monoallelic deletions of *RB1* appear to be a frequent and early event in the pathogenesis of MM, without an obvious relevance for disease progression.[269] Retinoblastoma gene deletion as a sole abnormality does not lead to a shorter survival of MM patients.[270] Plasma cells with a deletion of at least one of the 13q14 loci can also be seen in patients with MGUS (~45%).[271] The complete or partial deletion of chromosome 13 or translocations involving 13q by conventional cytogenetics in MM is associated with poor outcome even with high-dose treatment and autologous stem cell transplantation.[267,272–275] The del(13q) is detected more frequently by FISH than by metaphase cytogenetic. The overall survival of patients with monosomy 13/del(13q) by cytogenetics (median: 35.2 months) and del(13q) by FISH only (median: 33.2 months) is not significantly different and is shorter than in patients with diploid chromosome 13 by either technique.[276] The latter is associated with prolonged survival (65.6 months).[276] Figure 2.10 shows monosomy 13 using a FISH probe.

Rearrangements of 13q occur in non-CML CMPDs, including P. vera (~13%), ET, and especially chronic idiopathic myelofibrosis.[277–284]

del(17p)/monosomy 17

- B-cell lymphomas
- T-cell lymphomas
- B-CLL
- MDS
- AML
- CML (disease progression/clonal evolution)

Chromosome 17p is the site for *p53* (*TP53; see below*). Loss of the short arm of chromosome 17 is associated with a *p53* mutation on the remaining allele in several hematopoietic malignancies. Deletion of 17p (Figure 2.11) is associated with a poorer prognosis in many hematopoietic malignancies. It is usually associated with other chromosomal rearrangements, most often complex karyotypes.[161,285,286] AML patients > 60 years old with abnormalities of 17p, 5q–, –7, +8, and complex aberrations had a more adverse prognosis when compared with patients with a normal karyotype.[161] MDS with del(17p) may be associated with dysgranulopoiesis (pseudo Pelger–Huet nuclei), small vacuoles in the cytoplasm of neutrophils, and a poor prognosis.[287] In patients with MDS and AML, the presence

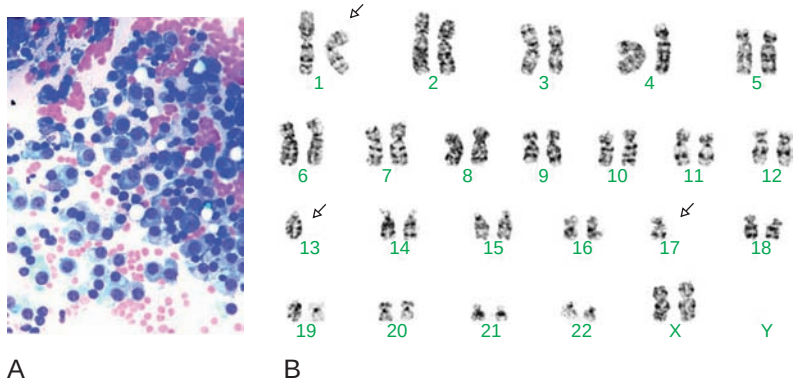


Figure 2.9 Multiple myeloma with complex karyotype including monosomy 13 and monosomy 17. A, cytomorphology; B, cytogenetics

of *MLL* copy gains/amplifications or losses of the short arm of chromosome 17, in association with 5/5q, has been found to be an indicator of an extremely poor prognosis.[288,289] However, in the series of MDS patients reported by Sole et al, MDS with del(17p) as well as rearrangements of 3q21q26, trisomy 8, trisomy 9, and translocations of 11q had an intermediate prognosis and those with complex karyotype, –7/7q–, and i(17q) had a poor prognosis.[247] The *p53* (*TP53*) mutations are found in the majority of MDS patients with 17p deletion.

Aberrations of chromosome 17 are observed in CML undergoing clonal evolution, accelerated phase (AP), or blast phase (BP; blast crisis).[290–292]

The del(17p) is common in follicular lymphoma (~23%) and it is associated with an adverse clinical outcome.[293,294] Other lymphomas which may be positive for del(17p) include MCL marginal zone B-cell lymphoma, DLBCL, post-transplant lymphoproliferative disorders, and T-cell lymphomas (including the NK-cell type).[220,226,229,295–299] Del(17p) is often associated with a poorer response to chemotherapy in

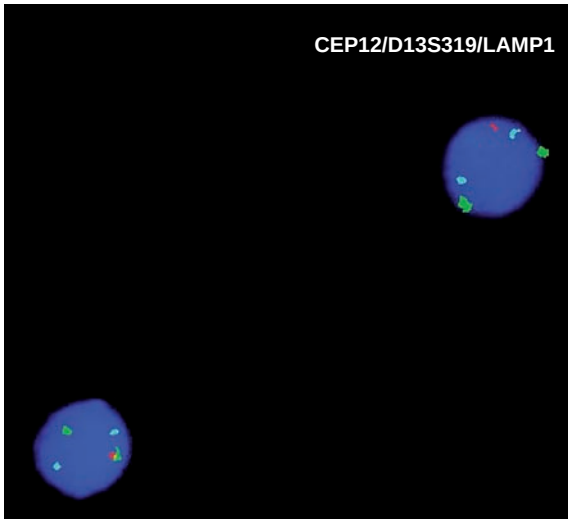


Figure 2.10 Deletion 13; FISH

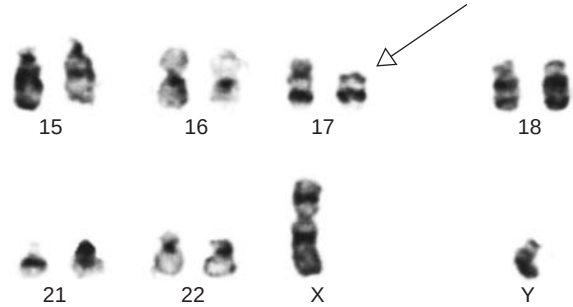


Figure 2.11 Del(17p)(11.1); cytogenetics (partial karyotype)

lymphomas. Loss of 8p and 17p and gain of 3q21, 6p, 7p, and 8q23 are associated with a high S-phase fraction in non-Hodgkin's lymphoma.[300]

In B-CLL, 17p deletion is associated with a lack of *IGVH* mutations and a poor prognosis.[301] Abnormal *p53* predicts for shorter survival, is more common in refractory B-CLL, and correlates with an aggressive disease and transformation. B-CLL patients with del(17p) do not respond to rituximab.[302]

Drach et al, using FISH, reported deletions of *p53* (which were predominantly monoallelic) in ~33% and ~55% of MM patients with newly diagnosed and relapsed disease, respectively.[303] In a recent study, Avet-Loiseau et al, identified del(17p) in 11% of MM patients.[304] MM patients with deletions of chromosome 17/*p53* (Figure 2.9) have a poor prognosis, but trisomy 17 is associated with prolonged survival.[112,303–305] A multivariate analysis on 513 patients reported by Avet-Loiseau et al, showed that genomic aberrations t(4;14) and del(17p), together with β_2-microglobulin level are important independent predictors of survival.[304] Also Gertz et al, showed that time to progression and overall survival are significantly shorter for patients with t(4;14) and del(17)(p13.1).[306] Chromosome 17p13.1 deletions are frequent in multiple myeloma involving the central nervous system.[307]

del(20q)

- AML
- MDS
- CMPDs (P. vera, ET, CIMF)
- Angioimmunoblastic T-cell lymphoma (rare cases)
- Waldenström macroglobulinemia (rare cases)
- ALL (rare cases)
- CML (rare cases)

Deletions of the long arm of chromosome 20 [del(20q)] are typical for myeloid disorders, and occur in AML, MDS, and chronic myeloproliferative disorders (with or without hypereosinophilia), but have also been reported occasionally in lymphomas (including angioimmunoblastic T-cell lymphoma and Waldenström macroglobulinemia) and Ph chromosome$^+$ ALL.[13,21,107,308–318]

The del(20q) is known to be a favorable prognostic factor in MDS when it is the sole change. In a subset of patients, del(20q) is accompanied by other chromosomal abnormalities. The appearance of del(20q) as an additional change, even if only as a minor clone, indicates disease progression and a poor prognosis.[308] Morphologically, myeloid disorders with del(20q) are characterized by prominent dyserythropoiesis and dysmegakaryopoiesis.[315]

Del(20q) is often observed in non-CML CMPDs: P. vera, essential thrombocythemia (ET), and chronic idiopathic myelofibrosis (CIMF).[319] Najfeld et al, using conventional cytogenetics reported del(20q) in ~19% of P. vera patients.[278] Recurrent abnormalities of chromosome 20 including partial deletion of its long arm have been reported in chronic neutrophilic leukemia (CNL).[320,321] Rare cases of CML may be positive for del(20q), especially during disease progression.[322]

Inversions

Chromosomal inversions are rare and account for ~0.5% of chromosomal aberrations in hematologic tumors. An inversion consists of two breaks in one chromosome with the area between the breaks being inverted and reinstated. Most common inversions include paracentric inv(3)(q21q26) in AML, a pericentric inv(16p13q22) in acute myelomonocytic leukemia with eosinophilia (AML-M4Eo), and paracentric inv(14)(q11q32) in T-PLL. A pericentric inversion involves part of the short and the long arms as well as the centromere; a paracentric inversion does not include the centromere.

inv(3)/t(3;3)

- MDS/tMDS
- AML/tAML

Patients with MDS and AML, especially secondary AML or AML with myelodysplasia and prominent

dyspoiesis in megakaryocytic lineage (dysmegakaryopoiesis), often have cytogenetic aberration of 3q21 and 3q26 involving the paracentric inversion [inv(3)(q21q26)] or a reciprocal translocation [t(3;3)(q21;q26)]. These abnormalities frequently cause inappropriate expression of the *EVI1* gene at 3q26. Inversion of chromosome 3 (Figure 2.12) or t(3;3) is associated with a poor prognosis in AML.[323]

inv(7)(p15q34)/t(7;7)(p15;q34)

- T-ALL

Inv(7)(p15q34) and t(7;7)(p15;q34) involve the T-cell receptor β gene (*TCRB*) on 7q34 and the *HOXA* gene locus on 7p15, and are identified in 3.3–5% of patients with precursor T-lymphoblastic leukemia (T-ALL).[324–327] Other molecular and/or cytogenetic aberrations frequently found in subtypes of T-ALL (*SIL/TAL1*, *CALM/AF10*, *HOX11*, *HOX11L2*) were not detected in the *TCRB/HOXA* rearranged cases except for deletion 9p21 and *NOTCH1* activating mutations, which were present in 64 and 67%, respectively.[324] Clinical findings are not significantly different from T-ALL in general.[324,328] Most T-ALL cases with *TCRB/ HOXA* rearrangement are $CD2^-$, $CD4^+$, and $CD8^-$.

inv(11)(p15q22)

- MDS/tMDS
- AML/tAML
- CML, disease progression/clonal evolution

The inv(11)(p15q22) is observed in *de novo* or therapy-related AML (tAML) and MDS (tMDS), and in patients with imatinib-resistant CML disease undergoing clonal evolution.[329–331] The inv(11)(p15q22) creates the fusion between the *NUP98* and *DDX10* genes.

inv(12)

- MDS
- AML
- ALL
- CMPDs
- Lymphomas (B-CLL, MCL, T-cell lymphomas)
- CML, blast crisis

Aberration of chromosome 12 often involves the *ETV6* gene (previously *TEL*). In contrast to rearrangements of the short arm of chromosome 12, which are commonly seen in various hematologic malignancies,[332] inversions involving chromosome 12 are rare.[333]

In contrast to 12p aberrations, abnormalities involving the long arm of chromosome 12 are rare and are seen most often in MDS.[261] Inv(12) can occur as a sole abnormality, but is usually part of a complex karyotype. Welborn et al, reported a group of 46 patients with a variety of myeloid and lymphoid malignancies with a pericentric and paracentric inversion of chromosome 12: AML (14 cases), blast phase (BP; blast crisis) of CML (1 case), MDS (11 cases), CMML (2 cases), chronic myeloproliferative disorders (5 cases), ALL (6 cases), B-CLL (2 cases), MCL (1 case), and T-cell lymphoma (1 case).[333] The majority of AML in that report comprised the AML-M2 subtype.

The presence of inv(12) is associated with a poor prognosis in AML,[334–337] and an intermediate prognosis in ALL.[338] The majority of patients with

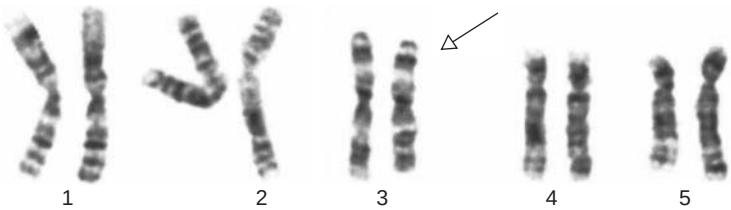


Figure 2.12 Inversion 3; cytogenetics (partial karyotype)

inv(12) reported by Welborn et al, had complex karyotypic changes, and many were refractory to chemotherapy and had a short overall survival.[333]

inv(14)

- T-PLL

Abnormalities of chromosome 14 occur in T-PLL, an aggressive lymphoproliferative disorder of mature T-cells. Figure 2.13 presents the inversion of chromosome 14 in T-PLL. In a series reported by Maljaei et al, 71% had inv(14) (q11q32)/t(14;14)(q11;q32) and 4/21 patients had abnormalities involving Xq28 (MTCP-1 locus) resulting from t(X;14)(q28;q11) or t(X;7)(q28;q35).[339]

inv(16) or t(16;16)

- AML, especially AML-M4Eo
- CML

Positional cloning revealed the breakpoint of inv(16) or t(16;16) to lie within the introns of *CBFβ* and *MYH11*.[340] *CBFβ* at 16q22 encodes the β-subunit of the core binding factor (CBF) and *MYH11* at 16q13 encodes the smooth muscle myosin heavy chain (SMMHC). CBF is a family of transcription factors containing a common β-subunit and one of three CBFα subunits, CBFα1, *RUNX1* (also known as *AML1* or *CBFα2)*, and *CBFα3*, all of which encode the so-called runt domain. The fusion gene (*CBFβ/SMMHC*) impairs hematopoietic differentiation typical for AML by disrupting the normal transcription factor activity of CBF. In addition, nearly 70% of patients with inv(16)$^+$ AML are known to possess mutually exclusive mutations of the receptor tyrosine kinases, c-*KIT* and *FLT3*, as well as *RAS* genes.

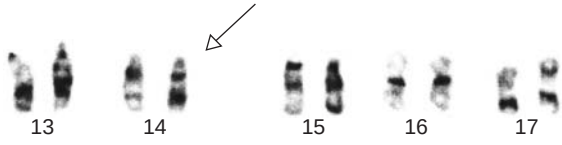


Figure 2.13 Inversion 14; cytogenetics (partial karyotype)

Abnormalities of chromosome 16 in AML have been associated with high complete remission and survival rates, and a favorable prognosis.[323,341] Inversion (16)(p13.1q22), t(16;16)(p13.1;q22), and del(16)(q22) are nonrandom abnormalities associated with acute myelomonocytic leukemia with eosinophilia (AML-M4Eo), and rarely with other subsets of AML. The t(16;16) or inv(16) (Figure 2.14) results in the fusion of *MYH11* at 16p13 (SMMHC) with part of the *CBFβ* gene at 16q22. The prognosis of AML with del(16q) differs from that in AML with inv(16)/t(16;16).[342] Additional karyotypic abnormalities may be present in up to 50% of AML with t(16;16)/inv(16) and most often include trisomy 8, 21, and 22. These additional karyotypic changes do not influence the overall outcome compared to patients with sole chromosome 16 aberrations.[323,342] The co-existence of t(9;22) and inv(16) in CML appears to correlate with a more rapid transformation into the blast phase (BP).[343]

The inv(16) may be difficult to detect by classic cytogenetics studies, partly due to the fact that changes are subtle and partly due to technically suboptimal metaphases. Other methodologies available for detection of inv(16)(p13q22) include FISH (dual-fusion probes or the break-apart probe) and RT-PCR. Rao et al applied the molecular cytogenetic technique of chromosome painting using chromosome 16 p-arm paint and showed pericentric inversion within chromosome 16 even in poor-quality metaphase spreads.[344]

Isochromosomes

i(3)

- Persistent polyclonal B-cell lymphocytosis

Persistent polyclonal B-cell lymphocytosis (PPBL) is diagnosed predominantly in women, usually cigarette smokers, characterized by an increase in the

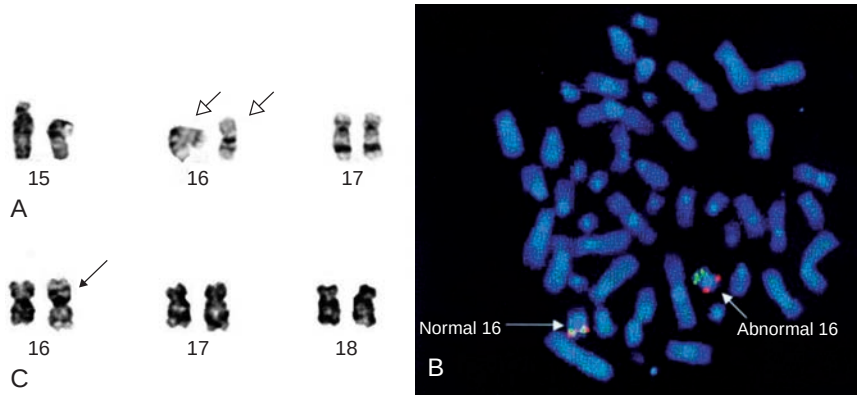


Figure 2.14 The translocation t(16;16) (A, cytogenetics; B, FISH) and inversion 16 (C cytogenetics)

number of polyclonal B lymphocytes, with *IGH-BCL2* gene rearrangements and 3q+/i(3)(q10), or trisomy 3 within a significant proportion of B cells.

i(7q)

- Hepatosplenic γδ T-cell lymphoma

The isochromosome 7q is frequently found in hepatosplenic γδ T-cell lymphoma.[345–348] The prognosis of this lymphoma is poor with a median survival time of 16 months.[345]

i(17)

- CML/CML in blast crisis
- Other hematologic malignancies

An isochromosome of the long arm of chromosome 17 is the most frequent genetic abnormality observed during the disease progression of CML, and has been described as the sole anomaly in various other hematologic malignancies.[349]

Translocations

t(1;3)

- tMDS/tAML/AML
- ALL
- MDS

Chromosome bands 1p36 and 3p21 are known to be recuring breakpoints in therapy-related leukemias and MDS. Sato et al identified recuring t(1;3)(p36;p21) translocation in eight patients with various hematologic malignancies: three patients with ALL, one with CML in the accelerated phase, two with MDS, and two with AML (five patients had a history of chemotherapy).[350]

t(1;4)

- Hepatosplenic γδ T-cell lymphoma

The t(1;4), along with isochromosome 7q and trisomy 8, is reported in hepatosplenic γδ T-cell lymphoma.[351]

t(1;6)

- B-CLL

Translocation t(1;6) (Figure 2.15) occurs rarely in B-CLL and is associated with unmutated *IGVH* and advanced clinical stage.[352]

t(1;14)

- Marginal zone B-cell lymphoma (MALT type)

A few recurrent translocations have been associated with an extranodal marginal zone lymphoma of

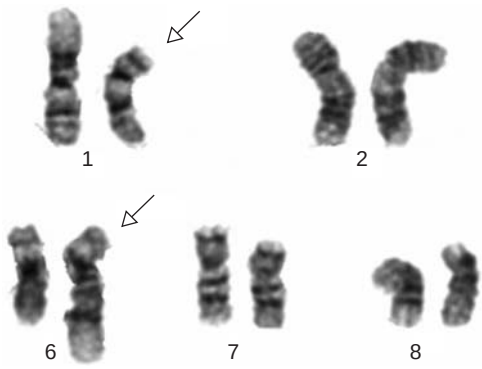


Figure 2.15 The translocation t(1;6); cytogenetics (partial karyotype)

the MALT type, including t(11;18)(q21;q21) and t(1;14)(p22;q32). The t(1;14)(p22;q32) leads to overexpression of *BCL10*.[353,354] Through t(1;14)(p22;q32), the *BCL10* gene is entirely transfered to the *IGH* gene, resulting in its overexpression. Wild-type *BCL10* is implicated in the activation of NF-κB. In normal tissue bcl-10 is localized in the cytoplasm, but a subset of MALT lymphomas with or without the t(1;14) translocation shows strong bcl-10 expression in both the nucleus and cytoplasm;[355,356] the bcl-10 expression is stronger in translocation-positive tumors, however. *BCL10* mutations are rare in gastric MALT-type lymphomas and are not related to the aberrant nuclear expression of bcl-10. Abnormal nuclear expression of bcl-10 and the t(11;18) translocation tend to appear together, operate in a common mechanism involving NF-κB-mediated inhibition of apoptosis, and may be associated with advanced MALT lymphomas.[357,358] *API2/MALT1* fusion is associated with aberrant nuclear bcl-10 expression. The translocation t(1;14) is seen most often in pulmonary and gastric MALT. In contrast to t(11;18), MALT lymphomas with t(1;14) often show other chromosomal aberrations, such as trisomies 3, 12, and 18.[359] The t(1;14)-positive cases, similarly to t(11;18) cases, are resistant to *H. pylori* eradication therapy.

t(1;19)

- B-ALL

Approximately 6% of pediatric patients with precursor B-lymphoblastic leukemia (B-ALL; more often in the pre-B category) harbor a rearrangement involving the gene regions of *PBX1* on 1q23 (belonging to the category of a homeobox gene; *HOX*) and *E2A* on 19p13.3, which can be visualized cytogenetically either as a der(19)t(1;19)(q23;p13.3) or the less common balanced t(1;19)(q23;p13.3). A dual-fusion FISH probe is also available.[360] The t(1;19) translocation occurs in B-ALL. The blasts are often positive for CD10, CD19, TdT, and CD22 and negative for CD20 and CD34. The t(1;19)(q23;q13) has been associated with an adverse prognosis in children with B-ALL.[95,361] However, with recent more intensive chemotherapy regimens, the t(1;19) did not correlate with inferior outcome, suggesting that effective treatment can offset the negative impact of chromosomal rearrangements in cases of childhood B-ALL.[97] Cases of adult ALL carrying the t(1;19)(q23;p13) are characterized by an aggressive clinical course and a short survival.[362]

t(1;22)

- Acute megakaryoblastic leukemia

The t(1;22)(p13;q13) is present in acute megakaryoblastic leukemia (AML-M7) in infants and children (<6 months old) and is associated with extensive infiltration of abdominal organs, poor response to therapy, and a dismal prognosis.[363] The t(1;22) leads to the fusion of the *MAL* (megakaryocytic acute leukemia) gene on chromosome 22 and the *OTT* (One Twenty Two) gene on chromosome 1.

t(2;3)

- AML

The t(2;3) is a recurrent translocation having an approximate 0.5% incidence in adult AML (either as the sole abnormality or accompanying additional

aberrations) and involves the 5′ region of the *EVIl* gene at 3q26, and the *BCL11A*, the *THADA* gene, or other regions at 2p16.1-21 (cryptic deletions distal to the 3q26 breakpoint may occur in some cases).[364] The clinical outcome in these cases is poor.

t(2;5)

- Anaplastic large cell lymphoma

The t(2;5)(p23;q35) is the specific aberration occurring in anaplastic large cell lymphoma (ALCL) and is associated with *NPM/ALK* fusion (*see below*), which can be detected on the molecular (FISH, PCR) and immunohistochemical levels. FISH appears to be more sensitive, with a higher rate of detection when compared to RT-PCR.[365] While t(2;5)$^{NPM/ALK}$ represents the majority of ALK rearrangements (75%), a subset of cases has variant translocations which include t(1;2) [*TPM3* gene on 1q25], t(2;3) [*TFG* gene on 3q35], t(2;2) [*ATIC* gene on 2q25], and t(2;17) [*CLTC* gene on 17q23].[366–369] Positive nuclear and/or nucleolar immunostaining for ALK-1 (Figure 2.16) usually indicates t(2;5), whereas cytoplasmic staining suggests variant translocations. Figure 2.17 shows *ALK* translocation by FISH analysis.

The t(2;5)$^+$ ALCL is characterized by a younger age of onset, lower serum LDH, better performance status, good response to chemotherapy, and favorable prognosis. The prognosis of ALCL without t(2;5) is unfavorable, similarly to peripheral T-cell lymphoma, unspecified.

A rare variant of DLBCL may express ALK by immunophenotyping (cytoplasmic localization) without the t(2;5) translocation. This protein appears to be a full-length ALK receptor (and not a chimeric molecule characteristic for ALCL).[370,371] The majority of ALK$^+$ DLBCL display t(2;17)(p23;q23) involving the clathrin gene (*CLTC*).[371]

t(2;8)

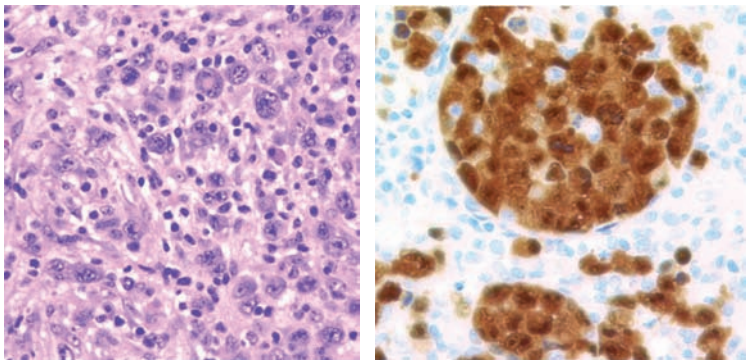
- Burkitt lymphoma
- B-ALL

The t(2;8)(p21;q24) occurs in a subset of Burkitt lymphoma and sporadically in precursor B-ALL.

t(2;17)

- DLBCL

Gascoyne et al described six cases of ALK$^+$ DLBCL characterized by a simple or complex t(2;17)(p23;q23) involving the clathrin gene (*CLTC*) at chromosome band 17q23 and the *ALK* gene at chromosome 2p23.[371] Similar findings were reported by DePaepe et al[372] (three cases) and Stachurski et al[373] (one case).[372,373] DLBCLs with t(2;17) display granular cytoplasmic expression of ALK by



A B

Figure 2.16 Anaplastic large cell lymphoma, ALK+. A, histology section shows highly pleomorphic large cell infiltrate. B, immunohistochemistry shows strong nuclear, nucleolar, and cytoplasmic staining with ALK. This immunophenotypic pattern is typical for t(2;5)

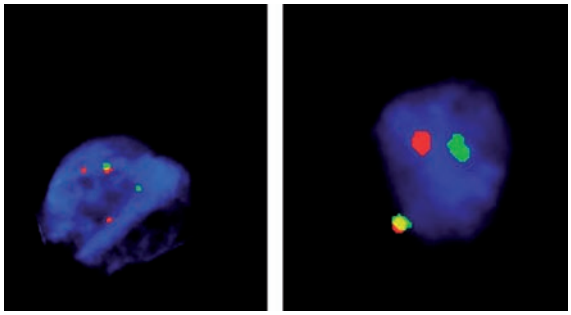


Figure 2.17 ALK translocation; FISH

immunohistochemistry (in contrast to nuclear/nucleolar staining in ALCL associated with *NPM/ALK* fusion). DLBCLs with ALK expression are aggressive.

t(2;19)

- ALCL

The translocation t(2;19)(p23;q13.1) was identified as a new variant of t(2;5)(p23;q35) in a case of Ki-1$^+$ ALCL with a null cell phenotype.[12]

t(3;3)(q26;q21)/inv(3)

- AML
- MDS

The t(3;3)/inv(3) is seen in AML (except the M3 type) and MDS and is often associated with marked thrombocytosis, trilineage dysplasia, and poor outcome.[374–376]

t(3;5)

- MDS
- AML

The t(3;5)(q25;q35) is an uncommon karyotypic aberration in AML and occurs in MDS and AML following MDS.[377,378] With the exception of APL (AML-M3), t(3;5) has been reported in every other subtype of AML, being most frequently associated with AML M6.[377]

t(3;8)(q26;q24)

- AML/tAML
- MDS/tMDS
- CML, blast crisis

The most common recurrent translocations involving 3q26 are t(3;12)(q26;p13), t(3;21)(q26;q22), t(3;3)(q21;q26), t(2;3)(p15~23;q26), t(3;7)(q26;q21), and t(3;8)(q26;q24).[379–382] The t(3;8)(q26;q24) is a recurrent translocation associated with therapy-related MDS/AML or *de novo* AML, and is frequently associated with monosomy 7, but may occur as a sole abnormality or with trisomy 13.[382]

t(3;8)(q27;q24.1)

- DLBCL

The translocation t(3;8)(q27;q24.1) simultaneously involving both *BCL6* and c-*MYC* without any involvement of the *IG* locus has been described in DLBCL.[383]

t(3;14)

- DLBCL

The t(3;14)(q27;q32) occurs in 7–14% of DLBCL cases.[117,384–386]. Au et al reported three patients (one was HIV$^+$) with nonclonal t(3;14) or t(3;22); their lymphadenopathy resolved spontaneously, and none had progressed to lymphoma at 4–6 years of follow-up.[387]

t(3;21)

- AML
- CML, blast crisis
- ALL

The t(3;21) involves *RUNX1* on 21q and *EVI1* on 3q26.[388] *RUNX1* corresponds to *CBFA2*, and encodes one of the DNA-binding subunits of the enhancer core binding factor (*CBF).*[389] The mechanism by which *EVI1* operates in the transformation

of hematopoietic cells is not known, but it upregulates cell proliferation, impairs cell differentiation, and induces cell transformation.[390] Two recuring chromosomal translocations involving the *RUNX1 (*also known as *AML1)* gene associated with myeloid leukemias are the t(8;21)(q22;q22) and the t(3;21)(q26;q22) (Figure 2.18). The latter occurs in chemotherapy-related myeloid leukemias and MDS (primarily following treatment with topoisomerase II inhibitors) and occasionally in the blast phase (BP; blast crisis) of CML.[389,391] *De novo* AMLs with t(3;21) are rare. Bacher et al described $t(8;21)^{+}$ AML which was followed by a secondary therapy -related AML with t(3;21)(q26;q22).[392] Both $t(8;21)^{RUNX1/ETO}$ and t(3;21), as well as t(16;16)/inv(16), disrupt core binding factor (CBF), a hematopoietic transcription factor, and therefore belong to so-called CBF^{+} AMLs. AML with either t(8;21) or t(16;16)/inv(16) are generally associated with a good prognosis.

t(3;22)

The nonclonal t(3;22) may occur in reactive lymph nodes.[387]

t(4;11)

- ALL

The translocations involving the *MLL* gene (also known as the *ALL1* gene) on 11q23 are common in leukemia and include several different partners: 1p32, 4q21 and 19p13.3 in ALL and 1q21, 2q21, 6q27, 9p22, 10p11, 17q25, 19p13.3 and 19p13.1 in AML [t(4;11) being the most common].[64,67,393,394] Those leukemias are characterized by higher WBC, but only those with t(4;11) show a female predominance. The t(4;11) occurs usually in early pre-B-ALL (pro-B-ALL), whereas other 11q23 translocations may occur in both B- and T-ALL. The t(4;11) translocation leads to the fusion of the *MLL* and *AF4* genes. B-ALL with t(4;11) is associated

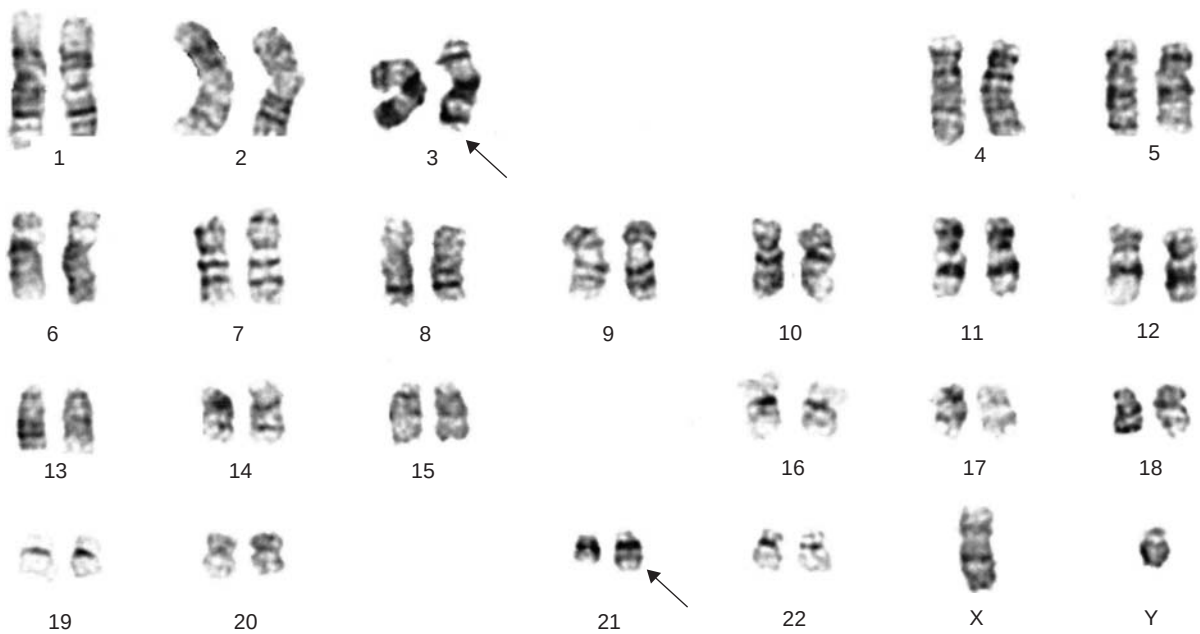


Figure 2.18 The translocation t(3;21), cytogenetics

with a poor prognosis.[64,224,395,396] Similarly to patients with Ph chromosome$^+$ ALL, the presence of t(4;11) is associated with an increased percentage of MRD and, therefore, an increased risk of relapse. The absence of the *MLL/AF4* fusion gene correlates with a significantly better clinical outcome after intensive polychemotherapy treatment without hematopoietic stem cell transplantation.[397] In a series reported by Moorman et al the majority of patients with t(4;11) relapsed within a year of diagnosis, with a median time to relapse of 6.5 months.[224] Adult patients with other *MLL*/11q23 translocations did not have a significantly inferior outcome compared with other Ph chromosome$^-$ patients.[224]

t(4;14)

- Plasma cell myeloma (MM)

The t(4;14)(p16;q32) translocation involves the subtelomeric regions of chromosome arms 4p and 14q, is karyotypically silent, and causes overexpression of *FGFR3* and *MMSET.*[398] The t(4;14)*$^{FGFR3/IGH}$* is detected most often by interphase FISH, but can be detected also by metaphase FISH, RT-PCR with capillary electrophoresis, gene expression profiling (microarray assay), and immunohistochemistry. The t(4;14)*$^{FGFR3/IGH}$* is found in approximately 15% of patients with MM,[398–400] and is much less common (~3%) in MGUS patients.[401–404] Patients with t(4;14) have a poor prognosis with significantly shorter progression-free (median 9.9 vs 25.8 months) and overall survival (median 18.3 vs 48.1 months) than patients without this translocation despite intensive chemotherapy and autologous stem cell transplants.[270,306,400,405,406] There is no association between t(4;14) and other biologic parameters, such as age, β_2-microglobulin, serum creatinine and hemoglobin, or percentage of bone marrow plasma cells. The strong correlation between t(4;14) and the chromosome 13 deletion makes it difficult to determine which factor plays a more important role in prognosis. Multivariate analysis adjusted for −13 suggests an independent prognostic significance of t(4;14).[406, 407] In the series reported by Gutierrez et al, retinoblastoma deletion as a sole abnormality did not lead to a shortening in the survival of MM patients, whereas t(4;14) confered the worst prognosis in MM patients treated with high-dose chemotherapy.[270]

t(5;10)(q22;q24)

- ALL

The t(5;10)(q22;q24) translocation was described in adult patients with B-ALL (FISH and Southern blot hybridization studies have eliminated likely involvement of the *APC* and *MCC* genes on chromosome 5, and the *PAX2*, *TLX1*, and *NFKB2* genes on chromosome 10).[408]

t(5;12)

- Chronic myelomonocytic leukemia (CMML)
- CMPDs (non-CML) with eosinophilia
- Chronic eosinophilic leukemia (CEL)

Chromosome 5 is a site for platelet-derived growth factor receptor β (*PDGFRB*). The t(5;12) (Figure 2.19) leads to the disruption of *PDGFRB* in which the 5′ end of *ETV6* (earlier known as *TEL*) is juxtaposed to the 3′ end of *PDGFRB*. *PDGFRB* is disrupted by other translocations, and to date, four additional partner genes have been reported (*H4*, *HIP1*, *CEV14,* and *Rab5*). CMPDs associated with t(5;12) are infrequent and represent a minority of patients within the *BCR*/*ABL*-negative group. Clinically, most patients present with either a myeloproliferative disorder with eosinophilia, CEL,

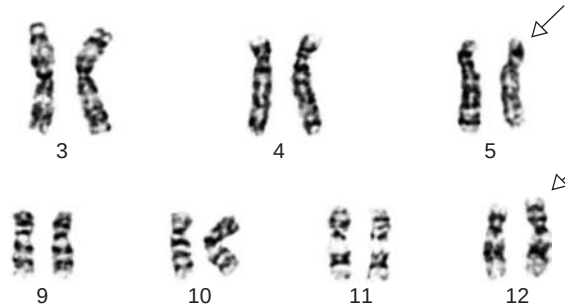


Figure 2.19 The translocation t(5;12), cytogenetics (partial karyotype)

or CMML.[409] Most patients are male with a 2-year survival of 55%.[409,410] In a series of *PDGFRB* fusion gene-positive and *BCR/ABL*-negative CMPDs reported by David et al, out of 12 patients treated with imatinib 11 had prompt responses with normalization of peripheral blood cell counts and disappearance of eosinophilia; 10 had complete resolution of cytogenetic abnormalities and a decrease or disappearance of fusion transcripts by nested RT-PCR.[410]

t(5;17)

- Acute promyelocytic leukemia
- MDS (rare cases)
- AML-M4eo (rare cases)

The t(5;17) is a rare translocation, in which the *RARα* gene recombines with the *NPM* (*nucleophosmin*) gene on chromosome 5 (*NPM/RARα*). This translocation occurs in occasional cases of acute promyelocytic leukemia (APL). The APLs with the *NPM/RARα* fusion transcript have a similar biology to those associated with t(11;17). They are refractory to treatment with ATRA. Sakai et al reported *de novo* acute myelomonocytic leukemia with eosinophilia (AML-M4Eo) with t(5; 17)(q13;q11) as a secondary chromosomal aberration to typical inv(16)(p13q22).[411]

t(6;7)(p24;q21)

- CML

Gozzetti et al reported a novel t(6;7)(p21;q23) that developed in a CML patient in complete cytogenetic remission during imatinib therapy.[412]

t(6;9)

- AML
- MDS

The t(6;9)(p23;q34) translocation leads to the fusion of the *DEK* gene located on 6p23 (coding for a nuclear DNA-binding protein) and the *CAN* (for Cain, also known as NUP_{214}) gene on 9q34 (encoding for nucleoporin).[413] This is a rare translocation occuring in AML (often with basophilia) and MDS.[414] The t(6;9) is associated with a poor prognosis, especially in younger patients.[323]

t(6;11)

- T-PLL

Wong et al reported a case of T-PLL with t(6;11)(q21;q23) as the sole chromosomal abnormality and suggested that the cytogenetically identified translocation implicates the *ATM* gene.[415]

t(7;7)

See inv(7).

t(7;11)

- AML
- CML, disease progression/clonal evolution (blast crisis)

The t(7;11) is a rare abnormality found in AML patients. It is usually associated with FAB M2 morphology (Figure 2.20). The t(7;11)(p15;p15) leads to the fusion transcript *NUP98/HOXA9*. It can be seen also in blast phase/clonal evolution of CML.[416,417]

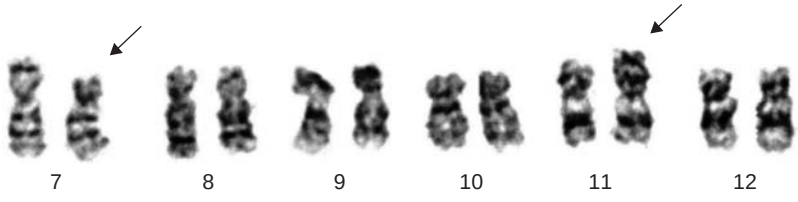


Figure 2.20 The translocation t(7;11), cytogenetics (partial karyotype)

t(7;12)

- AML (pediatric)
- ALL (pediatric)

Slater et al described two rare recurrent translocations affecting *ETV6* (previously *TEL*), t(7;12)(q36;p13) and t(7;12)(q32;p13), in pediatric AML.[418] Rare cases of infants with t(7;12)$^+$ ALL have been reported as well.[419]

t(8;9)(q24;p13)

- B-ALL

The t(8;9)(q24;p13) occurs in occasional cases of precursor B-ALL with t(14;18).[420–423]

t(8;9)(p22;p24)

- CML
- CEL
- AML
- ALL
- MDS

Translocation t(8;9)(p22;p24) leads to the fusion between *PCM1* (human autoantigen pericentriolar material) and *JAK2*. Reiter et al identified t(8;9)(p21-23;p23-24) in seven male patients with atypical chronic myeloid leukemia/chronic eosinophilic leukemia ($n = 5$), secondary AML ($n = 1$), and pre-B-ALL (*BCR-ABL*$^+$; $n = 1$).[424] Murati et al reported the t(8;9) translocation in erythroid leukemia.[425] Apart from *PCM1/JAK2*, another two genes that involve *JAK2* are *ETV6/JAK2*, which arises as a consequence of a t(9;12) reported in few cases of AML, and *BCR/JAK2*, reported in patients with atypical CML.

t(8;14)$^{MYC/IGH}$

- Burkitt lymphoma
- B-ALL
- DLBCL
- Other B-cell lymphomas (including FL, B-PLL, and MCL), often undergoing transformation to aggressive lymphoma
- Plasma cell myeloma (MM; rare cases)

The t(8;14)(q24;q32) translocation involves the c-*MYC* and *IGH* genes (Figure 2.21). The *IGH* gene is the most frequent partner of c-*MYC* rearrangements (>80%), followed by the *IG* kappa gene [*IGK*; t(2;8)(p12;q24)] and *IG* lambda gene [*IGL*; t(8;22)(q24;q11.2)], light chain genes (10–15%). Translocations involving 8q24 are most commonly associated with Burkitt lymphoma and result in c-*MYC* overexpression. Rearrangement of the c-*MYC* gene may be identified by FISH (single- or double-fusion probes, break-apart probe) or by classic cytogenetics. Positive c-*MYC* translocation combined with classic histomorphologic features (monomorphic 'high-grade' infiltrate with starry-sky appearance) and immunophenotype (CD10$^+$, bcl-6$^+$, bcl-2$^-$, CD43$^+$) is diagnostic of Burkitt lymphoma. In cases with more pleomorphic cytologic features or atypical phenotype, concurrent testing for *BCL2* and *BCL6* rearrangement should be always considered to exclude non-Burkitt lymphomas with c-*MYC* expression. Those 'dual-positive' lymphomas (*MYC+BCL2* or *MYC+BCL6*) are characterized by a very aggressive clinical course with a poor response to chemotherapy and therefore a poor outcome.[426–429]

Apart from BL, the t(8;14)(q24;q32) may be seen in a subset of DLBCL, FL, MCL, plasma cell myeloma (MM), B-PLL, and low-grade non-Hodgkin lymphomas transformed to high-grade tumors.[430–433] Alterations of c-*MYC* in non-Burkitt lymphomas may represent secondary changes associated with disease progression, similar to *p53* abnormalities. The t(8;14) occurs in a subset of B-ALL (FAB L3), often with CNS and abdominal organ involvement at presentation. The presence of an 8q24 aberration in a variety of NHL is associated

Figure 2.21 The translocation t(8;14) [*MYC/IGH*]; FISH

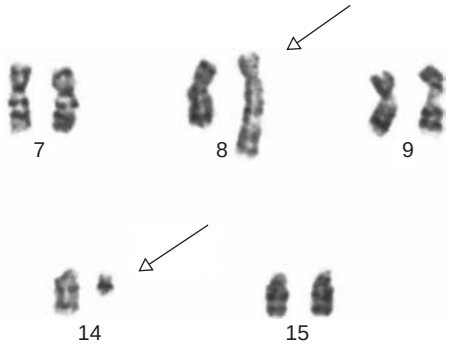


Figure 2.22 The translocation t(8;14); cytogenetics (partial karyotype)

with an inferior prognosis in all histologic groups, independent of clinical prognostic factors, when analyzed both at diagnosis and at relapse.[434] The presence of the t(8;14) translocation in FL is often associated with an aggressive clinical course and a higher risk for transformation into ALL. Figure 2.22 shows the t(8;14) by conventional cytogenetics.

t(8;16)$^{MYST3/CREBBP}$

- AML/tAML

AML with translocation t(8;16)(p11;p13) is an infrequent leukemia subtype with characteristic clinicobiologic features. This translocation leads to fusion of the *MYST3* (*MOZ*; monocytic leukemia zinc finger protein) gene on chromosome 8p11 and the *CREBBP* (*CBP*) gene on chromosome 16. The t(8;16)(p11;p13) is associated with *de novo* or therapy-related AML, often displaying monocytic differentiation (either the M4 or M5 subtype), erythrophagocytosis, extramedullary infiltration, and disseminated intravascular coagulation.[435] The study by Camos et al showed the distinctive gene expression profile of *MYST3/CREBBP*$^{+}$ AML, with overexpression of *RET* and *PRL* and a specific pattern of HOX gene expression.[436] AMLs with inv(8)(p11q13) display similar hematologic features to AMLs with *MYST3/CRBBP* fusion.[437]

t(8;21)$^{ETO/RUNX1}$

- AML

The t(8;21)(q22;q22.3) translocation (Figure 2.23) is seen in AML and is characteristically associated with the FAB M2 subtype and frequent co-expression of CD34, CD19, and CD56.[1,438–441] This translocation is one of the most frequent structural chromosomal abnormalities seen in AML and is reported in 6–20% of AML cases. The t(8;21) results in fusion between the *RUNX1* gene (runt-related gene family; formerly termed the *AML1* gene) and the *ETO* gene (for eight twenty-one), producing a chimeric protein, *RUNX1/ETO.*[442] AML with t(8;21)$^{RUNX1/ETO}$ belongs to a group of CBF leukemias. CBF is also rearranged in AML with t(3;21) and t(16;16)/ inv(16); *see below*.

The AML with t(8;21) is found more frequently in children and young adults and displays

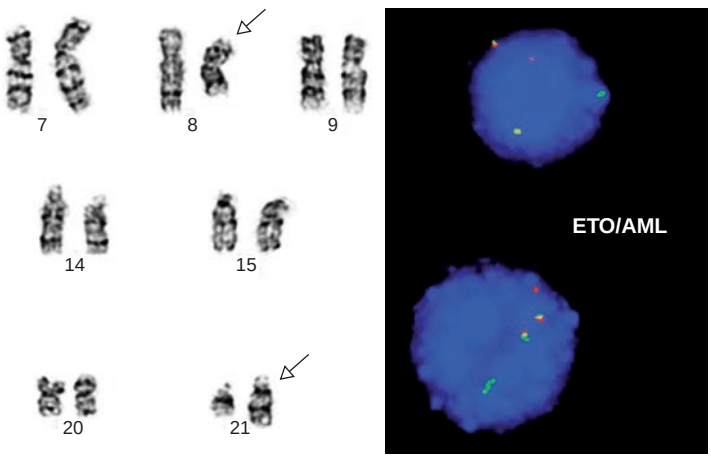


Figure 2.23 The t(8;21) [*RUNX1/ETO*] translocation (A, cytogenetics; B, FISH)

a predisposition for extramedullary localization.[443,444] This subtype of AML is associated with a favorable prognosis.[323,443,444] Complete remission rates and long-term event-free survival are high, particularly when treatment incorporates high-dose cytosine arabinoside.[445]

Despite the favorable prognosis of patients with AML with t(8;21), relapses occur in about 30% of cases. Several recent studies suggested that monitoring MRD may identify patients at risk of relapse. In a series by Leroy et al, levels of *RUNX1/ETO* transcripts showed large variations with a trend for a higher relapse rate for patients with high pretreatment expression levels.[446] After induction therapy, absolute transcript levels ($<10^3$ compared to the control cell line, or > 3-log decrease of the level at diagnosis) were significant predictors of the absence of relapse.[446] MDR levels after consolidation therapy were also significant indicators of relapse.

Additional cytogenetic abnormalities in AML with *RUNX1/ETO* are common (75%) and may include –X, –Y, del(9)(q22), +8, and +4.[447,448] Approximately 28% of patients show more than one chromosomal abnormality.[447] Loss of the Y chromosome may be seen as an age-related phenomenon, but is often present in AML with t(8;21). The influence of additional chromosome aberrations on the clinical outcome of patients with t(8;21) is unclear. Byrd et al reported that neither complex karyotype nor secondary aberrations affected the outcome of patients with t(8;21).[323] However, in the study by Schoch et al, the median overall survival of patients with del(9q) was significantly shorter than in patients with only t(8;21) or with t(8;21) and sex chromosome loss.[449] It appears that loss of a sex chromosome has no influence on prognosis, but the prognosis of patients with t(8;21) worsens when there is extramedullary disease, CD56 expression, or del(9q).[449]

t(8;22)$^{MYC/IGL}$

- Burkitt lymphoma
- B-ALL

The t(8;22)(q24;q11) occurs in a subset of Burkitt lymphoma and sporadically in pre-B-ALL.

t(9;11)

- AML

The translocations of 11q23 involve the *MLL* gene (also known as *ALL1* or *HRX*); they are common in leukemias and include several different partners: 1p32, 4q21, and 19p13.3 in ALL and 1q21, 2q21, 6q27, 9p21, 10p11, 17q25, 19p13.3, and 19p13.1 in AML. The t(9;11)(p21;q23) is associated with an intermediate prognosis in AML.[1,323] Other translocations involving 11q23 usually have a poor prognosis. The t(9;11), similarly to t(11;19), is often associated with monoblastic differentiation, but other variants of AML (e.g. AML-M2) have been reported as well. In childhood AML the t(9;11) is a favorable genetic factor.[450]

t(9;12)

- AML
- ALL
- CML, disease progression

The t(9;22) involves the *ETV6* gene (also known as *TEL*) on chromosome 12p12 and occurs in acute leukemias and during progression of CML.

t(9;14)(p13;q32)

- Lymphoplasmacytic lymphoma/Waldenström macroglobulinemia

The t(9;14)(p13;q32) is a translocation that occurs in B-cell lymphomas with plasmacytic differentiation with indolent presentation followed by gradual clinical progression of disease.[451] In a more recent series reported by Cook et al, the incidence of this translocation in lymphoplasmacytic lymphoma/Waldenström macroglobulinemia was less common than previously reported.[452] The t(9;14) results in the juxtaposition of the *PAX5* (paired homeobox-5) gene with the *IGH* on chromosome 14. The *PAX5*

gene encodes the BSAP (B-cell-specific activator protein) transcription factor, which is expressed throughout the process of B-cell development, except in terminally differentiated plasma cells.

t(9;22)$^{BCR/ABL}$

- CML
- AML
- ALL

The t(9;22) translocation occurs in CML, a subset of ALL, and in rare cases of AML. Ph chromosome, a shortened chromosome 22 (Figure 2.24 and 2.25), results from a reciprocal translocation between the *BCR* gene on chromosome 22 and the *ABL* gene on chromosome 9. The t(9;22) or its molecular equivalent can be detected in virtually all cases of CML by PCR, FISH, conventional cytogenetics or Southern blot. The fusion gene, *BCR/ABL* (Figure 2.26), encodes a chimeric protein with tyrosine kinase activity. Depending on the position of the *BCR* breakpoint, fusion genes are generated that encode 190 kD (minor breakpoint cluster region), 210 kD (major breakpoint cluster region), or 230 kD forms of the bcr-abl tyrosine kinase. The p230 is seen in patients with chronic neutrophilic leukemia. CML expressing p190 is relatively rare. Patients with p190 have a worse prognosis than those with p210. A quantitative molecular assay for bcr-abl RNA (qRT-PCR) can be used to monitor patients with CML after treatment.[453]

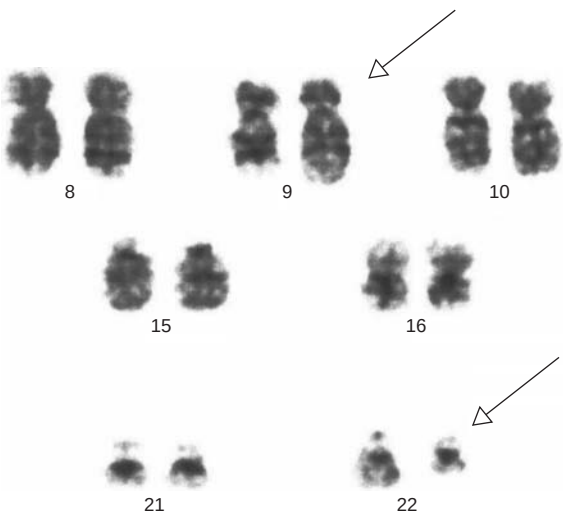


Figure 2.24 The t(9;22) [*BCR/ABL*]; cytogenetics (partial karyotype)

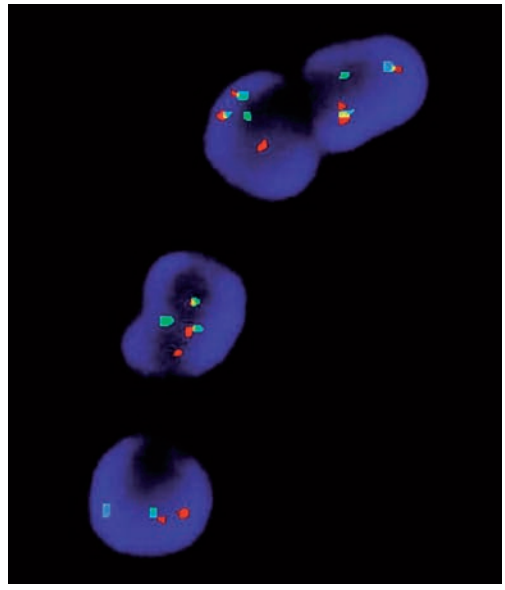


Figure 2.25 *BCR/ABL*; FISH

Deletions of the derivative chromosome 9 have recently been reported in CML. These deletions are large, occur at the time of the Philadelphia translocation, span the translocation breakpoint, and represent a powerful indicator of poor prognosis. Patients with deletions lack expression of *BCR/ABL* and do not exhibit an increased frequency of secondary cytogenetic changes following disease progression.[454] Allogeneic stem cell transplantation reverses the poor prognosis of CML patients with deletions in derivative chromosome 9.[455]

The t(9;22) can be observed in AML or ALL. Acute leukemia (Figure 2.27) with t(9;22) can be either *de novo* or a result of transformation from

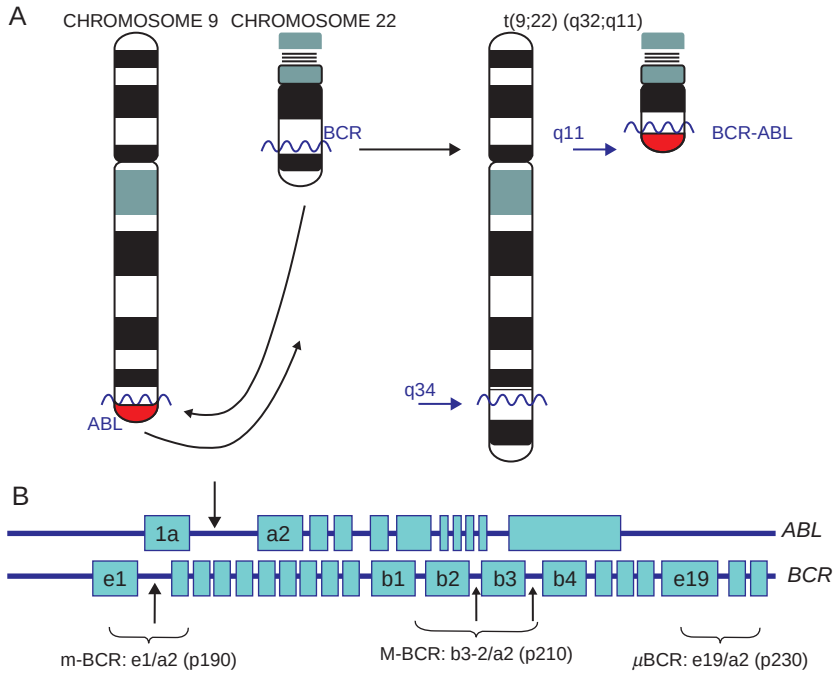


Figure 2.26 A, Philadelphia chromosome due to t(9;22); B, *BCR/ABL* translocation break points. The *ABL* gene break point is between exons 1a and a2, and the *BCR* gene break points are between e1, b2/b3, or e1, creating fusion products of varying sizes (p230, p210, and p190, respectively)

CML (blast phase, myeloid, or lymphoblastic). Progression of CML, including accelerated phase (AP) or BP (blast crisis), is often associated with chromosomal evolution, i.e. the appearance of chromosomal abnormalities in addition to the Ph chromosome. Those may include additional copies of the Ph chromosome, isochromosome 17 [i(17)(q10)], a gain of chromosomes 8 or 19, and less often −7, −17, +17, +21, −Y, and t(3;21)(q26;q22).

The t(9;22) is a frequent and prognostically unfavorable translocation in B-ALL despite intensified chemotherapy.[456,457] *BCR/ABL* (irrespective of the breakpoint site, e.g. p190 or p210) is the leading factor for a poor prognosis in comparison to clinical risk criteria and is associated with a lower chance of initial treatment response (68.4% vs 84.6%) and a lower probability of disease-free survival at 3 years (0.13 vs 0.47).[456] Among ALL patients, the t(9;22)

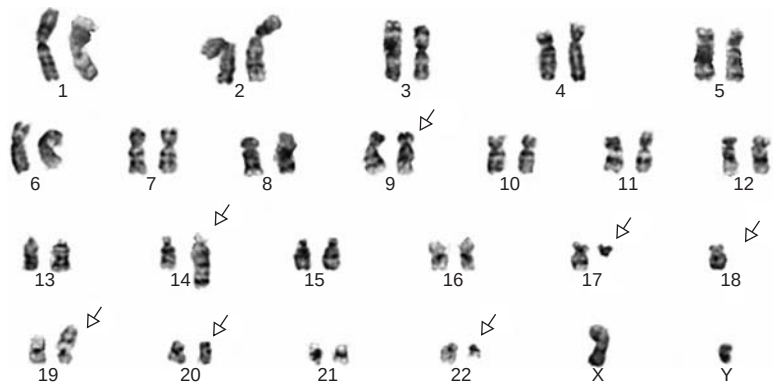


Figure 2.27 Acute lymphoblastic leukemia (ALL) with complex chromosomal abnormalities, including t(9;22); cytogenetics

occurs more often in adults than in children. The *BCR/ABL*$^+$ subtype of ALL in children is associated with high drug resistance and an increased percentage of MRD$^+$ patients and increased MRD levels at the end of induction therapy.[458] In adults, residual disease and hence drug resistance is higher than in children, and the presence of the Ph chromosome has no obvious additional negative effect on MRD.[458]

t(10;11)

- T-ALL
- Peripheral T-cell lymphoproliferative disorders
- AML

The t(10;11) is seen in patients with T-ALL and a subset of T-cell lymphomas. The *MLL/MLLT10* fusion gene as a result of t(10;11)(p12;q23) is often seen in AML.[65] In pediatric AML, t(10;11) is associated with an unfavorable prognosis.

t(10;14)

- T-ALL
- Peripheral T-cell lymphoproliferative disorders

The t(10;14) is seen in patients with T-ALL and T-cell lymphomas.

t(10;17)(p15;q21)

- AML
- B-ALL

The t(10;17)(p15;q21) is a rare abnormality reported in rare cases of poorly differentiated or undifferentiated AML (M1 subtype),[459] and occasional cases of B-ALL. The breakpoint on chromosome 17 is distal to *RARα* gene.

t(10;22)

- AML-M7

The t(10;22) translocation occurs rarely and was reported in acute megakaryoblastic leukemia by G-banding cytogenetics. FISH analysis revealed that it is a variant of Philadelphia translocation involving chromosome 10q.[460]

t(11;14))(q13;q32)$^{CCND1/IGH}$

- MCL
- Plasma cell myeloma (MM)

The t(11;14)(q13;q32) is the hallmark of MCL (Figure 2.28), where it can be detected in virtually all cases.[121,384,461–463] In this translocation, the gene encoding cyclin D1 protein at 11q13 (*CCND1* or *BCL1* gene) is relocated to an *IGH* gene on 14q32, resulting in upregulation of cyclin D1 expression. Apart from MCL, the t(11;14)(q13;q32) is also observed in a subset of multiple myeloma (3–20%).[464,465] In multiple myeloma, cyclin D1/bcl-1 upregulation is detected more often that t(11;14)(q13;q32), suggesting that other chromosome 11 abnormalities, as well as additional mechanisms, must be responsible for the cyclin D1 overexpression. Panani et al showed that t(11;14) had a worse impact on disease outcome as compared to t(14q32) with an unidentified partner chromosome.[466]

Cyclin D1 expression is also frequently found in hairy cell leukemia (HCL) by immunohistochemistry, but its expression is unrelated to translocations or amplification of the *CCND1* gene.[467]

The *CCND1* abnormalities can be confirmed by immunohistochemical staining on routine histologic section, FISH, RT-PCR, or classic (metaphase) cytogenetics, the first two being the most often used. The results of immunohistochemical staining on

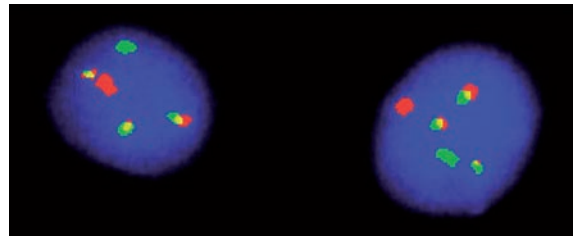


Figure 2.28 Translocation t(11;14); FISH

decalcified specimens, such as trephine bone marrow biopsy, however, are not always reliable.

t(11;14)(p13;q11) and t(11;14)(p15;q11)

- T-ALL

The t(11;14)(p13;q11) and t(11;14)(p15;q11) are frequently seen in precursor T-ALL.

t(11;17)

- APL

The t(11;17)(q23;q21) is present on a subset of patients with APL. This translocation results in fusion of the *RARα* gene (17q21) with the *PLZF* (promyelocytic leukemia zinc finger) gene (11q23). The t(11;17)$^{PLZF/RAR\alpha}$ APL differs from classic APL with t(15;17) by poor response to chemotherapy, ATRA resistance, and poorer prognosis, when treated with ATRA alone.[468,469] Patients treated with combination chemotherapy can achieve a complete remission, however.[16] In the t(11;17(q13;q21), the *RARα* gene partners with the *NuMA* (nuclear mitotic apparatus) gene. Another translocation of the *RARα* gene involves the *STAT5b* gene on chromosome 17(q11). APLs with either t(11;17)(q13;q21)$^{NuMa/RAR\alpha}$ or t(17;17)(q11;q21)$^{STAT5b/RAR\alpha}$ present with morphologic and clinical features similar to the classic type with t(15;17)$^{PML/RAR\alpha}$.

t(11;18)(q21;q21)$^{API2/MALT1}$

- Marginal zone B-cell lymphoma (MALT type)

There are two translocations involving the *MALT1* gene at 18q21: the t(11;18)(q21;q21) involving the *API2* and *MALT1* genes, and t(14;18)(q32;q21) involving *IGH* and *MALT1*. The t(11;18)(q21;q21) resulting in the *API2/MALT1* fusion transcript is an exclusive finding in extranodal marginal zone lymphoma (MZL) of the MALT type.[470] Within the gastrointestinal tract, extranodal MALT lymphomas occur most often in the stomach and rarely in the small and large intestine and esophagus. Gastric MALT lymphoma is associated with *H. pylori* infection. Apart from t(11;18)(q21;q21), other chromosomal aberrations in MZL lymphoma include t(1;14)(p22;q32), t(14;18)(q32;q21), trisomies 3, 12, and 18; *p53* LOH/mutation, and *fas* gene mutation. The cases with t(11;18) usually do not show other genetic aberrations,[471] such as trisomies 3 and 18, frequently seen in t(11;18)-negative tumors.[122,472–474]

The t(11;18) has been reported in 40–50% of MZLs arising in the stomach (MALT lymphoma).[475,476] Gastric MALT lymphomas positive for t(11;18) are more often associated with involvement of the lymph node and distal sites than t(11;18)-negative cases.[358] The presence of the t(11;18) translocation is a negative predictor for response to *H. pylori* eradication therapy.[477] The t(11;18) positive MALT lymphomas are distinct from other MALT lymphomas, including those with t(1;14) or t(14;18). The t(11;18) positive MALT lymphomas rarely undergo high-grade transformation,[122,478] despite more advanced stage and lack of response to *H. pylori* eradication therapy. Gastric MALT with the t(11;18)(q21;q21), however, does not adversely affect the response of gastric MALT lymphomas to chemotherapy with cladribine (2CdA).[479] This makes the cladribine an attractive agent for treatment of gastric MALT lymphomas unresponsive to *H. pylori* eradication. (*See also API2/MALT1* fusion gene.)

t(11;19)(q23;p13.1)

- AML
- B-ALL

The 11q23 breakpoint involves the *MLL* gene (Figure 2.29). The t(11;19(q23;p13.1) is one of the translocations involving 11q23 occuring in B-ALLs [including also t(4;11)]. Those B-ALLs are often characterized by a high WBC count, CALLA negativity, aberrant pan-myeloid marker expression (e.g. CD13 or CD33), and aggressive clinical behavior. The t(11;19) translocation is seen also in AML, most often in AML with monocytic differentiation.

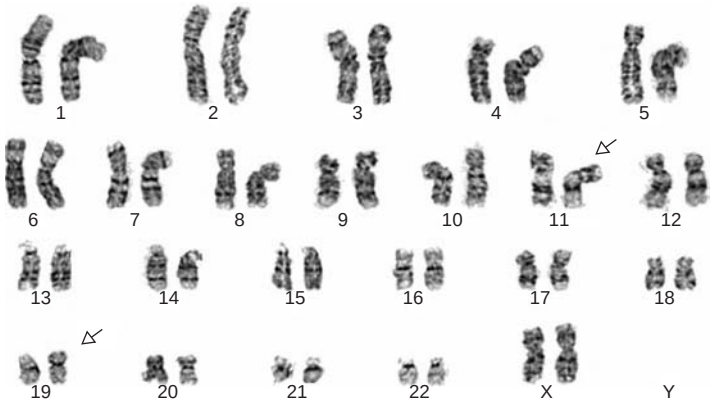


Figure 2.29 The translocation t(11;19); cytogenetics

Patients with AML and t(11;19)(q23;p13.1) and associated abnormalities of chromosome 11q23 have an intermediate survival.[1]

t(12;12)

- ALL

The t(12;21)(p13;q22) is a cryptic abnormality observed in 25% of children with B-ALL, and is associated with a favorable prognosis. The t(12;21) in most cases is not associated with hyperdiploidy or with t(1;19), t(4;11), or t(9;22) ALL.[480]

t(12;17)

- ALL

The balanced t(12;17)(p13;q12) is a rare but recurrent aberration in ALL.[481]

t(12;21)

- ALL

Rearrangements of 12p, resulting from deletions or translocations, are common findings in hematologic malignancies. In many cases, these rearrangements target the *ETV6* gene (previously called *TEL*) located at 12p13. Various partner genes have been implicated in the formation of fusion genes with *ETV6*. These include *PDGFRB*, *JAK2*, *NTRK3*, *ABL2*, and *ABL1*, each of which encodes for proteins with tyrosine kinase activity.

The t(12;21)(p12;q22) is commonly found in pediatric ALL.[7] This translocation generates the *ETV6/RUNX1* chimeric gene. *ETV6/RUNX1*$^+$ ALLs often express CD10, occur between age 1 and 10 years and have a favorable prognosis. The *ETV6* gene is also involved in t(5;12), t(9;12), and t(12;22), which occur in other hematopoietic tumors. Raimondi et al analyzed 815 children with newly diagnosed ALL and found 94 cases (11.5%) with 12p abnormalities involving *ETV6*, which was associated with a favorable treatment outcome.[338] The t(12;21) can be seen in a healthy individual.[482]

t(13;17)

- AML

The t(13;17) (Figure 2.30) is a rare translocation, which has been reported in AML.[483]

t(14;16)

- Plasma cell myeloma (MM)

The translocation t(14;16) is associated with fusion between the *IGH* gene on chromosome 14q and the c-*MAF* gene on chromosome 16q23. The c-*MAF* is translocated in approximately 5–10%

Figure 2.30 The translocation t(13;17); cytogenetics (partial karyotype)

of MM. The c-*MAF* is an oncogene that stimulates cell cycle progression, and promotes pathologic interactions between tumor and stroma cells.[484,485]

t(14;18)(q32;q21)$^{IGH/BCL2}$

- FL
- DLBCL
- ALL

The t(14;18)(q32;q21) juxtaposes the *BCL2* gene at 18q21 with the immunoglobulin heavy chain locus (*IGH* gene) at 14q32. It is characteristic for FL (Figure 2.31) in which the breakpoints of t(14;18) occur in the major breakpoint region (mbr) or in the minor cluster region (mcr) at the 3′ end of *BCL2*. The frequency of t(14;18)(q32;q21) in nodal FL by metaphase cytogenetics and/or interphase FISH has been shown to vary between reports, most often

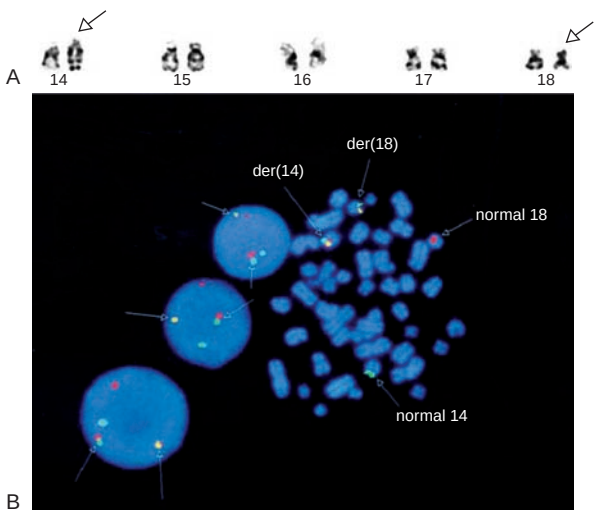


Figure 2.31 The t(14;18) [*IGH/BCL2*]; A, cytogenetics (partial karyotype); B, FISH

ranging between 80 and 100%.[384,431,432,463,486,487] The t(14;18) is also present in a subset of DLBCL, being detected in 12 to 35% of cases.[384,488,489] The incidence of t(14;18) is higher in lower grade FL (grade 1 or 2), when compared to grade 3.[108,490] Cytogenetic analysis is not always successful due to the low proliferative activity of lymphomatous cells. Although FISH methodology is more sensitive when compared to conventional cytogenetics standard cytogenetic analysis is helpful in identifying accompanying chromosomal abnormalities or unusual changes, such as three-way translocation involving two major lymphoma-specific abnormalities, 3q27 and t(14;18)(q32;q21).[491]

The t(14;18) translocation results in overexpression of bcl-2 protein, whose function is to inhibit apoptosis (programmed cell death). The overexpression of bcl-2 is also observed in other lymphomas, including B-CLL and MALT lymphoma, and is not always associated with t(14;18).

Rare cases of *de novo* B-ALL display t(14;18).[420,492–495] B-ALL with t(14;18) may arise as a blastic transformation of a preceding lymphoma[492,495,496] or as *de novo* ALL.[420] D'Achille et al resented a series of 12 cases of *de novo* ALL with translocation t(14;18)(q32;q21) with a median age of 65.5 years and no past history or any clinical evidence of lymphoma.[420] In this series, a t(8;14) was identified in 4/12 cases by conventional cytogenetics, and in 3 additional cases by FISH [one with a cryptic t(8;14) involving the der(14)t(14;18), one showing c-*MYC* translocation to a marker chromosome, and one associated with a t(8;9)(q24;p13)].[420] There was a close association between the t(14;18) and the t(8;9), with the latter translocation present only in conjunction with t(14;18).[420,422,423] The B-ALL with t(14;18) are associated with a poor prognosis.[420,492,495]

Aamot et al evaluated the pattern and frequency of secondary chromosome abnormalities in t(14;18)$^{+}$ lymphomas (112 cases of FL and 37 cases of DLBCL),[180] 94% of cases (140/149) showed secondary aberrations, with the most frequent being

−1p, −6q, and +7 (26%). DLBCL showed −1q, +7, and +12, more frequently whereas −1p, −Xp, and −16 were more frequent in FL grade 3 than in FL grades 1 and 2. Patients with <6.0 secondary cytogenetic aberrations had a better prognosis than did those with a higher number of aberrations. Trisomy 21 was associated with a shorter survival.[180]

The t(14;18)$^{BCL2/IGH}$ can be detected by cytogenetic, FISH, and PCR studies: FISH using either a single-fusion or dual-fusion strategy and PCR using a consensus primer targeting the *IGH* joining region in combination with one of three primers flanking the major, minor, and intermediate break-point cluster regions on chromosome 18q21. Break-apart FISH probes to detect *BCL2* gene rearrangements are also available.[497] Quantification of t(14;18) can be achieved by real-time PCR, especially using a multiplex real-time PCR protocol that allows amplification of control and target genes in the same reaction and precise size determination of *BCL2*/JH fusion sequences by capillary electrophoresis to evaluate treatment efficacy and minimal residual disease.[498] Variant translocations (e.g. involving either kappa or lambda light chain genes) can be detected by FISH and cytogenetic studies. Comparisons of FISH and PCR tests have shown higher detection rates using FISH methodology, especially in routinely fixed, paraffin-embedded tissues.[116,499,500] Belaud-Rotureau et al evaluated the applicability and sensitivity of FISH and PCR in FL using commercially available reagents and found FISH to be superior to PCR (using the BIOMED-2 protocol) in detecting *IGH/BCL2* fusion.[116] With the use of *IGH/BCL2* dual-fusion, dual-color probes, t(14;18) translocation was detected in 92% of cases with concordant results between isolated nuclei and frozen cytologic imprints.[116] The BIOMED-2 protocol allowed the detection of an *IGH/BCL2* fusion in 64% of fixed specimens and 71% of frozen specimens. Out of the FISH$^+$ cases, PCR results were negative in 31% of fixed specimens and 20% of frozen specimens.

The analysis of t(14;18) [*IGH*/*BCL2*] by FISH is most useful in distinguishing between FL and atypical follicular hyperplasia. Although most cases can be easily diagnosed by morphology combined with immunohistochemistry, in a subset of cases the histomorphologic and immunophenotypic features are equivocal, and therefore require additional testing either by FISH (*IGH/BCL2* rearrangement) or PCR (*IGH* rearrangements for clonality). FISH may also be useful to differentiate FL from other lymphomas with a nodular growth pattern, although morphologic and immunophenotypic features are sufficient in the majority of cases. FISH evaluation of *IGH/BCL2* and *IGH/MALT1* is also useful to distinguish between FL and marginal zone lymphoma with t(14;18) [conventional cytogenetics cannot distinguish the t(14;18)(q32;q21) involving *IGH* and *BCL2* from t(14;18)(q32;q21) involving *IGH* and *MALT1*]. Demonstration of t(14;18) in DLBCL is less important from the diagnostic point of view, but confirms the germinal center phenotype, and therefore has prognostic implications (patients with the germinal center phenotype have a better prognosis than those with the activated type; *see DLBCL, Chapter 3*).

Clonal *BCL2/IGH* rearrangements are frequently observed in healthy individuals.[501–503] In the study by Schmitt et al, using a classic two-step, nested, semi-quantitative PCR as well as a sequence-specific real-time quantitative PCR (qRT-PCR), 24% (39/204) of healthy individuals carried the t(14;18) translocation.[502] The breakpoint location distribution pattern appeared to be comparable to the pattern seen with follicular lymphoma patients. Liu et al reported that the frequency of t(14;18) (*IGH/BCL2* fusion) increases with age (patients >60 years old had a 13 times greater frequency of *BCL2* rearrangements in blood when compared to patients ≤20 years old) and smoking.[501] Schmitt et al, however, using a classic two-step, nested, semi-quantitative PCR as well as a hybridization probe-based real-time quantitative PCR, found no correlation between the frequency of *IGH/BCL2* rearrangements and age or gender.[502] *BCL2* rearrangement are also seen in patients with hepatitis C infection, who show a disappearance of t(14;18) translocation after antiviral

treatment [the t(14;18) translocation became negative in 6 of 7 patients treated with interferon and ribavirin for 6 to 12 months compared with 1 of 6 nontreated patients).[504,505] Persistent polyclonal B-cell lymphocytosis (PPBL) is another disorder associated with *IGH/BCL2* rearrangement.[506] It is diagnosed predominantly in women, usually cigarette smokers, and is characterized by an increase in the number of polyclonal B lymphocytes, often with binucleated morphology, the presence of multiple *IGH/BCL2* gene rearrangements, chromosomal instability [del(6q), +der(8), +8] and the finding of an additional long arm chromosome 3q+ (i3)(q10) or trisomy 3 within a significant proportion of B cells.[506–508] Himmelmann et al suggested that chronic antigenic stimulation plays a role in the pathogenesis of PPBL with the accumulation of $IgD^+/CD27^+$ memory B cells which might harbor a certain number of t(14;18) translocations.[509]

t(14;18)$^{IGH/MALT1}$

- Marginal zone B-cell lymphoma (MALT type)

The breakpoint on chromosome 18, involving the *MALT1* gene (Figure 2.32), is seen in MZLs of the MALT type. The *BCL2* and *MALT1* genes lie in very close proximity at the 18q21 locus. FISH studies for *BCL2* and *MALT1* can help to differentiate between MZL and FL, since both may be positive for t(14;18). In MALT lymphomas with t(14;18), there appears to be an interaction between the *MALT1* and *BCL10* genes. Both genes and their products can be detected by FISH and/or immunohistochemistry. The t(14;18)$^{IGH/MALT1}$ cases typically involve ocular adnexae and lung and are not reported in a gastric location. Another translocation involving the *MALT1* gene at 18q21 of MZL is associated with t(11;18) and involves the *API2* gene [*see above*, t(11;18)].

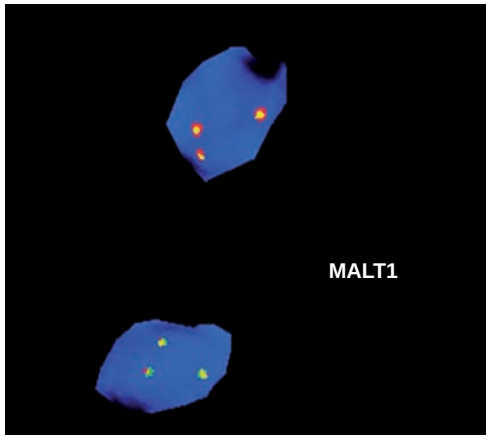


Figure 2.32 *MALT1* translocation (FISH)

t(14;19)(q32;q13)

- B-CLL

The t(14;19)(q32;q13) involves the *BCL3* locus at chromosome 19q13 and the *IGH* gene locus at 14q32. The t(14;19) is a rare chromosomal aberration occuring in B-CLL.[72,387,510–512] In a series reported by Michaux et al, B-CLL with t(14;19) was characterized by a high proportion of patients aged <50 years, lymphadenopathy, rapidly progressive disease, and overall poor prognosis.[510,511] The t(14;19) is rarely the sole cytogenetic aberration: trisomy 12 is the most frequent associated abnormality (50% of cases).[510,511] Huh et al reported the aberrant phenotype in a subset of B-CLL patients with t(14;19) (lack of CD23 expression in 6/7 and positive FCM7 in 5/7).[511]

t(15;17)

- APL

The reciprocal translocation t(15;17)(q22;q21) is the diagnostic hallmark of APL (AML-M3; Figure 2.33).[468] The t(15;17) translocation creates two chimeric genes, the *PML/RARα* gene is formed on derivative 15, whereas the reciprocal *RARα/PML* fusion is located on derivative 17 (Figure 2.34). The *PML* (promyelocytic leukemia) gene possesses growth suppressor and proapoptotic activity.[513,514] RARα (retinoic acid receptor α) is a transcription

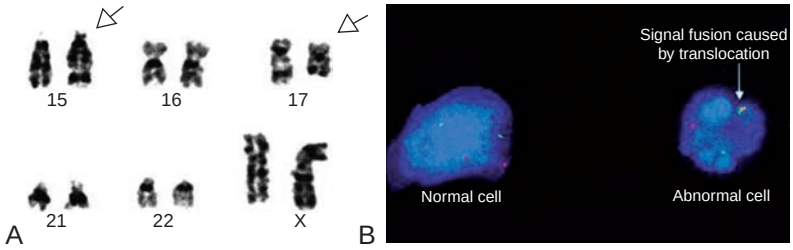


Figure 2.33 The t(15;17) [*PML/RARα*]; A, cytogenetics (partial karyotype); B, FISH

factor that mediates the effect of retinoic acid. *PML/RARα* fusion plays an important role in leukemogenesis by impairing the growth suppressor and proapoptotic activities of *PML*. It is also important in mediating the differentiation response to all-trans retinoic acid (ATRA) treatment.[164,468,515] The introduction of novel targeted therapies in the form of ATRA and arsenic trioxide changed the clinical course of APL over the last 25 years from one that was fatal for the majority of patients to the most curable subtype of AML. Reciprocal translocation of 17q21 is found in more than 95% of APL. The remaining APL cases show complex or variant translocations involving chromosomes 15 or 17 and other chromosomes as well as masked (cryptic) insertions.

To date, five different fusion partners of *RARα* have been identified. The vast majority of cases are characterized by the presence of the t(15;17)(q22;q12-21), which involves the *PML* gene. The other chromosomal aberrations seen in APL include t(11;17)(q23;q21)$^{PZL/RARα}$, t(5;17)(q35;q12-21)$^{NPM/RARα}$, t(11;17)(q13;q21)$^{NuMa/RARα}$, t(17;17)(q11;q21)$^{STAT5b/RARα}$, and der(17). APL associated with t(15;17), t(5;17) and t(11;17)(q13;q21) appears to be sensitive to ATRA. In contrast, APL associated with t(11;17)(q23;q21) leading to *PZL/RARα* rearrangement is typified by a lack of differentiation response to retinoids, and patients treated with ATRA alone have a poor prognosis.[469] In *PML/RARα*-positive APL about 70% of patients are expected to be cured with a combination of ATRA and anthracycline-based chemotherapy. Additional chemotherapy at the time of molecular relapse improves survival compared to patients when treatment is delayed at the point of hematologic relapse. Quantitative RT-PCR technology is expected to improve the predictive value of MRD monitoring and therefore to guide therapy in order to reduce the rate of relapses and to increase rates of cure in high-risk patients.[515]

Additional chromosomal changes have been reported in 25–40% of APLs, with trisomy 8 being the most frequent secondary change (46% of the cases with secondary changes).[516] No significant differences were seen with regard to age, sex, initial white blood cell count, percent of circulating blasts, platelet count, fibrinogen level, or incidence of microgranular variants between patients with or without additional rearrangements. Outcome was also similar between patients with t(15;17) alone and patients with t(15;17) and other clonal abnormalities for complete remission (92% vs 93%, respectively), event-free survival at 2 years (76.1% vs 78.1%, respectively), relapse at 2 years (16.7% vs

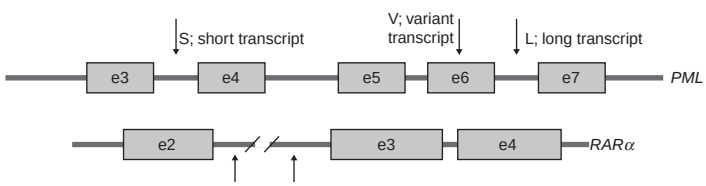


Figure 2.34 *PML/RARα* translocation break points. Breaks in intron 6 are termed bcr-1 or L (long); breaks in exon 6 are termed bcr-2 or V (variable) and in intron 3 are termed bcr-3 or S (short)

11.6%, respectively), and overall survival at 2 years (79.9% vs 79.5%, respectively).[516] Other additional chromosomal abnormalities involve chromosomes 6, 7, 12, 16, 21.[516–518] Owatari et al described t(2;7) in APL.[517] The prognostic significance of chromosomal abnormalities in addition to t(15;17) in APL is uncertain. De Botton et al reported a lack of prognostic significance of secondary chromosomal changes in APL patients treated with ATRA and chemotherapy.[516]

Standard cytogenetics can be used to identify t(15;17) and molecular techniques can be used to identify the fusion product of the translocation (either FISH or RT-PCR).[519,520] Rennert et al described a rapid and simple RT-PCR assay that identifies the *PML*/*RARα* chimeric messenger RNA (mRNA).[520] A set of primer pairs specific for intron 3 (bcr 3, long [L] form mRNA transcript) and intron 6 (bcr 1, short [S] form)/exon 6 (bcr 2, variant [V] form) breakpoints in *PML*, respectively, could readily identify RNA transcripts (S, L, or V forms) by agarose gel electrophoresis with the sensitivity of 1 in 10 000 to 1 in 100 000. The separate amplification of a β_2-microglobulin transcript control was used to assess for adequate RNA and cDNA preparation. The ratio of cases with the L form to those with the S form *PML/RARα* fusion transcript was 2:1, whereas three cases (10%) had fusion sites in exon 6 of the *PML* gene (V forms). Among 221 *PML*/*RARα*-positive cases, there were 82 S form cases (37%), 121 L form cases (55%), and 18 V form cases (8%).[519]

t(17;20)(q21;q12)

- APL

Rare cases of APL harbor simple or complex (three-way) translocations involving the *RARα* locus on chromosomes 17 and 20, including t(13;15;17;20) (q22;q22;q12;p13) and t(15;17;20) (q22;p13;q21). The t(17;20)(q22;q21) may mask the classic t(15;17), when part of the genetic material from chromosome 15 is transferred to chromosome 20.[521, 522]

Trisomies

trisomy 2/duplication 2p

- DLBCL
- Other B- and T-cell lymphomas

In non-Hodgkin's lymphomas trisomy 2 or duplications of 2p predict a poor prognosis.[22] In DLBCL duplication of chromosome 2p or *BCL2* oncogene rearrangement is associated with a relatively poor prognosis.[523]

trisomy 3/duplication 3p

- Marginal zone B-cell lymphoma/splenic marginal zone lymphoma
- Other non-Hodgkin's lymphomas
- Persistent polyclonal B-cell lymphocytosis

Trisomy 3 (Figure 2.35) represents the most common recuring abnormality in marginal zone B-cell lymphoma (MZL; MALT lymphoma).[217,431,524–526] In non-Hodgkin's lymphomas trisomy 3 or duplications of 3p predict a favorable clinical outcome.[22] Based on the most frequent recurrent abnormalities in splenic marginal zone lymphoma, Sole et al divided it into two groups, one with a gain of 3q and the other with deletions at 7q.[217]

trisomy 5

- AML
- ALL

Trisomy 5 (Figure 2.36) is rare abnormality and may occur in both AML and ALL, usually as part of

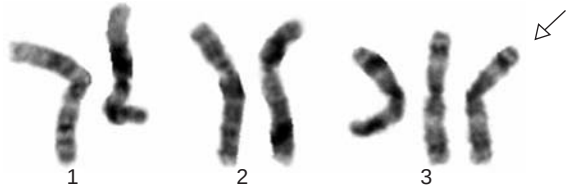


Figure 2.35 Trisomy 3, cytogenetics (partial karyotype)

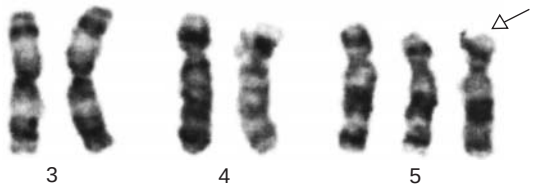


Figure 2.36 Trisomy 5, cytogenetics (partial karyotype)

complex karyotypic changes. In ALL, trisomy 5 is more common in cases with hyperdiploidy (>50 chromosomes). In AML it may accompany t(8;21) or trisomy 8.

trisomy 8

- AML
- APL
- MDS
- T-cell lymphoma
- CML (disease progression; blast crisis)
- P. vera
- B-CLL

Trisomy 8 (Figure 2.37) is the most common trisomy in *de novo* AML. The impact of trisomy 8 on AML patients is best predicted by the presence and nature of other chromosomal abnormalities.[108,323] Patients with sole +8 and +8 with additional abnormality other than t(8;21), inv(16)/t(16;16), and t(9;11) have significantly inferior overall survival, while patients with +8 and a complex karyotype with ≥3 abnormalities have a significantly inferior complete remission rate and overall survival.[323] As a sole abnormality, the frequency of +8 varies among different FAB subtypes, being most frequent in acute monoblastic leukemia (AML-M5), followed by acute megakaryoblastic leukemia (AML-M7), AML without maturation (AML-M1), and AML with maturation (AML-M2) types. Trisomy 8 is the most common additional chromosomal abnormality in APL (46% of APL with secondary changes).[516] Outcome is similar between patients with t(15;17) alone and patients with t(15;17) and other clonal abnormalities.[516]

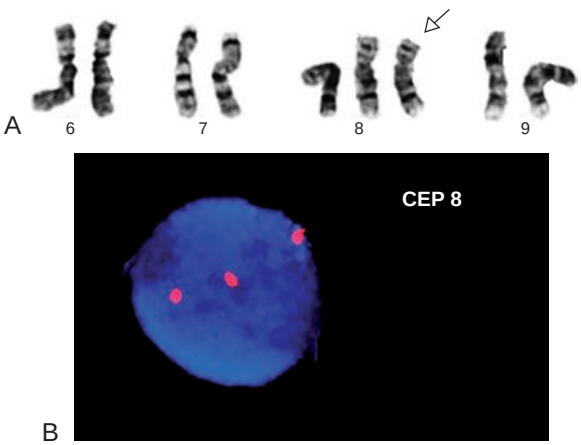


Figure 2.37 Trisomy 8. A, cytogenetics; B, FISH

In MDS, trisomy 8 occurs most frequently in chronic myelomonocytic leukemia (CMML) and refractory anemia with ringed sideroblasts (RARS). Gain of 8q in cutaneous T-cell lymphoma is associated with a significantly shorter survival.[192] Trisomy 8, alone or in combination with a gain of chromosome 19 and additional copies of the Ph chromosome, is often observed during clonal evolution of CML (accelerated phase or blast phase). Gain of chromosome 8 is often observed (15%) in the chronic phase of P. vera. Lau et al described trisomy 8 as the sole cytogenetic abnormality in a B-CLL patient.[527]

trisomy 9

- P. vera

Gain of chromosome 9 (Figure 2.38) is often observed (20%) in the chronic phase of P. vera.

trisomy 11

- AML
- MDS

Trisomy 11 occurs in AML (~3%; usually M1 or M2 types) and MDS (~1%).[528–531] Trisomy 11^+ AMLs are associated with prominent trilineage dyspoiesis and unfavorable outcome. The majority

Figure 2.38 Trisomy 9; cytogenetics (partial karyotype)

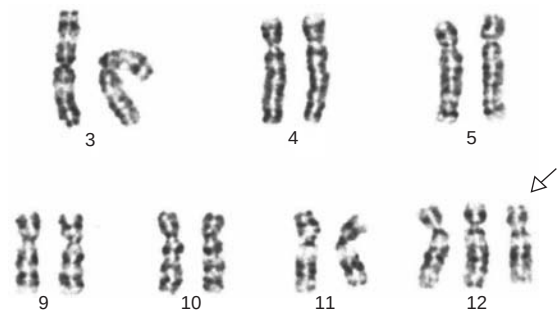


Figure 2.39 Trisomy 12 in B-CLL; cytogenetics (partial karyotype)

of leukemias show additional chromosomal changes, such as trisomy 8, loss of the long arm of chromosome 5, or complex karyotype. AMLs with trisomy 11 often display duplication of the *MLL* gene (*see* MLL).

trisomy 12

- MCL
- B-CLL
- FL
- Marginal zone B-cell lymphoma (MZL; MALT lymphoma)

In MCL, trisomy 12 is the only single cytogenetic parameter predictive of a poor prognosis.[105] Trisomy 12 is a common abnormality in B-CLL, followed by 14q+, 13q, and 11q. Patients with trisomy 12 have advanced clinical stage, atypical morphology and immunophenotype, and shorter survival.[19,532] In a series reported by Geisler et al, however, an additional copy of chromosome 12 without other chromosomal abnormalities was compatible with classical CLL, and had no prognostic influence.[301] Trisomy 12 is more often observed in patients with Richter syndrome than in the overall population of patients with B-CLL.[533] FLs with del(17p) and +12 have an adverse clinical outcome.[293] Trisomy 12 occurs frequently in marginal zone B-cell lymphomas.[431] Figure 2.39 presents trisomy 12 on conventional cytogenetic analysis.

trisomy 15

- AML
- MDS
- Non-Hodgkin's lymphomas

Trisomy 15 as the sole karyotypic aberration is an uncommon clonal cytogenetic aberration in hematologic malignancies, making its significance unclear. The analysis of cytogenetic and FISH results by Batanian et al revealed the presence of two types of trisomy 15 clones: a minor clone that could be transitory or indolent (MDS, lymphoma) and a major clone that could be of a neoplastic nature (AML).[534] Trisomies of chromosomes 15, 8, and 11 are seen in AML developing as a secondary malignancy.[535]

trisomy 17

- CML, disease progression/clonal evolution

A gain of chromosome 17 (similarly to its loss) may be seen during progression (karyotypic evolution) of CML.

trisomy 18

- DLBCL
- Plasma cell myeloma (MM)
- Other B-cell lymphomas, e.g. MZL

DLBCLs with 18q gains are significantly associated with a high number of chromosomal imbalances, primary nodal presentation, high serum LDH levels, high IPI, shorter cause-specific survival, and a high risk of relapse.[296] In the gastrointestinal tract, trisomy 18q21 appears to be associated with advanced tumor stage and may be a predictor of poor outcome

in surgically resected primary gastrointestinal B-cell lymphomas.[536] It is associated with a shorter survival of patients with lymphomas with a large cell component, but not with the low-grade lymphomas of the MALT type.[536] Figure 2.40 presents MM with a complex karyotype including trisomy 18.

trisomy 21

- AML
- ALL
- CML, disease progression/clonal evolution

Trisomy 21 is frequently observed in myeloid malignancies, but can also be seen in ALL. Children with Down's syndrome have a 150-fold increased risk of myeloid leukemia in the first 5 years of life. Leukemias in patients with Down's syndrome are associated with +8 or −7. In addition, increased white blood cell counts and circulating myeloblasts (almost invariably megakaryoblasts by phenotypic studies) may be seen in up to 10% of newborns with Down's syndrome (transient myeloproliferative disorder; TMD). Trisomy 21 in AML is associated with an intermediate risk factor for induction therapy success and overall survival.[323]

Children in whom ALL is associated with Down's syndrome (trisomy 21) have a significantly lower rate of remission, a higher mortality rate during the induction phase, and a poorer long-term survival rate in contrast to children with Down's syndrome and AML.

trisomy 22

- AML

Trisomy 22 is often identified as a recurring secondary abnormality in AML, particularly associated with inv(16)(p13q22).[537] It occurs more often in AML with monocytic differentiation (e.g. acute myelomonocytic leukemia and acute monocytic leukemia).

Molecular markers

Hematologic malignancies are frequently associated with translocations and mutations of genes encoding tyrosine kinase. Abnormalities involving the *ABL*, *ARG*, *PDGFRs*, *JAK2*, *SYK*, *TRKC*, *FGFRs*, *FLT3*, *KIT*, and *JAK2* genes, including fusion, gain of function mutation, or overexpression (e.g. due to internal tandem duplication) can be detected in both acute and chronic myeloproliferations. Approximately 50% of newly diagnosed AMLs have a normal karyotype and identifying molecular markers could help to improve the prognostic stratification of those patients. Mutation of the nucleophosmin (*NPM*) gene has been reported as the most frequent mutation in AML, especially in the presence of a normal karyotype.[538] AML with a normal karyotype also

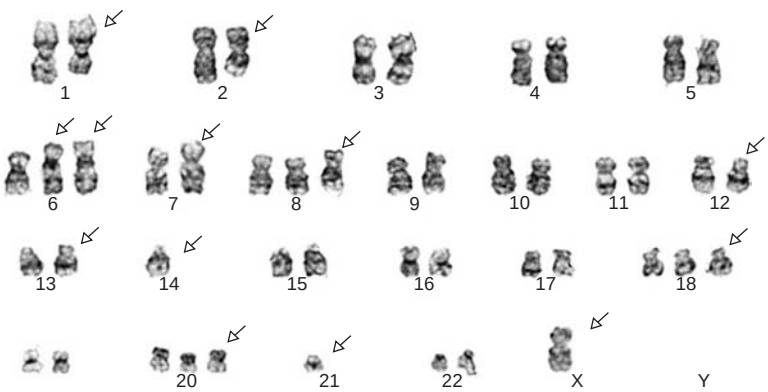


Figure 2.40 Complex chromosomal aberrations including trisomy 18 in multiple myeloma (cytogenetics)

shows mutation in *CEBPA* and internal tandem duplications of *FLT3*.[539] Several genetic and phenotypic characteristics of APL provide relevant targets for tailored treatment, including *PML/RARα* fusion, the expression of the CD33 antigen, absence of the multidrug resistance-related phenotype, and a frequently mutated and constitutively activated *FLT3* receptor.[540] Molecular analysis of *BCR/ABL* fusion by qRT-PCR and *JAK2* mutation analysis has become routine in the management of patients with chronic myeloproliferative disorders. In B-CLL, in addition to the mutation status of the immunoglobulin variable heavy-chain region gene (*IGVH*), a well-established predictive factor, new molecular markers associated with the biology of the disease have been identified, including telomere function, DNA repair, *ATM*, and *p53*.[541]

ABL (ABL1)

- CML
- AML
- ALL

The t(9;22) involving *BCR* and *ABL* genes is seen in CML and occasional cases of AML and ALL (Figure 2.24 and 2.25); *see also* t(9;22). The breakpoint in the *ABL* gene (*ABL1)* is variable over a region of 200 kb, often between the two alternative exons 1b and 1a, sometimes 5′ of 1b or 3′ of 1a, but always 5' of exon 2 (Figure 2.26). The t(9;22) results in three different fusion protein products which differ in size: p190, p210, and p230. The *BCR/ABL* fusion proteins have been shown to exhibit oncogenic potential associated with unregulated tyrosine kinase activity, leading to increased cell proliferation and decreased programmed cell death (apoptosis). The p190 results from e1a2 junctions and is most often associated with ALL. The p210 results from b2a2 or b3a2 and is most commonly seen in CML. The p230 results from e19a2 junctions and is associated with occasional cases of CNL. Methylation of the proximal promoter of the *ABL* oncogene is a common epigenetic alteration associated with clinical progression of CML. Imatinib mesylate (Gleevec, STI571, or CP57148B) is a direct inhibitor of *ABL* (ABL1), *ARG* (ABL2), *KIT*, and *PDGFR* tyrosine kinases. A small group of patients in chronic-phase CML and even more patients with advanced-phase disease may be either initially refractory to imatinib or eventually develop imatinib resistance.[542–556] In many cases (40–50%) this is attributed to the development of mutations that impair the ability of imatinib to bind to and inhibit the constitutively active *BCR/ABL* kinase. To date, more than 40 different point mutations that code for distinct single amino acid substitutions in the *BCR/ABL* kinase domain have been isolated from imatinib-resistant patients.[44,542–556] These mutations affect amino acids involved in imatinib binding or in regulatory regions of the *BCR/ABL* kinase domain, resulting in decreased sensitivity to imatinib while retaining aberrant kinase activity.[542–545] New tyrosine kinase inhibitors (Dasatinib, Nilotinib) inhibit *BCR/ABL* more potently than imatinib and maintain activity against an array of imatinib-resistant *BCR/ABL* mutants. Figures 2.41 to 2.43 show the molecular detection of *ABL* mutations.

ABL (*ABL1*) amplification, due to a cryptic episomal translocation *NUP214/ABL1*, is a novel finding in T-ALL.[557,558] *ABL* amplification can be identified by FISH. *NUP214/ABL1* expression defines a new subgroup of T-ALL patients, who could benefit from treatment with imatinib. De Keersmaecker et al described another *ABL1* fusion, *EML1/ABL1*, in a T-ALL patient with a cryptic t(9;14)(q34;q32) associated with deletion of *CDKN2A* (p16) and expression of *TLX1* (*HOX11*).[559]

ALK

- Anaplastic large cell lymphoma (ALCL)
- DLBCL

The t(2;5) (Figure 2.17) disrupts the nucleophosmin (*NPM*) gene on 5q35 and the anaplastic lymphoma kinase (*ALK*) gene on 2p23, generating a novel *NPM/ALK* gene. *NPM/ALK* fusion leads to a

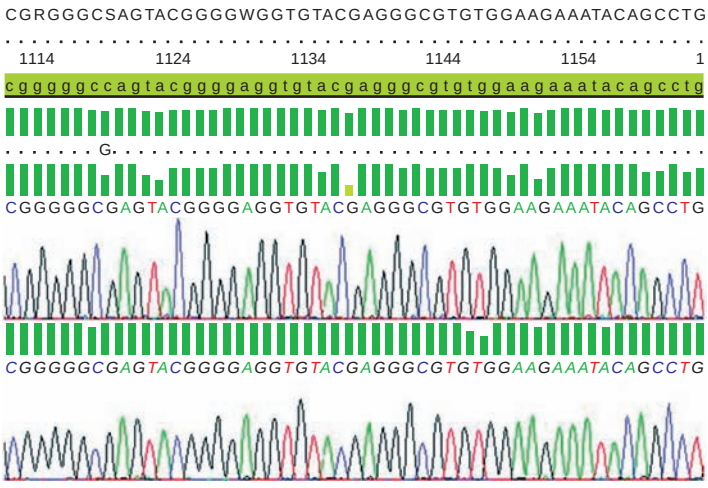


Figure 2.41 Molecular analysis of ABL mutation: negative sample (no mutation is identified)

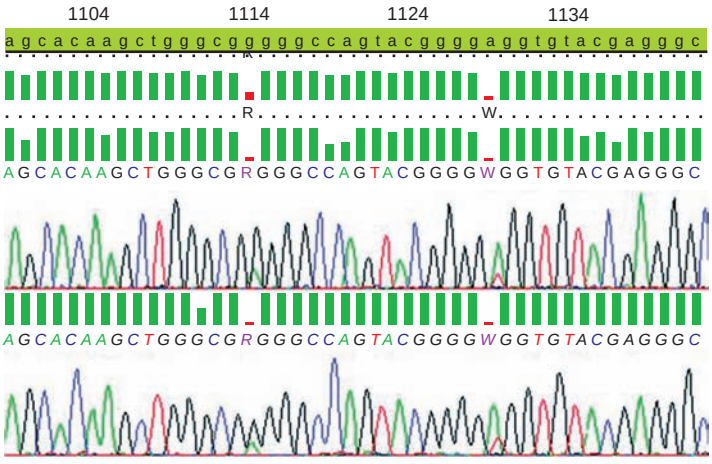


Figure 2.42 Molecular analysis of ABL mutation: mutation is identified (G250R/E255V)

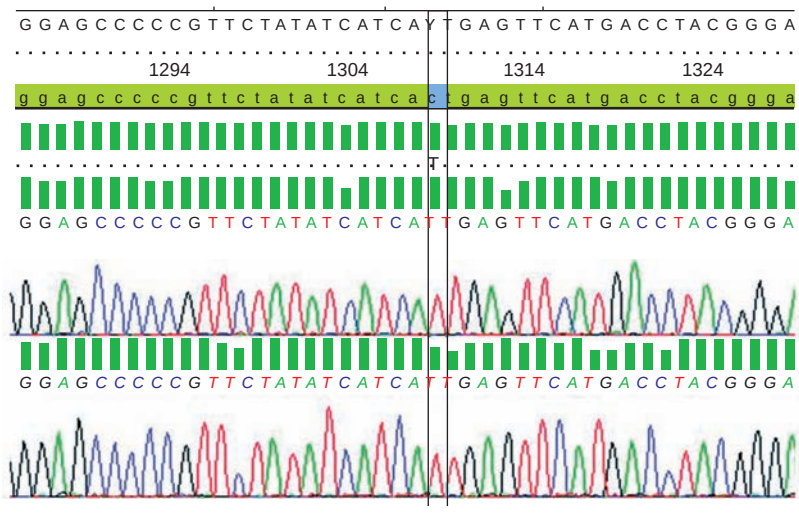


Figure 2.43 Molecular analysis of ABL mutation: mutation is identified (1309C>T change)

chimeric mRNA molecule and a unique 80 kDa *NPM/ALK* fusion protein referred to as p80.[560] The *NPM/ALK* fusion induces constitutive, ligand-independent activation of the *ALK* tyrosine kinase leading to aberrant activation of cellular signaling pathways. c-*MYC* may be a downstream target of ALK signaling and its expression a defining characteristic of ALK$^+$ ALCLs.[561]

The *NPM/ALK* fusion is present in ALCL, a T-cell malignancy characterized by CD30 expression. A subset of T-cell lymphomas with anaplastic features may be negative for *ALK* expression; those patients have a worse prognosis and a poor response to chemotherapy when compared to patients with *ALK*$^+$ tumors. Microarray gene expression profiling showed that *ALK*$^+$ ALCL and *ALK*$^-$ ALCL have different gene expression profiles, further confirming that they are different entities.[562] The t(2;5)$^{NPM/ALK}$ can be identified by FISH or PCR. qRT-PCR of *NPM/ALK* is a promising and rapid approach for monitoring MRD in patients with ALCL.[563] Several cytogenetic and molecular studies have demonstrated that chromosomal aberrations other than the t(2;5)(p23;q35) may give rise to *ALK* fusion genes in ALCL. These alternative partners to the *NPM* gene include *TPM3* (nonmuscle tropomyosin) associated with t(1;2)(q21;p23), *TFG* (*TRK*-fused gene) associated with t(2;3)(p23;q21), *CLTC* (clathrin heavy chain gene) associated with t(2;17)(p23;q23), and *MSN* (moesin).[564–566] The moesin (*MSN*) gene at Xq11-12 acts as an alternative fusion partner for activation of ALK in ALCL and these lymphomas exhibit a distinctive membrane-restricted pattern of ALK labeling.[567]

ALK$^+$ DLBCLs are characterized by aggressive clinical behavior, granular cytoplasmic expression of ALK by immunostaining, and the presence of *ALK/CLTC* fusion as a result of t(2;17) or, rarely, cryptic *ALK* insertions to chromosome 4q22–24.[371–373] A few cases of ALK$^+$ DLBCL with t(2;5) have also been reported.[568,569]

AML1

See RUNX1 (below).

API2

- Marginal zone B-cell lymphoma (MALT type)

The t(11;18) translocation results in fusion between *API2* (also known as the c-*IAP2* or *BIRC2* gene) on 11q21 (a member of the *IAP* family with caspase-inhibitory functions) and *MALT* gene 1 (*MALT1*) on 18q21, encoding the paracaspase. The t(11;18)(q21;q21) is a characteristic chromosomal translocation in extranodal marginal zone B-cell lymphoma (mucosa-associated lymphoid tissue lymphoma; MALT). The *API2/MALT1* fusion protein has been shown to enforce activation of NF-κB signaling and increase inhibition of apoptosis,[570] partially by neutralizing apoptosis promoted by Smac.[571] There also appears to be interaction of some of the product of *API2/MALT1* fusion and *BCL10*, involving NF-κB-mediated inhibition of apoptosis, and hence promotion of MALT lymphomas.[355,356]

ATM

- B-CLL
- T-PLL
- MCL

The *ATM* gene (ataxia teleangiectasia mutated) located on chromosome 11q22~23 (Figure 2.7) participates in activation of *p53/TP53* after DNA damage and initiates cell cycle checkpoints in response to double-stranded DNA breaks by phosphorylating *p53*, *BRCA1*, *H2AX*, *cAbl*, IkB-alpha, and chk1, as well as other targets. The *ATM* gene encodes a large protein that belongs to a family of kinases which function in DNA repair and cell cycle checkpoint control (mainly intra S phase) following DNA damage. The *ATM*/*p53*-dependent DNA damage response pathway plays an important role in the progression of lymphoid tumors. Frequent inactivating mutations of the *ATM* gene have been reported in patients with rare sporadic T-PLL, B-CLL, and MCL. Inactivation of the *ATM* or *TP53* gene in B-CLL leads to aggressive disease.[572] The p53 dysfunction in B-CLL can occur in the absence of *TP53*

mutation and such dysfunction is associated with mutation of the gene encoding *ATM*.[573,574]

B-CLL patients with *ATM* deficiency have significantly shorter survival times (35 vs 97 months) and more aggressive disease, suggesting that *ATM* is involved in the leukemogenesis of B-CLL.[572,575,576] In occasional patients, the deletions of *ATM* can extend to the *MLL* gene locus. *ATM* gene mutations do not play a pivotal role either in the pathogenesis of FL or in its transformation to DLBCL.[577]

BCL1 (CCND1; PRAD1)

- MCL
- Plasma cell myeloma (MM)

The *BCL1* gene (major genomic breakpoint cluster region designated as *BCL1* for B-cell lymphoma/leukemia 1; syn: *CCND1* and *PRAD1*) is located on chromosome 11q13 and codes for cyclin D1. It is activated by its juxtaposition near the enhancer region of the IG heavy chain locus on chromosome 14. This phenomenon is caused by the t(11;14)(q13;q32) translocation, and defines MCL. The *CCND1* transcript or its encoded protein (cyclin D1/bcl-1) can be detected by PCR, cytogenetics/FISH (Figure 2.28), or immunohistochemical staining. Cyclin D1 forms a complex with cell cycle-dependent kinase (CDK)4 (and CDK6), which inactivates the retinoblastoma protein (pRb) via phosphorylation.

All cases of MCL (both typical and blastoid) contain t(11;14)(q13;q32). Hairy cell leukemia may express cyclin D1/bcl-1 at the mRNA or protein level in occasional cases, but the levels of expression are lower than in MCL and the expression is not associated with t(11;14), *CCND1* rearrangements, or *CCND1* gene amplification. Cyclin D1 expression is also found in MM patients and is associated either with t(11;14)(q13;q32) or extra copies of chromosome 11. Myeloma patients with the t(11;14) or trisomy 11 significantly overexpress cyclin D1 in comparison with patients without 11q abnormalities, who have cyclin D1 mRNA levels similar to healthy donors. Cyclin D1 overexpression/11q abnormalities identifies a subset of MM patients who are more likely to have a prolonged duration of remission and event-free survival following autologous transplantation.[578] Most authors reported no significant difference regarding overall survival between cyclin $D1^+$ and cyclin $D1^-$ MM patients.[579,580] In the series by Hoechtlen-Vollmar et al, however, the amplification of the cyclin D1 gene in MM was a significantly unfavorable parameter with regard to overall survival and progression-free survival by univariate analysis.[581] In multivariate analysis, cyclin D1 amplification and serum β_2-microglobulin were independent and well-suited parameters for predicting survival.[581] The relatively high frequency of cyclin D1 expression, compared with the chromosome 11 abnormalities, suggests that upregulation of cyclin D1 protein might be the result of other mechanisms as well.[582,583] *De novo* $CD5^+$ diffuse large B-cell lymphomas do not express cyclin D1/bcl-1 and are negative for t(11;14)$^{CCND1/IGH}$.

BCL2

- FL
- DLBCL

The *BCL2* family of proto-oncogenes is a critical regulator of apoptosis whose expression frequently becomes altered in human cancers, including some of the most common types of lymphomas and leukemias.[584] The *BCL2* gene (B-cell lymphoma/leukemia 2) on chromosome 18q21 encodes a 26 kDa protein that inhibits apoptosis through the mitochondrial pathway. Apart from bcl-2, anti-apoptotic proteins include Bcl-X_L and mcl-1, and pro-apoptotic proteins include BAX, Bak, Bcl-X_S, Bid, and Bik. The *BCL2* gene was originally discovered in FLs (Figure 2.31) with the t(14;18)(q32;q21) translocation.[585] The t(14;18) places *BCL2* under the control of the immunoglobulin heavy chain (*IGH*) Eμ enhancer which induces production of high levels of bcl-2 protein. Apart from t(14;18), variant translocations leading to juxtaposition of the

BCL2 with either *IGK* on 2p11 or *IGL* on 22q11 have been recognized in B-cell lymphomas. Clonal *BCL2/IGH* rearrangements are a very frequent observation in healthy individuals.[501,502]

Bcl-2 protein can easily be detected by routine immunohistochemistry. It is located in the mitochondrial inner membrane where it functions as a major negative regulator of apoptosis. Bcl-2 is widely expressed in normal lymphoid tissues, but is absent in germinal center B-cells. Overexpression of bcl-2 due to the t(14;18) represents the example of oncogenesis mediated by decreased cell death. Bcl-2 expression has been detected frequently in aggressive non-Hodgkin's lymphomas, regardless of t(14;18), and is associated with an unfavorable prognosis.[586–589] The *BCL2* gene amplification is another important mechanism for Bcl-2 protein overexpression in DLBCL.[590–592]

High bcl-2 protein expression is more frequent in B-cell lymphomas (51%) than in T-cell NHL (17%) and is heterogeneously distributed among the different histological subtypes.[587] DLBCLs display bcl-2 expression in 30–60% of cases, more frequently in nodal than in extranodal tumors.[590,593–595] Bcl-2$^+$ DLBCLs with t(14;18) probably represent a progression from FL. In DLBCL, bcl-2 protein-positive cases significantly outnumbered cases with t(14;18), suggesting that mechanisms other than translocation are operative in DLBCL.[588] Alternative mechanisms for bcl-2 protein expression include, among others, increased *BCL2* copy number (e.g. 18q+), or transcriptional deregulation by NF-κB.[596] The presence of a *BCL2* translocation at diagnosis has no impact on prognosis in patients with DLBCL,[588,597,598] but the bcl-2 protein expression (>50% positive tumor cells) is associated with decreased disease-free or overall survival.[23,588,589,598–600] Cases with t(14;18) and additional chromosomal aberrations have a worse prognosis than DLBCLs with t(14;18) only. Hermine et al reported the independent effect of bcl-2 protein expression to be predictive of poor diease-free survival, in agreement with the role of bcl-2 in chemotherapy-induced apoptosis.[587] Multivariate analysis confirmed the significant benefit for survival and event-free survival when rituximab is added to the standard chemotherapy regimen in bcl-2$^+$ DLBCL, suggesting that rituximab is able to prevent chemotherapy failure in patients with bcl-2 protein overexpression.[601] Maartense et al, reported a difference in the impact of bcl-2 overexpression on prognosis between elderly patients (>65 years) and younger patients (<65 years) with DLBCL; a negative prognostic value of overexpression of bcl-2 and p53 is not of concern for patients older than 65 years of age.[602] Some FLs do not express bcl-2, suggesting inhibition of apoptosis due to other factors (e.g. Bcl-X_L) rather than bcl-2 overexpression.[603]

Bcl-2 family proteins are expressed in a subset of peripheral T-cell lymphomas and the level of expression correlates with some histologic types, apoptotic rate, and proliferation.[604] In ALCL, bcl-2 and ALK expression are mutually exclusive.[605] ALK$^+$ ALCLs are sensitive to chemotherapy, therefore it is suggested that bcl-2 expression may be associated with chemoresistance. In systemic ALCL the expression of apoptosis regulating proteins, bcl-2 and P19, and the activation of caspase 3 are strongly related to ALK status.[606] Ten Berge et al suggested that the difference in clinical outcome between ALK$^+$ and ALK$^-$ cases may be due to differences in levels of apoptosis inhibition.[606] A high number of bcl-2$^+$ tumor cells in ALCL predicts an unfavorable prognosis.

Bcl-2 is expressed in ~60% of cases of classic Hodgkin lymphoma, more often in the nodular sclerosis type than in the mixed cellularity type.[607] Although simultaneous expression of bcl-2, mcl-1, and LMP-1 in classic Hodgkin lymphoma was reported to correlate with an excellent survival,[608] the expression of bcl-2 in patients with HL treated with adriamycin, bleomycin, vinblastine, and dacarbazine is associated with inferior failure-free survival and overall survival.[607]

Among AMLs, the percentage of bcl-2$^+$ cells is higher in M4 and M5 types, according to the FAB classification, and in cases with high white blood cell

counts.[609] High expression of bcl-2 is associated with a low complete remission rate after intensive chemotherapy (29% in cases with ≥20% positive cells vs 85% in cases with <20% positive cells) and with a significantly shorter survival. In multivariate analysis, the percentage of bcl-2$^+$ cells, age, and the percentage of CD34+ cells are independently associated with poor survival in AML patients.[609]

Increased bcl-2 expression in childhood ALL is inversely related to the presence of chromosomal translocations. However, it does not reflect increased disease aggressiveness or resistance to chemotherapy.[610]

BCL6

- DLBCL
- FL
- Other types of lymphoma (rare cases)

The proto-oncogene B-cell lymphoma 6 (*BCL6*; *LAZ3*) on chromosome 3q27 (Figure 2.44) encodes a transcription protein, with a POZ/zinc finger motif that is a involved in cell cycle control, proliferation, lymphocyte differentiation, immunologic response, and repression of genes involved in lymphocyte activation and differentiation, and inflammation.[611–613] The *BCL6* gene was initially identified through its involvement in the DLBCL translocation between 3q27 and the immunoglobulin gene at 14q32.[614,615] It is implicated in differentiation blockade of B-cells during the germinal-center (GC) reaction and in the pathogenesis of B-cell lymphomas.

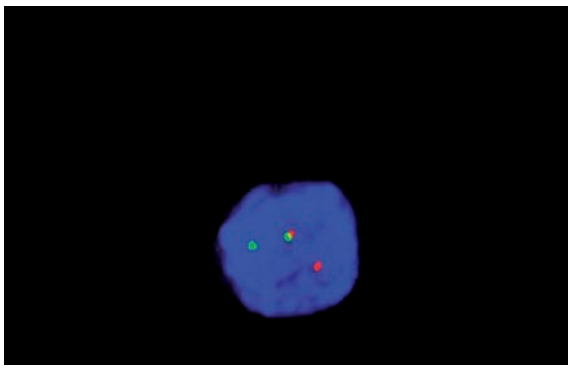


Figure 2.44 *BCL6* (3q27) rearrangement; FISH

The *BCL6* rearrangements are diverse and include translocations, microdeletions, point mutations, and hypermutation. It can be translocated to a number of translocation partners, most commonly immunoglobulin heavy (*IGH*/14q32) and light chain (*IGK*/2p12; *IGL*/22q11) genes. The other partners include 1q21, 2q21, 4p11, 5q31, 6p21, 7p12, 8q24, 9p13, 11q13, 11q23, 12q11, 13q14–21, 14q11, 15q21, and 16p11. Because of the large number of translocation partners, FISH analysis of *BCL6* is best achieved by using a break-apart probe. *BCL6* translocations are found in approximately 40% of DLBCLs and 5–15% of FLs.[432,616–618] Rearrangement of *BCL6* can be identified in other types of lymphoma, including marginal zone lymphoma and MCL.[244,619,620]

The *BCL6* gene rearrangement is more common than the occurrence of 3q27 translocations, suggesting alternative ways of gene rearrangement. A block in the normal downregulation of *BCL6* might favor differentiation arrest, continuous cell proliferation, survival, and genetic instability, all of which could lead ultimately to neoplastic transformation. This includes the requirement for *BCL6* gene expression for GC formation, and an immune reaction characterized by a high proliferation rate, oligoclonal expansion of B-cells, and the presence of active somatic mutations, and downregulation of *BCL6* expression in cells exiting the GC microenvironment. Downregulation of *BCL6* is necessary for lymphocytes within the GC to differentiate into memory B-cells or plasma cells, or to undergo selective apoptosis upon antigen stimulation. *BCL6* has been shown to directly suppress *p53* gene expression in GC B-cells.[621] Microarray analysis identified *BCL6* as a primary target of *p53*. The *BCL6* intron 1 contains a region in which three types of genetic alterations are frequent: translocation, point mutations, and internal deletions (TMDR; translocations, mutations, deletion regions) with a *p53* response element (p53RE) residing within the TMDR.[622]

Bcl-6 protein is selectively expressed by germinal center B-cells in normal lymphoid tissues. In lymphomas, bcl-6 is expressed in tumors arising from follicle center cells, including FL (~100%), Burkitt lymphoma (100%), the majority of DLBCLs (>80%), and nodular lymphocyte-predominant Hodgkin lymphoma (>80%).[623–626] Structural alterations of *BCL6*, including chromosomal translocations and somatic hypermutation, have been identified in several non-Hodgkin lymphoma with various frequencies. Biologic consequences of bcl-6 expression in DLBCL might vary according to the presence or absence and the nature of the underlying alteration of the *BCL6* gene (the partner involved in the translocation).[627] Mutations within the 5′ noncoding domain of the *BCL6* gene occur frequently in germinal center and post-germinal center lymphomas, including DLBCL, FL, and Burkitt lymphoma, and can contribute to the deregulation of *BCL6* expression.[613,627]

Most studies show a favorable prognostic impact of a high level of a *BCL6* expression (either by RT-PCR or immunohistochemistry). *BCL6* rearrangement has been described as an independent factor of favorable clinical outcome in DLBCL.[628] Also high *BCL6* mRNA expression appears to be a favorable prognostic factor in DLBCL.[629] As mentioned above, the predictive value of *BCL6* translocation on survival depends on the partner in *BCL6* translocation. Non-*IG/BCL6* translocations are an indicator of poor prognosis in DLBCL when compared with translocations involving immunoglobulin genes.[630] Through the use of gene expression profiling DLBCLs have been subdivided into three distinct categories: germinal center B-cell-like (GCB), activated B-cell-like (ABC), and type 3 DLBCL.[39,631,632] DLBCLs with a GCB profile express genes restricted to the germinal center such as *BCL6*, whereas DLBCLs with an ABC profile express plasma cell genes such as *XBP1*, *IL4*, *IRF,* and caspase 8.[632] GCB-type lymphomas are positive for CD10 and/or bcl-6, and are negative for post-germinal center cell markers typical for plasma cells, such as MUM1 or CD138. Patients with GCB type DLBCL had a significantly better overall survival than those with ABC type DLBCL.[631] Based on a central role of *BCL6* in the pathogenesis of DLBCL, Melnick et al predicted that targeting *BCL6* transcriptional repression complexes in malignant B-cells may constitute a novel form of transcription therapy for lymphomas and possibly other tumors.[633,634]

Although *BCL6* translocation is not necessary for large cell transformation of FL, *BCL6* rearrangement may constitute a subgroup with a higher risk of transforming into aggressive lymphoma.[635] Mutations of the *BCL6* gene are also observed in a subset of B-CLL, typically in cases harboring mutated *IGVH*.[636] In B-CLL, *BCL6* mutations did not appear to correlate with prognosis in the series by Sahota et al.[637] However, in a study reported by Sarsotti et al, the coexistence of *IGVH* and *BCL6* mutations was correlated with a shorter treatment-free interval compared to cases harboring only the *IGVH* mutation (median 55 months vs not reached), resembling the clinical course of unmutated *IGVH* cases (median treatment-free interval 44 months), indicating that *BCL6* mutations identify a subgroup of Binet stage A B-CLL patients with a high risk of progression despite the presence of the mutated *IGVH* gene.[636] As expected, in the same series, deletions of 17p13 (*TP53* locus) and 11q22 (*ATM* locus) were observed in cases with unmutated *IGVH*.[636]

BCL10

- Marginal zone B-cell lymphoma (MALT type)

At least three distinct chromosomal translocations, t(11;18)(q21;q21), t(1;14)(p22;q32), and t(14;18) (q32;q21), involving the *API2* (also known as c-*IAP2*), *BCL10*, and *MALT1* genes have been implicated in the molecular pathogenesis of MALT lymphoma. The t(11;18)(q21;q21) fuses the N-terminus of the *API2* gene to the C-terminus of the *MALT1* gene and generates a functional *API2/MALT1* product. The t(1;14)(p22;q32) and t(14;18)(q32;q21) bring the *BCL10* and *MALT1* genes, respectively, to the

IGH locus, deregulating their expression. In normal B-cell follicles, both *MALT1* and *BCL10* are expressed predominantly in the cytoplasm of centroblasts (high expression), centrocytes (moderate), and B-cells of the mantle zone (weak). In MALT lymphoma, *MALT1* and *BCL10* expression varied among cases with different chromosomal translocations. In MALT lymphomas with t(14;18)(q32;q21), tumor cells showed strong homogeneous cytoplasmic expression of both *MALT1* and *BCL10*. In cases with evidence of t(1;14)(p22;q32) or variants, tumor cells expressed *MALT1* weakly in the cytoplasm but *BCL10* strongly in the nuclei. In all MALT lymphomas with t(11;18)(q21;q21), tumor cells expressed weak cytoplasmic *MALT1* and moderate nuclear *BCL10*.[638] The *BCL10* gene encodes an amino-terminal caspase recruitment domain (CARD), an NF-κB-activating protein. The *BCL10* mutation may play a pathogenic role in B-cell MALT lymphoma development, particularly in aggressive and antibiotic unresponsive tumors. Pulmonary MALT lymphoma is commonly associated with t(11;18)(q21;q21)*API2/MALT1* and rarely with t(14;18)(q32;q21)*IGH/MALT1* and t(1;14)(p22;q32)*BCL10/IGH*.[639]

BCR

- CML
- AML
- ALL

The t(9;22) (Figures 2.24 and 2.25) leading to the fusion of the *BCR* and *ABL* genes is seen in CML and occasional cases of AML and ALL. The breakpoint in *BCR* (Figure 2.26) occurs in the M-bcr (for major breakpoint cluster region), m-bcr (minor breakpoint cluster region), or μ-bcr (micro-bcr) region. M-bcr is a cluster of 5.8 kb, between exons 12 and 16, also called b1 to b5 (most breakpoints being either between b2 and b3, or between b3 and b4) resulting in a 210 kDa chimeric protein (p210). In m-bcr breakpoints occur between exons 1 and 2, resulting in a 190 kDa protein (p190), and μ-bcr is associated with the presence of a larger protein (p230). (*See also t(9;22) and* ABL *above.*)

Core binding factor

- AML

Core binding factor (CBF), a heterodimer with subunits b (CBFb) and a (CBFa), is a hematopoietic transcription factor which binds to a core motif of the DNA (CBFb by itself does not contain any known DNA-binding motif or any transcriptional activation domain, but increases CBFa's affinity to DNA by 5- to 10-fold). CBF regulates the expression of myeloid and T-cell-specific genes such as *GM-CSF*, *M-CSFR*, *IL-3*, and T-cell receptors *TCRA-D*, *TCRB*, and *TCRG*. The most common type of AML with rearranged CBF is AML with t(8;21)*RUNX1/ETO*. Other types of CBF^+ acute myeloid leukemias (CBF-AMLs) include AML with *CBFB/MYH11* and t(16;16)/inv(16). CBF leukemias (CBF^+ AML), characterized by either inv(16)/t(16;16) or t(8;21), constitute AML subgroups with a favorable prognosis, but there is substantial biologic and clinical heterogeneity within these cytogenetic groups. Approximately one-third of patients with CBF^+ AML relapse. Bullinger et al, using gene expression profiling, identified a subgroup of CBF^+ AML cases (35/93) characterized by shorter overall survival times, and while there was no obvious correlation with fusion gene transcript levels, *FLT3* tyrosine kinase domain, *KIT*, and *NRAS* mutations, the newly defined inv(16)/t(8;21) subgroup was associated with elevated white blood cell counts and *FLT3* internal tandem duplications.[640] Illmer et al found a slightly higher incidence of $JAK2^{V617F}$ mutations in patients with a CBF leukemia (3.6%) when compared to all AML samples (1%).[641] The presence of $JAK2^{V617F}$ mutations in CBF leukemias was associated with an aggressive clinical course, with 80% of the patients relapsing.[641] qRT-PCR (RQ-PCR) for *RUNX1/ETO* or *CBFB/MYH11* fusion transcripts can be used for monitoring patients during therapy.[642]

CCND1

See BCL1, above

CEBP

- AML
- B-ALL

CCAAT-enhancer-binding-protein (CEBP) transcription factors play pivotal roles in proliferation and differentiation, including suppression of myeloid leukemogenesis. *CEBPA* is a single exon gene located on chromosome 19q. Mutations in *CEBPA* have been implicated in AML, most often in association with FAB types M1 and M2, although they have also been found in M4 and M5 types. Mutations in *CEBPA* occur in approximately 10% of all AMLs and are associated with a normal karyotype. In a series reported by Bienz et al, ~18% of normal karyotype AMLs had mutations in the *CEBPA* gene, and ~28% had *FLT3*-ITD.[643] Mutations in *CEBPA* tend to confer a favorable prognosis; the median disease-free survival and overall survival for patients with *CEBPA* mutations were 33.5 and 45.5 months, respectively, compared with 10 and 12 months for patients without the mutations.[643] Low levels of RNA expression of *CEBPA* have been noted in AML, where it may reflect adverse prognosis. *CEBPA* expression is downregulated in the presence of fusion protein *RUNX1/ETO* via inhibition of the *CEBPA* promoter.

Chapiro et al reported that CEBPA is activated by juxtaposition to the immunoglobulin gene enhancer upon its rearrangement with the immunoglobulin heavy-chain locus in precursor B-cell acute lymphoblastic leukemia harboring t(14;19)(q32;q13).[644] Akasaka et al showed that five *CEBP* gene family members are targeted by recurrent *IGH* chromosomal translocations in B-ALL: 10 cases with t(8;14)(q11;q32) involved *CEBPD* on chromosome 8, 9 cases with t(14;19)(q32;q13) involved *CEBPA*, 1 case involved *CEBPG*, located 71 kb telomeric of *CEBPA* on chromosome band 19q13, 4 cases with inv(14)(q11q32)/t(14;14)(q11;q32) involved *CEBPE*, and 3 cases with t(14;20)(q32;q13) involved *CEBPB*.[645]

ETO (CBFA2T1)

- AML

The *ETO* gene (for eight twenty-one; syn: *RUNX1T1*, *CBFA2T1*, *AML1T1*) is a transcription factor at chromosome 8q22 and *RUNX1/ETO* fusion acts as an inhibitor of transcription involving CBF DNA-binding sites. The *ETO* is a co-repressor that links the transcriptional pathogenesis of acute leukemias and B-cell lymphomas and offers a target for transcriptional therapy of hematologic malignancies.[646] *RUNX1/ETO* collaborates with other genetic alterations, such as mutations of receptor tyrosine kinases, to induce AML. Kuchenbauer et al analyzed 99 patients with a *RUNX1/ETO* rearrangement for additional aberrations: frequent chromosomal abnormalities included loss of a sex chromosome (56/99, 56.5%) and del(9q) (24/99, 24.2%), and the most frequent molecular aberrations included mutations of *KITD816* (3/23, 13%), *NRAS* (8/89, 8.9%), *FLT3* (3/87, 3.4%), *AML1* (1/26, 3.8%), and *PU1* (1/14, 7.1%) (*MLL*-PTD, *KRAS*, and *CEBPA* mutations were not found).[447] The *RUNX1/ETO* is generated by the t(8;21) translocation found in approximately 10–15% of acute myelogenous leukemias. Those AMLs usually represent FAB M2 morphology, often express CD19 and CD56 by flow cytometry analysis, and are characterized by a good response to chemotherapy and high remission rate. AML with t(8;21) belongs to a good prognostic group, similarly to those associated with t(15;17) and inv(16).[1] The *KIT*-D816 mutations confer a poor prognosis to *RUNX1*/*ETO*-positive AMLs and should therefore be included in the diagnostic workup.[440]

RUNX1/*ETO* transcript levels can be quantitatively assessed at diagnosis and during follow-up by RT-PCR.[647,648] Quantification of *RUNX1*/*ETO* transcript levels was shown to be a powerful tool for prediction of prognosis that is independent of pretreatment risk factors, and may be helpful for

directing therapeutic decisions. The median reduction of the initial *RUNX1/ETO* expression level was 4 log (range 0-5) after both induction and consolidation therapies.[647] The quality of molecular response after induction as well as consolidation therapies in patients with *RUNX1/ETO*-positive AML had a significant impact on the cumulative incidence of relapse, event-free survival, and overall survival (patient- or disease-related factors had no impact on the molecular response to induction or consolidation therapy).[647]

ETV6 (TEL)

- ALL
- MDS
- AML
- Non-Hodgkin's lymphomas

Rearrangements of the short arm of chromosome 12 are found frequently in hematologic malignancies, including MDS, AML, ALL, and lymphomas.[332,649] Aberration of chromosome 12 often involves the *ETV6* gene (previously *TEL*). The *ETV6* gene translocation partners involve more than 20 genes including *MDS2* (1p36), *ARNT* (1q21), *ARG* (1q25), *MDS/EVI1* (3q26), *FGFR3* (4p16), *BTL* (4q11~q12), *ACS2* (5q31), *PDGFRB* (5q33), *STL* (6q23), *MNX1* (7q36), *PAX5* (9p13), *JAK2* (9p24), *SYK* (9q22), *ABL* (9q34), *CDX2* (13q12), *TTL* (13q14), *TRKC* (15q25), *PER1* (17p12~p13), *CBFA2* (21q22), and *MN1* (22q11).[260,338,650–654]

Abnormalities of the short arm of chromosome 12 (12p) are found in about 5% of AMLs and MDS.[29] Additional, cryptic rearrangements of chromosome 12 can be identified by FISH studies (cryptic deletions of *ETV6* are rare in cytogenetically abnormal myeloid malignancies without 12p aberrations and tend to be more frequent in karyotypically normal AML, comprising 10% of the latter).[655] Raimondi et al analyzed 815 children with newly diagnosed ALL and found 94 cases (11.5%) with 12p abnormalities involving *ETV6*, which was associated with a favorable treatment outcome.[338]

The t(12;21)(p13;q22)$^{ETV6/RUNX1}$ is one of the most frequent genetic aberrations in childhood B-ALL, where it occurs in 25% of all cases. In contrast, the translocation is seen in only 3% of adult ALL cases. ALLs with *ETV6/RUNX1* fusion have a very good outcome with standard chemotherapy. This in part may be due to *ETV6/RUNX1* protein activity as a transcriptional repressor of the multidrug resistance gene (*MDR1*) expression.[656] *ETV6* deletions, trisomy 21, and an additional der(21) t(12;21) are seen in 55%, 14%, and 15% of *ETV6/RUNX1*$^+$ ALLs, respectively.[257] The 12p aberrations and near tetraploidy are more common in *ETV6/RUNX1*$^+$ patients, whereas the incidence of diploidy, pseudodiploidy, hypodiploidy, low hyperdiploidy, near triploidy, del(6q), chromosome 9, and 11q23 abnormalities is similar among *ETV6/RUNX1*$^+$ and *ETV6/RUNX1*$^-$ patients.[257] None of the *ETV6/RUNX1*$^+$ patients had a high hyperdiploid karyotype. Univariate analysis indicated that among *ETV6/RUNX1*$^+$ patients those with a deletion of the nontranslocated *TEL* allele had a worse prognosis than those without this abnormality.[257] The presence of *ETV6/RUNX1* gene fusion in childhood B-ALL does not seem to be associated with high in vitro drug sensitivity, except for L- as paraginase.[657]

The t(7;12)(q36;p13) a recurrent translocation involving the *ETV6* gene (12p13) and a heterogeneous breakpoint at 7q36, is almost exclusively present in infant AML (covers 30% of infant AMLs), while it is extremely rare in infant ALL and acute leukemia in older children.[418,659] The t(7;12) is associated with a poor outcome and an ectopic expression of HLXB9 is commonly involved in this genetic subtype of leukemia.[658]

FLT3

- AML
- B-ALL
- MDS

FMS-like tyrosine kinase 3 (*FLT3*) is a member of the class 3 receptor tyrosine kinase family that is preferentially expressed on hematopoietic progenitor

cells and mediates stem cell differentiation and proliferation. FLT3 is located at 13q12. *FLT3* is expressed at high levels in 70–100% of AMLs and in a high percentage of ALL, and is almost universally expressed in childhood B-ALL.[61,659–663] Mutations of *FLT3* have been detected in about 30% of patients with AML and a small number of patients with ALL or MDS.[58]

Cases of B-ALL with *MLL* gene rearrangements and those with high hyperdiploidy (>50 chromosomes) express the highest level of *FLT3*, with activating mutations of *FLT3* in 18% of *MLL*-rearranged and 28% of hyperdiploid cases.[663] The inhibitor, CEP-701, electively induces apoptosis in vitro in leukemic cells expressing high levels of *FLT3*.[663]

Two types of activating *FLT*-3 mutations have been described in AML: small internal tandem duplications (ITD; 20% of AMLs) and mutations in the critical Asp835 residue in the activation loop of *FLT3*. *FLT3*-ITD can range in size from three to hundreds of nucleotides. There is a statistically significant coincidence of invaginated nuclear morphology, loss of HLA-DR, and presence of the *FLT3*-ITD, suggesting that AMLs with these three features may represent a distinct AML subset.[664] *FLT3* mutations are indicative of a poor prognosis, independent of other risk parameters. Patients with *FLT3* mutations (especially ITD) have significantly shorter overall survival when compared to patients without mutations of *FLT3*.[57] The 5-year event-free survival rate for patients with an *FLT3*-ITD was 14%, significantly lower than that for patients without mutations (69%).[59] Chillon et al confirmed a high frequency of *FLT3* mutations in APL and in adult AML without recurrent cytogenetic translocations.[56] In the same series, the *FLT3* mutations were associated with some negative prognostic features at diagnosis (leukocytosis, high blast cell percentage, and elevated LDH values), but were not found to be an independent prognostic factor.[56] In the study by Schnittger et al, patients with *FLT3* length mutations had a significantly shorter event-free survival (7.4 vs 12.6 months) because of a higher relapse rate, but overall survival for patients with or without *FLT3* length mutations was similar.[55] Overexpression of *FLT3* ($>2 \times 10^5$ copies/μg RNA) is an unfavorable prognostic factor for overall survival in AML without *FLT3*-ITD.[665] A high mutant/wild-type *FLT3* ratio enhances the predictive value of *FLT3* for survival.[62] Patients with a high mutant/wild-type ratio (i.e., greater than 0.78) had significantly shorter overall and disease-free survivals, whereas survival in patients with a ratio below 0.78 did not differ from those without *FLT3* aberrations.[62] Whitman et al identified three genotypic groups of AML: normal $FLT3^{\text{wild-type/wild-type}}$, heterozygous $FLT3^{\text{ITD/wild-type}}$, and hemizygous $FLT3^{\text{ITD/–}}$. Disease-free survival and overall survival were significantly inferior for patients with hemizygous $FLT3^{\text{ITD/–}}$.[666] Although disease-free survival and overall survival for normal $FLT3^{\text{wild-type/wild-type}}$ and heterozygous $FLT3^{\text{ITD/wild-type}}$ groups did not differ, the overall survival of the hemizygous $FLT3^{\text{ITD/–}}$ group was worse than for the other groups.[666] Stirewalt et al showed that complete response rates were 35%, 67%, and 52% for patients with large (≥40), small (<40), and no ITDs, respectively.[667] Increasing ITD size was associated with decreasing overall survival (large, 13%; small, 26%, and no ITD, 21%).[667]

Alterations of the *FLT3* gene, in the form of ITDs and D835 point mutations, occur frequently in APL, but *FLT3* overexpression can be documented in patients without *FLT3* mutations.[540,668–670] In childhood APL, *FLT3*-ITD incidence was higher than in non-APL, although not statistically significant.[671] In a series reported by Au et al, 43% of the APL patients had an *FLT3* mutation (65 ITDs, 19 D835/I836, 4 ITD+D835/I836).[670] Patients with mutant *FLT3* had a higher rate of induction death (19% vs 9%), but no significant difference in relapse risk (28% vs 23%) or overall survival (59% vs 67%) at 5 years. *FLT3* length mutations were detected more frequently together with bcr3 compared with bcr1 (56.5% vs 19.4%) and in variant (hypogranular) APL (M3v) compared with classical (hypergranular) APL (64.5% vs 24.1%).[670]

In pediatric AML the presence of the *FLT3* mutations (ITD) is the single most significant, independent

prognostic factor for poor outcome.[61,672] *FLT3*-ITD predicts a poor outcome in MDS.[673,674] Shih et al showed that one-third of MDS patients acquire activating mutations of the *FLT3* or *NRAS* gene during AML evolution.[673] Also Georgiou et al showed that *FLT3* mutations (ITD and Asp835) are a critical additional event that transforms a subset of MDS patients to AML.[675]

FOXP1

- DLBCL
- Marginal zone B-cell lymphoma, extranodal (MALT type)

The human Forkhead-box (*FOX*) gene family consists of at least 43 members.[676] *FOXO3* and *FOXO4* genes are fused to the *MLL* gene in hematologic malignancies.[676] *FOXP1* (Forkhead box-P1) is a transcription factor that is differentially expressed in resting and activated B cells. The *FOXP1* gene has been mapped to chromosome 3p14.1 *FOXP1* expression has been demonstrated in a subset of DLBCLs, especially those of activated B-cell-like types (ABC). Streubel et al identified 9 cases (10%) of extranodal marginal zone B-cell lymphoma (MALT type) harboring t(3;14)(p14.1;q32), comprising tumors of the thyroid (3/6), ocular adnexae (4/20), and skin (2/20) (MALT lymphomas of the stomach, salivary gland, spleen, and lung and nodal MZLs were negative).[677] Most t(3;14)(p14.1;q32)$^+$ MALT lymphomas harbored additional genetic abnormalities, such as trisomy 3. Analysis by qRT-PCR showed upregulation of *FOXP1* in cases with t(3;14) (p14.1;q32) or trisomy 3.[677]

The genomic rearrangement of *FOXP1* can be identified by FISH in B-cell lymphomas with a t(3;14)(p13;q32) and t(2;3), and these aberrations are associated with a strong expression of FOXP1 protein in tumor cells, as demonstrated by immunohistochemistry.[678] Immunohistochemistry and FISH studies reported by Wlodarska et al of DLBCLs ($n = 98$) and extranodal MZLs ($n = 93$) showed a high expression of FOXP1 protein in ~13 and ~12% of cases, respectively (none of these cases showed *FOXP1* rearrangements by FISH).[678] Nakamura et al, using FISH, identified only one case of gastric MALT lymphoma with *IGH/FOXP1* rearrangement and extra copies of *FOXP1* in 10 of 59 cases (17%).[679] Barrans et al showed that high FOXP1 expression was almost exclusively confined to patients who lacked the germinal center phenotype, expressed MUM1 and bcl-2 in the absence of t(14;18), and had a particularly poor outcome in a group with an already poor prognosis.[680] The series reported by Banham et al showed that FOXP1 protein expression has prognostic significance in patients with *de novo* DLBCL.[681] The overall survival curves showed that patients grouped as FOXP1$^+$ (40%) had a significantly decreased overall survival (a median overall survival of 1.6 and 12.2 years in *FOXP1*$^+$ and *FOXP1*$^-$ cases, respectively). In addition, FOXP1$^+$ patients showed a clear trend to earlier progression in comparison to the FOXP1$^-$ patients. The analysis of FOXP1 expression with low, intermediate, and high IPI showed that FOXP1$^-$ patients had a better overall survival within each group, indicating that FOXP1 expression has a predictive value independent of the IPI.[681] Among primary cutaneous B-cell lymphomas, localization on the leg and expression of *FOXP1* are independent parameters associated with a poor prognosis.[682]

IGH

- B-cell lymphomas
- Plasma cell myeloma (MM)

The immunoglobulin heavy chain (*IGH*) gene locus is located on chromosome 14 at band 14q32.33, at the telomeric extremity of the long arm. It consists of 123 to 129 *IGHV* genes, depending on the haplotypes, 27 *IGHD* segments belonging to 7 subgroups, 9 *IGHJ* segments, and 11 *IGHC* genes. *IGH* is frequently involved in translocations occuring in B-cell malignancies (Table 2.3). *See also* immunoglobulin heavy chain genes rearrangements, *below*.

In patients with MM, 14q32 translocations and del(13) are the most frequent chromosomal

Table 2.3 Translocations involving the *IGH* gene on chromosome 14q32

Translocation	*Gene*	*Entity*
t(1;14)(p21;q32)	*BCL10* (1p21)	marginal zone B-cell lymphoma
t(3;14)(q27;q32)	*BCL6* (3q27)	diffuse large B-cell lymphoma
t(4;14)(p16;q32)	*FGFR3* (4p16)	multiple myeloma/plasma cell leukemia
t(5;14)(q31;q32)	*IL3* (5q31)	B-ALL
t(8;14)(q24;q32)	*MYC* (8q24)	Burkitt lymphoma, DLBCL (subset), B-ALL, multiple myeloma (rare), other B-cell lymphomas (rare)
t(9;14)(p13;q32)	*PAX5* (9p13)	lymphoplasmacytic lymphoma
t(11;14)(q13;q32)	*BCL1* (*CCND1*) (11q13)	mantle cell lymphoma, multiple myeloma
t(14;18)(q32;q21)	*BCL2* (18q21)	follicular lymphoma, DLBCL (subset)
t(14;18)(q32;q21)	*MALT1* (18q21)	marginal zone B-cell lymphoma
t(14;19)(q32;q13.1)	*BCL3* (19q13.1)	B-CLL

abnormalities, observed in 75% and 45% of cases, respectively.[403] Based on the presence of *IGH* rearrangements and del(13q), Avet-Loiseau et al defined four major genetic categories: (a) patients lacking any 14q32 abnormality (25%) and generally also lacking del(13); (b) patients presenting either t(4;14) or t(14;16), almost always associated with a del(13) (15% of patients); (c) patients with other 14q32 abnormalities and presenting del(13) (25%); and (d) patients with other 14q32 abnormalities but not presenting del(13) (35%).[403] The t(4;14)(p16;q32) is rarely observed in MGUS, whereas t(14;16)(q32;q24) is often associated with plasma cell leukemia.[403] In ~30% of the translocations involving *IGH*, the partner chromosomal gene is *CCND1* (11q13; *BCL1*).[430] The t(11;14)(q13;q32) (Figure 2.28) results in upregulation of the *CCND1* gene (cyclin D1/BCL1) and is the most common translocation detected in MM.[683]

IGVH gene mutations

- B-cell lymphomas
- B-CLL

Based on the *IGVH* mutations, B-cell lymphoproliferative disorders can be classified into 3 categories: (a) those with unmutated V_H genes, in which it was postulated that the cell of origin had not entered the germinal center; (b) tumors with ongoing V_H gene mutations, such as follicle center lymphomas, in which malignant cells remain under the influence of the germinal center reaction; and (c) B-cell tumors with mutated stable V_H genes, such as MM, which were postulated to have irreversibly traversed through the germinal center.[684]

Somatic hypermutations in rearranged immunoglobulin heavy chain variable region (*IGVH*) genes allow a division of CLL patients into two categories with significantly different outcomes: mutated *IGVH* and unmutated *IGVH* genes. The unmutated *IGVH* gene is a poor prognostic factor.[685,686] Other molecular markers associated with CLL biology and prognosis include *ATM*, *p53*/*TP53*, and genes involved in telomere function.[541] Most of those molecular markers, as well as morphologic and immunophenotypic parameters, correlate with the status of *IGVH* mutation. Positive ZAP-70 and CD38 expression is associated with poor prognosis and is more often identified in patients with unmutated *IGVH*.[686–701] Although present in both *IGVH* mutation subgroups, *ATM* mutations are more common in cases with unmutated *IGVH* genes and are associated with a poor prognosis.[702] Buhl et al identified a novel gene, *CLL* upregulated gene 1 (*CLLU1*) mapped to chromosome 12q22, that is highly upregulated in B-CLL cells without *IGVH* mutations.[703]

In MCL mutated *IGVH* was associated with a higher rate of complete remission, but there was no

correlation between *IGVH* mutation status and other clinical characteristics or overall survival.[704]

JAK2

- P. vera
- Essential thrombocythemia (ET)
- Chronic idiopathic myelofibrosis (CIMF)

The Janus family of cytosolic tyrosine kinases (JAK) plays an essential role in development and normal hematopoiesis.[705] *JAK2*, encoded on chromosome 9p24, plays a central role in nonprotein tyrosine kinase receptor signaling pathways, which could explain its involvement in malignancies of different hematologic lineages.[654,705] The fusion of *TEL* to *JAK2* has been reported in acute leukemia and blast phase of CML. Novel *JAK2* somatic mutations (e.g. a G–C to T–A transversion, at nucleotide 1849 of exon 14, resulting in the substitution of valine to phenylalanine at codon 617; *JAK2*^{V617F}) have been reported in subsets of classic non-CML (*BCR/ABL*-negative) chronic myeloproliferative disorders (CMPDs) such as P. vera, chronic idiopathic myelofibrosis (CIMF), and ET.[706–710]

Bidirectional sequencing of all *JAK2* exons revealed a nonsynonymous coding region mutation in the DNA template derived from peripheral blood granulocytes of P. vera patients: a guanosine to thymidine alteration in exon 14 of the gene. This results in valine to phenylanine substitution at a conserved residue (Val617Phe or p.V617F). Nearly 50% of all *BCR/ABL*-negative CMPD patients exhibit c2343 G>T by sequencing or ARMS (amplification-refractory mutation system). These include 97% of P. vera, 57% of ET, and 50% of CIMF.[710] Levine et al found the *JAK2*^{V617F} mutation in granulocytes from 74% of P. vera (25% homozygous), 32% of ET (3% homozygous), and 35% of CIMF (9% homozygous) patients.[707] With the PCR single nucleotide polymorphism genotyping assay performed in routine bone marrow biopsy specimens, Bousquet et al showed an incidence of *JAK2*^{V617F} mutation in 87% of P. vera cases, 67% of ET cases, and 66% of CIMF cases.[711] Buccal cell analysis showed wild-type *JAK2* in these patients. Molecular and cytogenetic analyses demonstrated that homozygous *JAK2*^{V617F} is the result of duplication of the mutant allele. An acquired, somatic *JAK2* mutation is strongly associated with *BCR/ABL1*-negative CMPD, most strikingly P. vera, and a specific mutation is probably a functionally important contributor to the clinical phenotype. Figure 2.45 shows *JAK2* mutation detection by PCR analysis.

The *JAK* family of nonreceptor tyrosine kinases (TKs) is known to include four members: JAK1, JAK2, JAK3, and TYK2. Each JAK member responds to an external signal in co-operation with a select subset of cell-surface receptors. These JAK-dependent receptors respond to particular cytokines and growth factors, including interferons, interleukins (e.g. IL-3 and IL-5), EPO, granulocyte-colony stimulating factor, granulocyte-macrophage colony stimulating factor, and thrombopoietin. Receptors that use JAKs are known as 'type I' cytokine receptors because they lack an intracellular TK domain of their own, and are strictly dependent on signaling through the JAKs to phosphorylate downstream targets upon ligand binding. JAKs have two kinase-like domains, in contrast to other TKs (and hence the name Janus after the

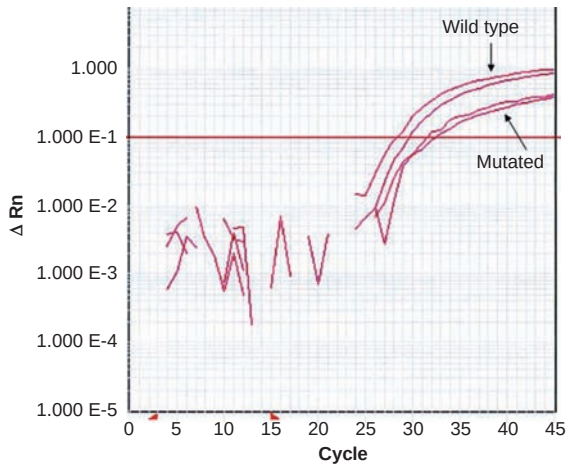


Figure 2.45 *JAK2* mutation (both wild type and mutated forms of *JAK2* are identified); PCR

two-headed Roman god of beginnings and doorways). JAK proteins also contain 7 JAK homology (JH) domains. *JAK2*^{V617F} alters a highly conserved residue within the 'pseudokinase' domain (JH2) of the *JAK2* TK protein, resulting in constitutive activation of the adjacent true kinase domain (JH1). JH2 looks similar to classical protein kinase domains, but lacks actual kinase activity, despite containing functionally important tyrosine residues. The JH1 domain, in contrast, has tyrosine residues which are phosphorylated when the protein becomes active, and also possesses kinase activity. These JH1 tyrosines are phosphorylated by another JH1 domain on a different JAK protein whenever JAK is activated by a receptor–ligand binding. Additionally, three inhibitory regions (IR1, IR2, and IR3) within JH2 have been identified; IR3 directly inhibits the JH1 domain. The V617F residue does not reside immediately within one of these directly inhibitory segments, but is near IR1 in a small control loop. Conformational change brings two JAK molecules into closer proximity than they were in the inactive state, leading to phosphorylation of each one. Next, the activated JAKs phosphorylate their associated receptors, which provide recruitment and docking sites for cytoplasmic transcription factors such as the STAT (signal transducers and activators of transcription) proteins. Activated STATs separate from the receptor complex and translocate to the nucleus to initiate transcription of diverse target genes.

Most studies have shown no differences in the clinical phenotype of patients with or without the *JAK2* mutation. However, a longer disease duration was associated with the 'homozygous' status.[707] In a study of patients with P. vera by Tefferi et al, a statistical comparison between *JAK2*^{V617F} heterozygotes and homozygotes did not reveal any significant association with regard to age, gender, leukocyte, or platelet count at the time of diagnosis, duration of disease, or the incidence of thrombosis or bleeding.[712] However, compared with their heterozygote counterparts, *JAK2*^{V617F} homozygote patients displayed a significantly higher hemoglobin level at the time of diagnosis, a higher rate of fibrotic transformation, an increased incidence of pruritus, and higher PRV-1 transcript levels in their blood granulocytes.[712]

When correlating the detection rate of the mutant *JAK2* with morphologic data, Bousquet et al found that the most robust marker for the diagnosis of CMPD was the presence of a prominent megakaryocytic proliferation with cluster formation and abnormally lobulated or naked nuclei.[711] Patients with the *JAK2*^{V617F} show abnormal nuclear megakaryocytic phospho-STAT5 (nMEG pSTAT5) expression by immunohistochemistry.[713]

c-KIT

- AML
- Systemic mastocytosis
- Chronic myeloproliferative disorders
- High grade MDS

Mutations in codon D816 of the *KIT* gene represent a recurrent genetic alteration in AML and are associated with a CBF^{+} AML. In a series reported by Boissel et al, c-*KIT* mutations were present in 17% of AML patients.[539] Among the pediatric AML patients, 40% had a mutation in *KIT* (11.3%), *RAS* (18%) or *FLT*-ITD (11.1%), while 70% of CBF^{+} leukemias were associated with a mutation of *KIT* or *RAS*.[714] c-*KIT* exon 8 mutations were more frequent in the inv(16) than in the t(8;21) subset (20 vs. 6%).[539] In AML c-*KIT* mutations have an independent negative impact on overall survival (median 304 vs 1836 days) and event-free survival (median 244 vs 744 days) in patients with t(8;21), but not in patients with a normal karyotype.[440] *KIT* mutations are associated with a shorter survival in t(8;21), but not inv(16) patients (in contrast, *RAS* mutations did not affect the prognosis).[539] The high frequency of the c-*KIT* gene exon 8 deletion plus insertion mutations in AML suggests an essential role for this region of the receptor's extracellular domain, and the association with inv(16) and t(8;21) suggests a link between these changes in the pathogenesis of AML.[715] Activating c-*KIT* mutations have been

identified also in patients with mastocytosis and other chronic myeloproliferative disorders, including P. vera.[716,717] Mutations of c-*KIT* have been reported in high-grade MDS, but not in refractory anemia (RA) and other low-grade myelodysplasias.[718]

c-MAF

See t(14;16), *above*.

MALT1

- Marginal zone B-cell lymphoma (MALT type)
- DLBCL (rare cases)

At least three distinct chromosomal translocations, t(11;18)(q21;q21), t(1;14)(p22;q32), and t(14;18)(q32;q21) involving *API2*, *BCL10*, and *MALT1*, respectively, have been implicated in the molecular pathogenesis of extranodal MZL of the MALT type. Two of those translocations involve the *MALT1* gene at 18q21: t(11;18) and t(14;18). The t(11;18)(q21;q21) fuses the N-terminus of the *API2* gene to the C-terminus of the *MALT1* gene and generates a functional *API2/MALT1* product. The *API2/MALT1* translocation occurs in up to 50% of gastric MALT lymphomas and in some cases of MZL of the lung, salivary gland, and orbit.[475,476] Nodal MZLs do not display t(11;18). The t(14;18)(q32;q21) involves the *IGH* and *MALT1* genes [a t(14;18) translocation in MZL cannot be distinguished from t(14;18) of FL based on classic cytogenetic studies alone]. The *IGH/MALT1* fusion has been reported in MZL occuring in liver, skin, orbit, lung, salivary gland, spleen, and stomach.

Approximately 10 to 20% of primary cutaneous MZLs have recurrent chromosomal translocations, including the t(14;18)(q32;q21)*IGH/MALT1*, t(11;18)(q21;q21), and t(3;14)(p14;q32).[719] The *IGH/MALT1* translocation has been described in primary cutaneous DLBCL[720] and the *API2/MALT1* translocation in DLBCL of the orbit.[721]

The t(1;14)(p22;q32) and t(14;18)(q32;q21) bring the *BCL10* and *MALT1* genes, respectively, to the *IGH* locus on chromosome 14, resulting in aberrant expression of BCL10 and MALT1 proteins. In normal B-cell follicles, both MALT1 and BCL10 are expressed predominantly in the cytoplasm of centroblasts (high expression), centrocytes (moderate), and B-cells of the mantle zone (weak). In MALT lymphoma, MALT1 and BCL10 expression varies among cases with different chromosomal translocations. In MALT lymphomas with t(14;18)(q32;q21), tumor cells show strong homogeneous cytoplasmic expression of both MALT1 and BCL10. In cases with evidence of t(1;14)(p22;q32) or variants, tumor cells expressed MALT1 weakly in the cytoplasm, but BCL10 strongly in the nuclei. In all MALT lymphomas with t(11;18)(q21;q21), tumor cells expressed weak cytoplasmic MALT1 and moderate nuclear BCL10.[638]

The *MALT1* gene translocations can be detected by FISH using a break-apart probe (which confirms the presence of translocation but does not specify the partner gene) or fusion probes for *API2/MALT1* and *IGH/MALT1*.

MDR

- Various hematopoietic tumors

Resistance to chemotherapy is a poor prognostic factor. The failure of treatment may be due to rapid efflux of the chemotherapeutic agents from the intracellular environment or the inability of cancer cells to undergo apoptosis. The expression of multidrug resistance (MDR)-related proteins and/or *MDR* genes has been correlated with tumor sensitivity to treatment and clinical outcome in many hematologic malignancies, including AML, ALL, B-CLL, and non-Hodgkin's lymphomas. The best known *MDR* gene, *MDR1*, encodes P-glycoprotein, a 170 kD membrane protein acting as a multispecific drug efflux pump. P-glycoprotein belongs to the adenosine triphosphate (ATP)-binding transporter family. P-glycoprotein confers long-term resistance to caspase-dependent (but not to caspase-independent) apoptotic stimuli.[722] The methods for identifying the MDR include molecular tests (e.g. PCR) for gene

expression and immunophenotyping. P-glycoprotein can be detected by flow cytometry and/or immunohistochemistry using monoclonal antibodies (e.g. MRK16, C219, and JSB-1). The MDR-phenotype is often associated with an increased expression of P-glycoprotein at the plasma membrane.[723] MDR-related proteins, including P-glycoprotein, may be activated in association with overexpression of mutant or inactivated *p53/TP53*. Other proteins that may confer drug resistance include multidrug resistance-associated protein 1 (MRP1), lung resistance protein (LRP), and breast cancer resistance protein (BCRP). Although the status of P-glycoprotein expression correlates with relapse rate and overall survival, some patients relapse in spite of low levels of *MDR1* or *MRP1*. Phosphorylation of P-glycoprotein, mutations of the MDR, and cell cycle stage can influence the functional status of the efflux pump.

MDR is frequently expressed in AML and is associated with a lower complete remission rate and, in some studies, with lower overall survival for patients on standard chemotherapy.[724–729] The three major candidates accounting for the development of MDR in AML are *MDR1*, *MRP1*, and the lung resistance protein gene (*LRP*). AML in the elderly is associated with an increased frequency of unfavorable cytogenetics and *MDR1* expression, both of which independently contribute to poor outcome.[724,728] Approximately 70% of AMLs in elderly patients express *MDR1*.[728] Complete remission in elderly AML patients ranges from 81% in *de novo* $MDR1^-$ AML with favorable to intermediate cytogenetics, to 13% in MDR^+ secondary AML with unfavorable cytogenetics. *MDR1*/P-glycoprotein expression is less common in younger patients with AML (35%) and is also associated with resistance disease and lower complete remission rates,[724] but in childhood AML expression of *MDR1* is not associated with a poor prognosis.[730] *MDR1* expression in AML proved to be an independent prognostic factor for the outcome of induction therapy and overall survival. MRP1 expression was an independent predictor for disease-free survival in the multivariate analysis, whereas LRP expression had no impact on treatment outcome.[731] Schaich et al showed that the proportion of *MDR1* expression correlated with the presence of aberrant phenotype: significantly more $MDR1^+$ AML patients were found within t(8;21), +8, +21, del(7q), del(5q), and −7 abnormalities of (3q), and multiple aberrations.[732] In contrast, no patient with inv(16) was positive for *MDR1*. Only 26% of $MDR1^+$ patients with aberrant karyotypes achieved complete remission (54% in $MDR1^-$ counterparts).[732] Furthermore, within abnormalities of 11q, +21, +22, −5, or 3q no $MDR1^+$ patient reached complete remission, whereas the $MDR1^-$ counterparts had complete remission rates comparable to the rate of patients with a normal karyotype.[732] List et al showed that addition of cyclosporine A to an induction and consolidation regimen containing infusional cytarabine and daunorubicin significantly reduces resistance to these drugs, prolongs the duration of remission, and improves overall survival in patients with high-risk AML,[733] implicating P-glycoprotein as an important cellular mechanism of resistance in AML patients.

P-glycoprotein expression adversely impacted the rate of complete remission or restored chronic phase in the blast phase of CML.[734] In MDS, P-glycoprotein expression increases as the disease progresses. MDR genes have been associated with prognosis in ALL.[735] Overexpression of *MDR1* and *MDR3* genes and P-glycoprotein has been correlated with resistance to therapy and poor prognosis in B-CLL.[736] Patients with MM refractory to alkylating agents frequently express P-glycoprotein (MDR phenotype). Also high-grade lymphomas frequently express P-glycoprotein (before treatment), which at least partially may be responsible for chemoresistance and poor prognosis.[737,738] Among HIV-associated lymphomas, response to treatment and overall survival were significantly lower in patients with positive P-glycoprotein expression.[737] Aggressive behavior of T/NK-cell lymphomas (nasal type) despite chemotherapy treatment may be explained in part by P-glycoprotein expression.[739]

MLL

- AML
- B-ALL
- T-ALL
- Biphenotypic (mixed lineage) acute leukemia
- MDS

The *MLL* gene (*for* Mixed Lineage Leukemia or Myeloid/Lymphoid Leukemia) on chromosome 11q23 is the human homolog of *Drosophilia trithorax* (trx), a master regulator of *HOX* genes, essential for patterning during embryogenesis.[740] Fusion transcripts involving the *MLL (ALL1)* gene are found in both AML and B-ALL (Figure 2.46 and 2.47), and other hematologic malignancies. Approximately 50 different chromosomal translocations of the *MLL* gene are currently known and are associated with high-risk acute leukemia. The *MLL* rearrangements are most often seen in AML with monocytic differentiation (AML-M4 and AML-M5), acute leukemias in infants, and leukemias related to treatment with DNA topoisomerase II inhibitors. In AML, the most frequent translocation partners include t(9;11)$^{AF9/MLL}$, t(10;11)$^{AF10/MLL}$, t(6;11)$^{AF6/MLL}$, and t(11;19)$^{MLL/ELL}$.

In the majority of cases, abnormalities involving the *MLL* gene are associated with a very poor prognostic outcome. The incidence of AML with *MLL* rearrangement was significantly higher in therapy-related AML (t-AML) than in *de novo* AML (9.4% vs 2.6%) and in patients younger than 60 years (5.3% vs 0.8%).[741] Compared with AMLs with intermediate karyotype, AMLs with 11q23/*MLL* rearrangement have a worse outcome (comparable with AML with an unfavorable karyotype).[741] In childhood ALL t(4;11)(q21;q23) was associated with a poor prognosis,[394,396,742,743] which was independent of other poor prognostic features, including the *BCR/ABL* fusion gene and persistence of MRD (0.01% or more at the end of the 6-week remission induction phase).[743] T-ALL with HOX11 expression of *MLL/ENL* fusion has a highly favorable outcome.[744] *MLL* amplification (Figure 2.46) is recognized as a recurrent aberration in AML and MDS, associated with adverse prognosis, poor response to chemotherapy, and karyotype complexity.

Partial tandem duplications of the *MLL* gene (*MLL*-PTD) have also been reported in karyotypically normal AML and in AML with +11.[68,745] In the past, *MLL*-PTD identified a subgroup of AML patients with an unfavorable prognosis.[68,745] Intensive consolidation therapy that includes autologous peripheral stem-cell transplantation, however, may change the prognostic significance of *MLL*-PTD. In a recent study by Whitman et al (238 adults aged 18–59 years with cytogenetically normal *de novo* AML), 24 (~10%) patients harbored a *MLL*-PTD, and 92% of these patients achieved complete remission compared with 83% of patients without *MLL*-PTD, and neither overall survival nor disease-free survival significantly differed between the two groups.[746] Thirteen *MLL*-PTD$^+$ patients relapsed within 1.4 years of achieving complete remission.[746]

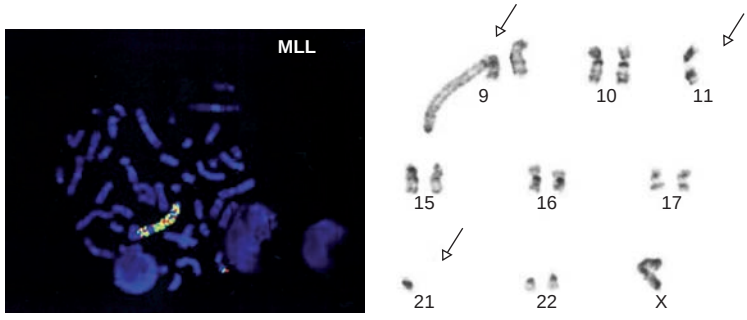


Figure 2.46 *MLL* gene amplification (chromosome 9 amplification); A, FISH; B, cytogenetics (partial karyotype)

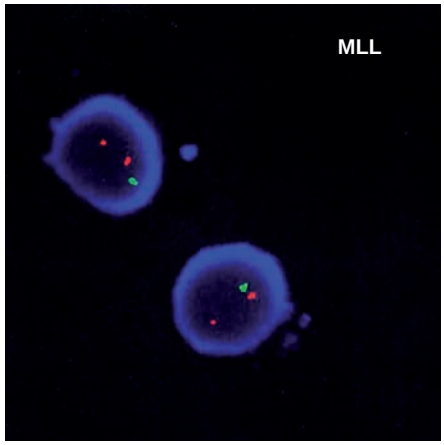


Figure 2.47 Deletion of the *MLL* gene; FISH

c-MPL

- ET
- Chronic idiopathic myelofibrosis (CIMF)

Thrombopoietin, the key growth factor in megakaryopoiesis, acts through its receptor, MPL, and the JAK-STAT signal transduction pathway. Recently, two mutations in the thrombopoietin receptor gene (c-*MPL*) have been found: W515L and W515K.[747,748] These mutations have been evident in patients with ET and CIMF, but not in P. vera.[748,749] *MPL*$^{W515L/K}$ and *JAK2*^{V617F} can co-exist in CIMF.[750] Multiple molecular abnormalities are involved in the pathogenesis of the CMPDs and aberrant *MPL* expression may be a common denominator of aberrant signaling in both the JAK2^{V617F}-positive and JAK2^{V617F}-negative CMPDs.[751]

MUM1 (IRF4)

- Plasma cell myeloma (MM)
- DLBCL
- Other B-cell lymphoproliferations

The *MUM1* (multiple myeloma 1, or interferon regulatory factor 4) is a protooncogene that is deregulated as a result of the (6;14)(p25;q32) chromosomal translocation in MM, and is also expressed in a variety of malignant lymphomas (melanoma-associated antigen, mutated 1, also named *MUM1*, is located on chromosome 19p). MUM1, which can be identified by immunohistochemistry, may provide a marker for the identification of transition from bcl-6 positivity (germinal center B-cells) to CD138 expression (immunoblasts and plasma cells). In normal B-cells, MUM1 expression is thought to denote the final step of intragerminal center B-cell differentiation and subsequent maturation towards the plasma cells. In DLBCL (Figure 2.48), MUM1 is detected in 50–75% of cases, and is seen both with and without bcl-6 expression.[752–754] The majority of DLBCLs expressing CD10 and bcl-6 are MUM1^{-}. In a study by Colomo et al, no association was found between MUM1 expression and outcome.[23] Among primary cutaneous DLBCLs, staining for BCL2, OCT2, and/or MUM1 is associated with a

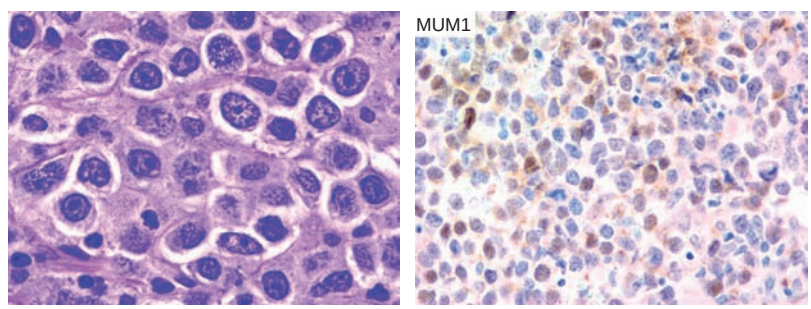


Figure 2.48 Diffuse large B-cell lymphoma with expression of MUM1 (A, H&E staining; B, immunohistochemistry staining for MUM1)

poor, and BCL6 with a favorable prognosis.[755,756] In the microarray study, the expression of MUM1 in at least 30% of tumor cells was associated with a significantly worse outcome.[757] The *MUM1* gene has been identified as an oncogene transcriptionally activated by t(6;14)(p25;q32) chromosomal translocation in MM. The published results about the prognostic significance of MUM1 expression in B-CLL are not conclusive.

c-MYC

- BL
- DLBCL
- B-ALL
- Follicular lymphoma (transformation)
- MCL, blastoid variant
- Plasma cell myeloma (MM)
- B-CLL/PLL (transformation)
- ALCL, ALK$^+$

The MYC is a leucine zipper transcription factor involved in regulation of the cell cycle, proliferation, differentiation, and cellular immortalization. The *MYC*/immunoglobulin rearrangements play a major role in the pathogenesis of B-lineage lymphomas by deregulation of c-*MYC* oncogene expression via juxtaposition to one of the immunoglobulin enhancers. The c-*MYC* gene is translocated to the *IG* loci in all BLs (Figures 2.21 and 2.22).[758,759] The t(8;14)(q24;q32) translocation which involves c-*MYC* (on chromosome 8q24) and *IGH* (on chromosome 14q32) is the most frequent translocation (80% of BL). The remaining (variant) translocations include t(2;8)(p11.2;q24) involving *IGK* (on chromosome 2p11.2) and t(8;22)(q24;q11) involving *IGL* (on chromosome 22q11). This results in overexpression of c-*MYC*, driving cell proliferation and expression of other genes involved in cell growth.[758]

The c-*MYC* translocations are characteristic of BL, but they have been reported also in a subset of DLBCL (6%)[760] and other hematolymphoid tumors including atypical Burkitt lymphoma/Burkitt-like lymphoma, FL, a blastoid variant of MCL, MM, prolymphocytoid transformation of B-CLL/SLL, Burkitt-type precursor B-lymphoblastic leukemia, and ALK$^+$ ALCL.[427,434,561,761–769] The 8q24^{MYC} abnormalities are usually associated with an aggressive clinical course, high IPI (International Prognostic Index), advanced stage, shorter overall survival, and generally poor prognosis.[770,771] The 8q24 (c-*MYC*) aberrations carry a negative prognostic significance, either at diagnosis or at disease progression, in a variety of nonHodgkin's lymphomas.[434] The overexpression of c-*MYC* is associated with a shorter overall survival in MCL.[772] and clinically aggressive forms of MM.[766]

Subsets of lymphomas may display dual translocations of t(14;18) and chromosome 8q24^{MYC} gene rearrangement. They are of high grade with morphologic features of BL/Burkitt-like lymphoma or DLBCL. Patients with concurrent t(14;18)$^{IGH/BCL2}$ fusion and 8q24^{MYC} translocations have an extremely poor outcome.[426,773] Overexpression of c-*MYC* has been shown to be a consistent finding in ALK$^+$ ALCL, but not ALK$^-$ ALCL, and the *MYC* gene is considered a downstream target of deregulated ALK signaling.[561,769,774] In addition to the characteristic t(2;5)(p23;q35) translocation, Monaco et al reported a t(3;8)(q26.2;q24) translocation and *MYC* gene rearrangement (confirmed by FISH analysis) in pediatric ALCL.[769] C-*MYC* may be a downstream target of ALK signaling and its expression a defining characteristic of ALK$^+$ ALCLs.[561] The *MYC* rearrangement may be responsible for the aggressive clinical behavior in a subset of ALK$^+$ ALCL.[769]

Detection of c-*MYC* translocations can be achieved by classic cytogenetics and FISH. Cytogenetics is limited by the need for fresh tissue and the difficulty in obtaining suitable metaphases from the neoplastic cells. Detection of c-*MYC* by FISH using the commercially available dual-fusion FISH (D-FISH) *IGH*/*MYC* probe might not always be possible, as some 8q24 break-points occur outside the region spanned by the relatively small (750 kb) locus-specific *MYC* probe.[766,775] Because there are only a few assays available for detecting light-chain translocations, most

of them are identified by conventional cytogenetics. Einerson et al developed and validated four FISH probes: two break-apart probes to detect *IGK* and *IGL* translocations, and two dual-color, dual-fusion FISH (D-FISH) probes to detect *IGK/MYC* and *IGL/MYC*.[776] Seven specimens, all of which lacked *MYC* rearrangements using a commercial *IGH*/*MYC* D–FISH probe, were found to have 8q24 breakpoints within a cluster region >350–645 kb 3′ from *MYC*, provisionally designated as the Burkitt variant rearrangement region 2 (BVR2).[776]

NPM1 (nucleophosmin)

- AML
- APL
- ALCL
- MDS
- CMML

The *NPM1* gene (5q35) encodes a multifunctional phosphoprotein (NPM) with oncogenic and tumor suppressor functions. NPM plays multiple roles in ribosome assembly and transport, cytoplasmic-nuclear trafficking, centrosome duplication, and regulation of the *ARF-p53* tumor-suppressor pathway.[777–779] Translocations involving the *NPM* gene cause cytoplasmic dislocation of the NPM protein. In hematologic malignancies, the *NPM1* gene is frequently involved in chromosomal translocation, mutation, and deletion. The *NPM1* gene on 5q35 is translocated with the anaplastic lymphoma kinase (ALK) gene in ALCL with t(2;5). The *MLF1* and *RARα* genes are fused with *NPM1* in myelodysplastic syndrome/AML with t(3;5) and APL with t(5;17), respectively.[25,778–783] The region of chromosome 5 to which *NPM1* maps is deleted in a proportion of *de novo* MDS, and loss of chromosome 5 is extremely frequent in therapy-related MDS.[777] Rare cases of CMML may display *NPM1* mutation.[784] Recently, mutations of exon 12 have been found in a significant proportion of *de novo* AML, especially in those with a normal karyotype.[25,778,782,783] Mutations in *NPM1* disrupt the nucleolar-localization signal and cause an aberrant cytoplasmic localization of the protein, which can be detected by immunohistochemistry.[782] It is suggested that loss of NPM function might impair the *p53* pathway. AML with *NPM1* mutation may display peculiar cytomorphologic features, such as nuclear invaginations.[785]

Cytoplasmic NPM (NPMc) is a characteristic feature of a large subgroup of patients with AML who have a normal karyotype, *NPM1* gene mutations, and responsiveness to induction chemotherapy.[782] Exon-12 *NPM1* gene mutations are reported in approximately 60% of AMLs with normal karyotype (about one-third of all adult AMLs).[782] The molecular signature of NPMc$^+$ AML includes upregulation of several genes putatively involved in the maintenance of a stem-cell phenotype, suggesting that NPMc$^+$ AML may derive from a multipotent hematopoietic progenitor.[786] *NPM1* mutations in AML predict a good response to induction therapy and a favorable prognosis,[781,787–789] and could serve to monitor MRD.[790] In a large series of AML patients, Falini et al showed that all 200 AML cases expressing cytoplasmic NPM (NPMc$^+$ AML) carried *NPM1* mutations, and none of the 250 cases with nucleus-restricted NPM (NPMc$^-$ AML) was mutated.[791] Pasqualucci et al showed NPMc$^+$ AML falling into different FAB categories and suggested that, for clinical use, NPMc$^+$ AML be provisionally regarded as a separate AML with prognostic significance.[783] *NPM1* gene mutations causing aberrant cytoplasmic localization of nucleophosmin have been demonstrated to be the most frequent submicroscopic alterations in cytogenetically normal AML and to confer improved prognosis, especially in patients without a concomitant *FLT3* gene internal tandem duplication (*FLT3*-ITD).[778,781–783,787,789] Based on *NPM1* and *FLT3* status, four groups of AML can be identified: *NPM1*$^+$/*FLT3*$^-$, *NPM1*$^+$/*FLT3*$^+$, *NPM1*$^-$/*FLT3*$^-$, and *NPM1*$^-$/*FLT3*$^+$. The first group identifies patients with an improved prognosis. In childhood AML, NPMc$^+$ did not abrogate the negative prognostic influence of *FLT3*-ITD mutations, but in children who lack *FLT3*-ITD NPMc$^+$

phenotype identified a group with a superior prognosis.[792] Sensitive qRT-PCR assays for *NPM1* mutations can now monitor and quantify MRD in AML patients with normal karyotype and *NPM1* gene mutations.[790]

PAX5

- B-ALL
- B-cell lymphomas
- Hodgkin lymphoma
- Plasma cell myeloma (MM)
- Marginal zone B-cell lymphoma

The *PAX5* (*pa*ired-homeobo*x*-5) gene on chromosome 9 is a member of the paired homeobox gene family that encodes a B-cell specific transcription factor that regulates B-cell genes (*CD19*, Ig genes) and plays a role in B-cell differentiation.[793,794] PAX5 protein is expressed in precursor and mature B-cell lymphoproliferations, Hodgkin lymphoma, and a small subset of plasma cell neoplasms (Figure 2.49). The t(9;14) results in the juxtaposition of the *PAX5* gene with the *IG* heavy chain locus of chromosome 14 (*IGH*). The t(9;14)(p13;q32) translocation is associated with approximately 50% of lymphoplasmacytic

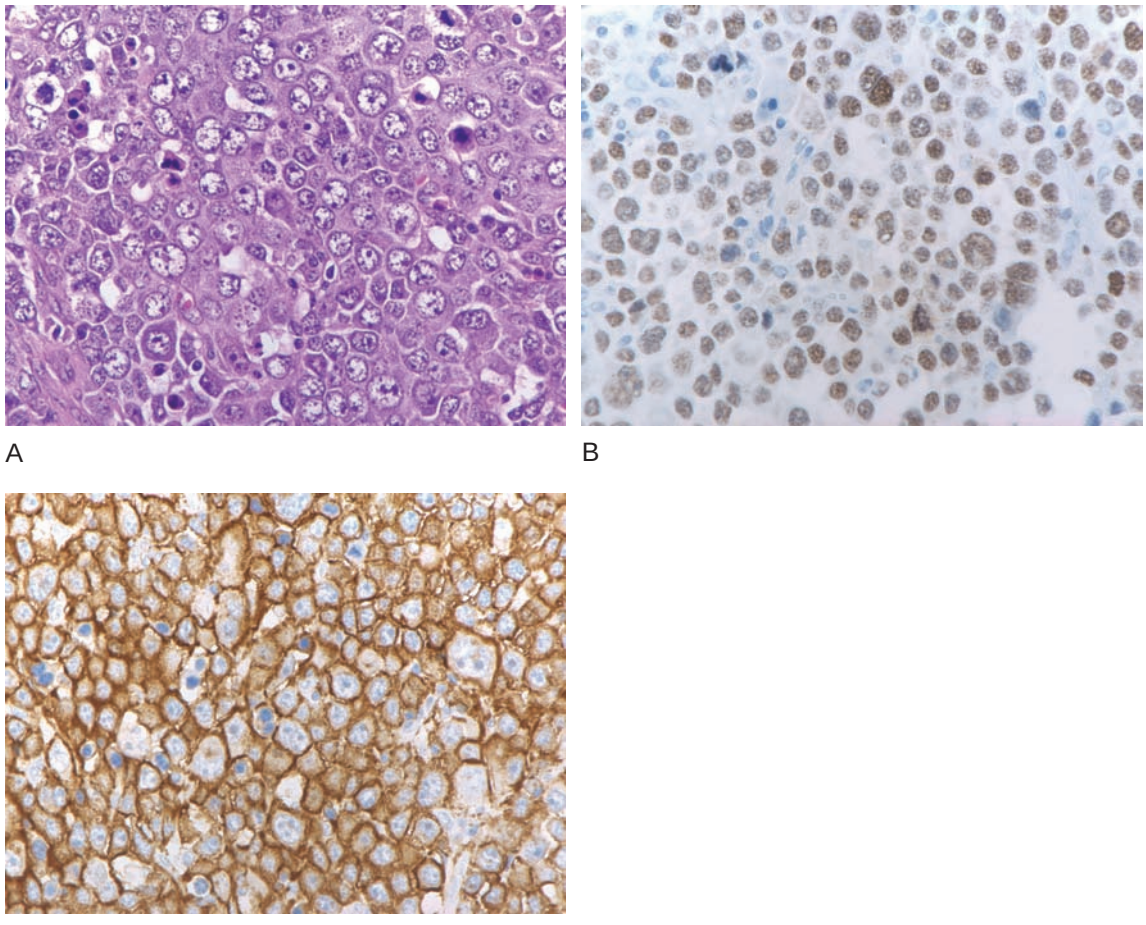


Figure 2.49 Diffuse large B-cell lymphoma. A, H&E section; B, immunohistochemical staining for PAX5 (nuclear staining); C, immunohistochemical staining for CD20 (membranous staining)

lymphomas.[795,796] A number of other reciprocal translocations involving 9p13 have been identified (1p25, 3q27, 7q11, 10p13, 12q13). Deregulation of *PAX5* expression has been reported in splenic marginal zone B-cell lymphoma.[797]

PDGFRA

- ET
- CIMF
- PV
- Systemic mast cell disease with eosinophilia
- Hypereosinophilic syndrome/chronic eosinophilic leukemia (HES/CEL)

Platelet-derived growth factor receptor α (*PDGFRA*; chromosome 4q12) and β (*PDGFRB*; chromosome 5q31-q32) are involved in mutations occurring in CMPDs. Activation of *PDGFRA* due to *FIP1L1/PDGFRA* fusion has been described in a subset of patients with systemic mastocytosis associated with eosinophilia and HES/CEL.[46,798–800] *FIP1L1/PDGFRA* fusion results from karyotypically occult interstitial deletion of part of chromosome 4, del(4)(q12). *PDGFRA* mutation can be identified by FISH or RT-PCR (*CHIC2* deletion can serve as a surrogate marker for *FIP1L1/PDGFRA* fusion).[46] Patients with this mutation are sensitive to imatinib treatment (although not all patients with systemic mastocytosis with eosinophilia respond to imatinib and, on the other hand, a subset of patients responding to imatinib lack *FIP1L1/PDGFRA* fusion).[46,801,802] Other rearrangements involving *PDGFRA* described in CMPD include t(4;22)(q12;q11) [*BCR/PDGFRA*], t(4;10)(q12;p11) [*KIF5B/PDGFRA*], and ins(9;4)(q33;q12q25) [*CDK5RAP2/PDGFRA*].[803–805]

PML

- APL

The translocation t(15;17)(q22;q11) results in the fusion of the retinoic acid receptor-α (*RARα*) gene on chromosome 17q and the *PML* gene on chromosome 15q22.[468] The t(15;17) generates two chimeric genes: *PML/RARα* is formed on derivative 15, whereas the reciprocal *RARα/PML* fusion is located on derivative 17 (Figure 2.34). Inactivation or loss of *PML* leads to a proliferative advantage for cells, impairs cellular senescence in response to oncogenic stimuli, and impairs cellular differentiation after exposure to differentiating agents such as vitamin D and retinoic acid. Depending on the *PML* gene breakpoint in chromosome 15, the transcript subtypes bcr1, bcr2, and bcr3 may be formed. The t(15;17) can be detected by classic cytogenetics in 70–90% of APL cases. A small proportion of patients with APL have complex or simple variants of this translocation. Masked (cryptic) t(15;17) can be generated by submicroscopic insertions of *PML* or *RARα*, or more complex chromosomal rearrangements.[16,806,807] Variant translocations reported in APL patients include: t(11;17)(q23;q21), t(5;17)(q35;q12-21), t(11;17)(q13;q21), and der(17) with *RARα* being fused to the *PLZF*, *NPM*, *NuMA*, and *STAT5b* genes, respectively.[808–812] APL patients with fusion genes involving *NPM* and *NuMA* are sensitive to ATRA,[811,812] whereas patients with a *PLZF/RARα* rearrangement do not response to retinoids and treatment with ATRA alone in these patients is associated with a poor prognosis.[469] Correlation with FISH and molecular studies is important in patients with clinical, morphologic, and/or immunophenotypic features of APL and lack of t(15;17) by classic cytogenetics.[16]

The balanced translocation t(17;20)(q21;q12) may mask a t(15;17)(q22;q21), and requires FISH and molecular studies for APL diagnosis.[521] The dual-fusion (dual-color) FISH probe set and RT-PCR can detect t(15;17)(q22;q21) and all variant forms of translocations associated with *PML* and *RARα*, and can be used for the diagnosis of APL and to monitor low levels of disease in treated patients.[16,807,813] In addition, FISH and molecular methods (RT-PCR and cDNA sequencing) can detect all alternate translocations involving *RARα*, but not *PML*. Mutations of the *PML* gene in APL are associated

with resistance to retinoid acid and a very poor prognosis.[814] Interestingly, mutations of the *PML* gene can be also found in nonAPL, AML, MDS, MM, and occasional lymphomas.[814]

PRV-1

- ET
- P. vera
- CIMF

The *PRV-1* gene is localized to chromosome 19q13.12-2 and is overexpressed in granulocytes from patients with P. vera and other nonCML CMPDs. Recent studies suggest that *PRV-1* and *NB1* are alleles of the same gene now referred to as *CD177*.[815] Changes in *CD177* expression may be a marker of increased or decreased myelopoiesis. Among patients with ET, *PRV-1* positivity predicts a significantly higher number of thromboembolic or microcirculatory events.[816]

RARα

- APL

The *RARα* gene is located on chromosome 17q12. The breakpoint in the *RARα* gene is located in the intron between the A and B domains (Figure 2.35). Three breakpoint clusters in the *PML* gene have been identified: bcr-1 (~70% of patients), bcr-2 (~10%), and bcr-3 (~20%), giving rise respectively to the long (L), intermediate (V), and short (S) length hybrid *PML*/*RARα* transcripts (the V form is linked to ATRA decreased sensitivity and the S form to an association with an excess of secondary chromosome changes). Chromosomal abnormalities with the *RARα* gene include t(15;17)(q22;q12), t(11;17) (q23;q12), t(5;17)(q35;q12), and t(11;17)(q13;q12) involving the *PML*, *PLZF*, *NPM*, and *NuMa* genes, respectively.

See also PML/RARα *and t(15;17), above.*

RAS

- AML
- MDS
- Plasma cell myeloma (MM)

Four alleles of *RAS* have been identified: H-*RAS* (Harvey), Ki-*RAS* (Kirsten; A and B), and N-*RAS* (neuroblastoma). Activation of Ras protein participates in the control of the cell proliferation, survival, and differentiation. Point mutations of *RAS* (codons 12, 13, and 61) are present in AML and in MDS, especially chronic myelomonocytic leukemia (CMML), and usually involve N-*RAS*, and, less commonly, Ki-*RAS*. Point mutations of *RAS* occur in ~20% of *de novo* AML patients.[61,817–819] Published data have not revealed an association of activating *RAS* mutations with an adverse prognosis.[61,817–819] In contrast to *TP53*, *RAS* mutations are less common in therapy-related MDS or AML than in *de novo* disease.[163] In a study reported by Stirewalt et al, AML patients with *RAS* mutations had higher WBC counts, a lower percentage of CD34+ blasts, and less resistant disease.[61] In the report by Shih et al, N-*RAS* mutations had no prognostic impact either in MDS or AML, but N-*RAS* mutations were more frequent in MDS progressing to AML than in MDS at the time of diagnosis.[673] *RAS* mutations are a recurrent genetic lesion in MM and may play a role in the progression of MGUS to MM.[820] In a study reported by Intini et al, *RAS* mutations, mostly involving the N-*RAS* gene, were detected in 20% of patients, and did not correlate with the presence of chromosome 13q deletion, trisomy of chromosome 11, 1q amplification, or hyperdiploidy.[821] Specific *RAS* mutations in patients with juvenile myelomonocytic leukemia may be associated with spontaneously improving disease.[822]

RB1 (retinoblastoma gene)

- ALL
- DLBCL
- Hodgkin's lymphoma
- ALCL
- AML
- MDS

Deletions of 13q14 are well known in several malignancies. The retinoblastoma gene (*RB1*) is located on 13q14, but other loci including D13S319

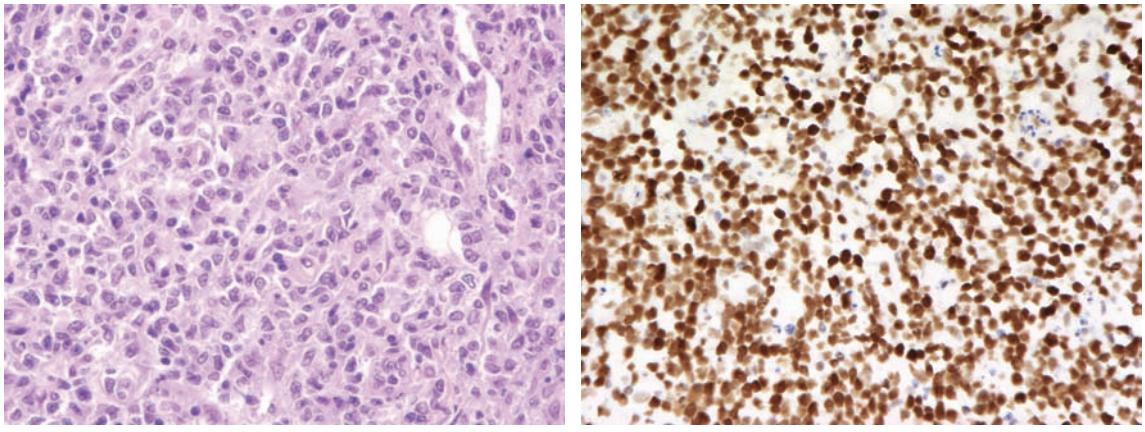
and D13S25 telomeric to this within 13q14.3 can deleted in hematologic malignancies, including B-CLL, MM, and non-Hodgkin's lymphoma, with varying clinical significance. The pRb regulates the G_1/S transition of the cell cycle.[599,823] Abnormalities in *RB1*, a tumor-suppressor gene, are responsible for retinoblastoma.[824] The pRb inactivation is a frequent phenomenon in tumors of different cell lineages including hematologic malignancies.[825–828] Loss of pRb is seen in adult precursor lymphoblastic leukemia (ALL), but pRb alterations alone are not strong independent predictors of outcome.[829] Concurrent expression of pRb and p53 may predict a poor response to therapy in ALL.[829]

In reactive lymphoid tissues, pRb is expressed by proliferating cells such as germinal center B-cells. In DLBCL, loss of pRb expression is an adverse prognostic factor, whereas high pRb expression (>80%) is associated with extended survival.[599] Figure 2.50 presents Rb expression in DLBCL. In Hodgkin lymphoma, high expression of pRb correlates with better survival and loss of pRb expression is associated with adverse outcome.[827,830]

Retinoblastoma protein is frequently absent or phosphorylated in ALCL, and the absence of Rb expression is associated with better clinical outcome in patients with ALCL.[831] In a study by Rassidakis et al, 5-year progression-free survival for patients with Rb-negative ALCL was 89.4% compared with 47.7% for patients with total Rb-positive ALCL.[831]

In AML the prognostic value of low *RB1* expression is controversial but the majority of published studies have found low *RB1* expression to be a negative prognostic predictor.[832] Jamal et al reported no significant differences in the frequency of complete remission or length of survival between AML patients with normal and abnormal pRb.[826] No mutations of *RB1* have been reported in AML or MDS, however *RB1* mutations occur in the accelerated phase and blast crisis of CML.

Deletions of the retinoblastoma gene are seen in ~50% of patients with MM.[833] Zojer et al observed monoallelic deletions of the *RB1* gene and the D13S319 locus in 48/104 patients (46.2%) and in 28/72 (38.9%) patients, respectively, with newly diagnosed MM.[268] FISH studies found that 13q14 was deleted in all 17 patients with karyotypic evidence of monosomy 13 or deletion of 13q, but also in 9 out of 19 patients with apparently normal karyotypes. Patients with a 13q14 deletion were more likely to have stage III disease, higher serum levels of β_2-microglobulin, and a higher percentage of bone



A B

Figure 2.50 Diffuse large B-cell lymphoma (A; H&E section) with expression of Rb (B, immunohistochemistry analysis)

marrow plasma cells than patients with a normal 13q14 status on FISH analysis.[268] The presence of a 13q14 deletion on FISH analysis was associated with a significantly lower rate of response to conventional-dose chemotherapy (40.8% compared with 78.6%) and a shorter overall survival (24.2 months compared with >60 months) than in patients without the deletion.[268] Multivariate analysis of prognostic factors confirmed the independent predictive value of 13q14 deletions for shortened survival. Zojer et al concluded that deletions of 13q14 are frequently detected by interphase FISH in patients with newly diagnosed MM, correlate with increased proliferative activity, and represent an independent adverse prognostic feature in MM.[268] Wu et al found a strong association between chromosome 1p and/or 1q abnormalities and deletion of chromosome 13 or 13q in MM, which, similarly to complex abnormalities and hypodiploidy, were associated with inferior overall survival.[834] As shown by Carlebach et al, monoallelic deletions of *RB1* appear to be a frequent and early event in the pathogenesis of MM, without obvious relevance for disease progression.[269] This was confirmed recently by Gutierrez et al, who reported that patients with *RB1* deletions without other abnormalities in FISH analysis displayed a similar outcome to those patients without genetic changes by FISH (46 vs 54 months).[270] *RB1* deletion as a sole abnormality did not lead to a shortening in the survival of MM patients, whereas t(4;14) conferred the worst prognosis in MM patients treated with high-dose chemotherapy.[270] Deletions of 13q14 are rare in monoclonal gammopathy of undetermined significance (MGUS).

RUNX1(AML1)

- AML/tAML
- MDS/tMDS
- CML, disease progression/clonal evolution (blast crisis/accelerated phase)
- B-ALL

The *RUNX1* gene (also known as *AML1*) located on chromosome 21q22 is one of the most important hematopoietic transcription factors. It is involved in a number of different chromosomal translocations including t(8;21)(q22;q22), t(X;21)(p22;q22), and t(19;21)(q13;q22) in AML,[835–837] t(3;21)(q26;q22) in MDS/AML (therapy-related) and blast crisis of CML,[839] and t(12;21)(p13;q22) in B-ALL.[840] The t(8;21)(q22;q22) translocation, present in 10–15% of AMLs, results in the production of the *RUNX1*/*ETO* fusion protein. This translocation is most often associated with FAB-M2 morphology and co-expression of CD19 and CD56 by $CD13^+/CD33^+$ myeloblasts. Figure 2.51 presents three signals for *RUNX1*. Gattenloehner et al showed that CD56 expression on AML cells correlated with an abnormal expression pattern of *RUNX1* isoforms: whereas full-length p48 *RUNX1* (p48) upregulated CD56 in AML cells, three newly identified shorter *RUNX1* isoforms, p38a, p30, and p24, suppressed CD56 expression.[840]

The two most frequent translocations involving the *RUNX1* gene on 21q22 in AML are t(8;21)(q22;q22) and t(12;21)(p13;q22), leading a fusion of *RUNX1* gene to *ETO* and *ETV6* (*TEL*), respectively. Jeandidier et al described 11 novel translocations rearranging band 21q22 with bands 1q25, 2p21, 2q37, 3p21, 3p23, 4q31, 6p24, 6p12, 7p15, 16p11, and 18q21.[841] In the same series,

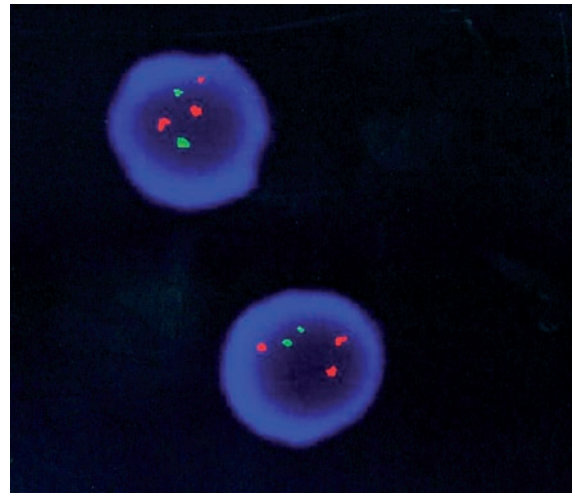


Figure 2.51 *RUNX1* (*AML1)* gene (3 signals); FISH

rearrangements of band 21q11 and 21q21 were detected in six novel translocations with 5p15, 6p21, 15q21, 16p13, and 20q11 and with 1p33, 3q27, 5p14, 11q11, and 14q11, respectively.

RUNX1 point mutations are common in high-risk MDS (RAEB) and in AML following MDS, but they are rare in chronic idiopathic myelofibrosis and in *de novo* AML.[842] *RUNX1* mutations in pediatric hematologic malignancies are infrequent, but are possibly related to AML-M0, acquired trisomy 21, and leukemic transformation. These patients may have a poor clinical outcome.[843] The frequency of *RUNX1* mutations in *de novo* AML is low and they have been detected with a substantially higher frequency in poorly differentiated AML-M0, some MDS cases, and in radiation-associated and therapy-related AML. Patients with *RUNX1* mutations are significantly associated with +13 and −7/7q−, whereas MDS/AML patients without *RUNX1* mutations showed a high frequency of −5/5q− and a complex karyotype.[844,845] Patients with *RUNX1* mutations showed more mutations of their *FLT3*, N-*RAS*, *PTPN11*, and *NF1* genes, resulting in a significantly higher mutation frequency for receptor tyrosine kinase (RTK)-*RAS* signaling pathways when compared to *RUNX1*-wild-type AML/MDS patients (38% vs 6.3%).[844] Conversely, *p53* mutations were detected only in patients without *RUNX1* mutations. Among AML M0 ($n = 90$), Dicker et al detected *RUNX1* mutations in 46% at diagnosis (this group included all trisomy 13+ cases; $n = 18$).[845] A high incidence of *RUNX1* mutations (80%) was detected in cases with trisomy 13 from other FAB subgroups ($n = 20$).[845] Quantitation of *FLT3* transcript levels revealed a highly significant 5-fold increase in AML with *RUNX1* mutations and trisomy 13 compared to samples without trisomy 13 (*FLT3* mutations were equally distributed between *RUNX1* mutated and unmutated samples).[845]

TCR

- T-cell lymphoproliferations

See T-cell receptor gene rearrangements, below.

TEL

See ETV6, *above*.

TP53 (p53)

- Various hematopoietic tumors

The *p53* (*TP53*) gene is a tumor suppressor that codes for a multifunctional DNA-binding protein (transcription factor) involved in cell cycle arrest, DNA repair, differentiation, and apoptosis. The *p53* gene accumulates in response to DNA damage and co-ordinates the cellular response to such damage by inducing apoptosis or cell cycle arrest.[846] Inactivation of *p53* by mutation or deletion occurs in approximately 50% of cancers and is associated with genomic instability,[846] and resistance to chemotherapy.[847] The negative regulation of cell cycle progression by *p53* is in part regulated by induction of $p21^{CIP1}$. The *p53* gene is one of the most frequently mutated genes in human cancer, mainly in solid tumors.[848] Based on the published data, Imamura et al reported *p53* abnormalities in hematopoietic malignancies with the following frequencies: B-CLL, 15%; Richter syndrome, 40%; low grade B-cell lymphoma, rare; high grade B-cell lymphoma, 30%; Burkitt lymphoma, 40%; Hodgkin lymphoma, 70%; MM, 5%; HCL, 10%; CML (chronic phase), rare; CML (blast crisis), 20–30%; MDS, 5%; AML, 15%; common ALL, 3%; mature B-ALL, 50%; and adult T-cell lymphoma/leukemia, 40%.[849] Deletions of one allele and mutations of *p53/TP53* are reported in 5% of AML and MDS. The wild-type p53 protein has a short half-life (6 to 20 minutes) and therefore does not accumulate in tissue in amounts allowing for immunohistochemical detection.[849] Missense mutations of *p53/TP53* result in the stabilization of the p53 protein and therefore increased levels of p53 usually reflect *p53/TP53* mutations.[850] However, since only a fraction of lymphomas with p53 overexpression have *p53* mutations, other mechanisms may also play a role in stabilization of the p53 protein.

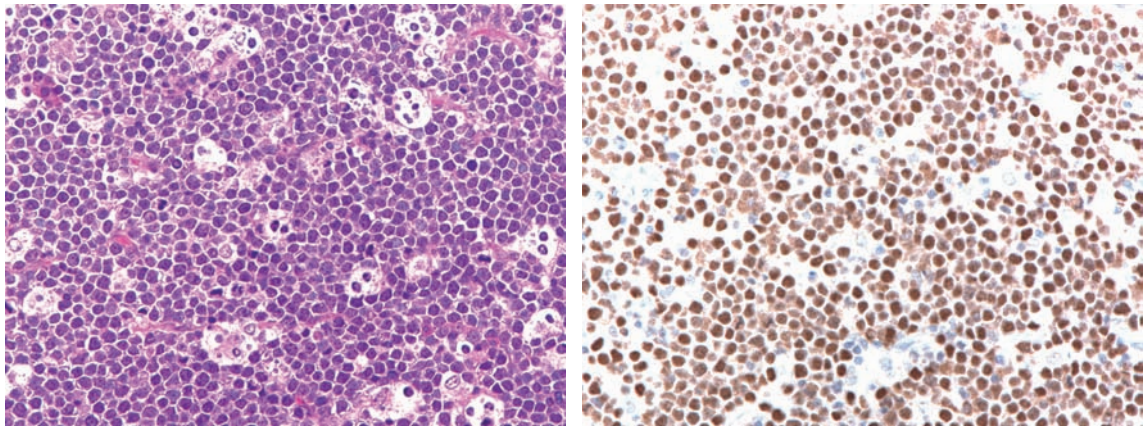
The *p53* gene mutations in lymphoid neoplasms have been detected mainly in high grade lymphomas

and have been associated with tumor progression in follicular and small lymphocytic lymphomas. B-cell lymphomas with *p53* mutations often have *MYC* activation. Deletions of *p53* in B-CLL are associated with disease progression and atypical morphologic and immunophenotypic features.[851] In B-CLL the presence of *p53* mutation or deletion is strongly associated with adverse clinical outcome and predicts a poor response to conventional therapy (drug resistance).[573,852–855] The *p53* mutations occur in only 10–15% of B-CLL patients.[852,854] However, *p53* dysfunction in B-CLL can occur in the absence of mutation and may be associated with mutation of the gene encoding ATM (ataxia-teleangiectasia mutated), a kinase implicated in *p53* activation.[573,575,576] Deletions affecting chromosome bands 11q22–q23 and 17p13 led to a reduced expression of the corresponding genes, such as *ATM* and *p53/TP53*. Inactivation of the *ATM* or *p53*/*TP53* gene is frequent in B-CLL and leads to aggressive disease. Patients with ATM deficiency had significantly shorter survival times (35.66 vs. 97.3 months) and more aggressive disease, suggesting that *ATM* is involved in the leukemogenesis of B-CLL. The *ATM* gene may also play a role in the reported 11q23 abnormality in B-CLL, which also characterizes an aggressive disease.[575] Approximately 14% of B-CLLs carry deletions of the long arm of chromosome 11 at 11q22–23. Loss of heterozygosity at 11q22–23 and, more recently, absence of ATM protein have been associated with a poor prognosis in B-CLL.[576] Functional impairment of the p53 pathway because of the inactivation of *p53* or *ATM* can be detected by exposing leukemic cells to ionizing radiation, culturing them overnight, and examining then by Western blotting for levels of p53 and p21 (a transcriptional target of *p53*).[573] In type A defect (associated with *p53* mutation) baseline p53 levels are increased, reflecting the prolonged half-life of mutant p53 as compared to the wild-type protein. In contrast, in the type B defect (associated with *ATM* mutation) baseline p53 levels are not increased, but there is impaired accumulation of *p53* in response to radiation.[573] Carter et all proposed a flow cytometry assay to analyze p53 dysfunction in B-CLL.[856]

The *p53* mutations are associated with an aggressive clinical course of splenic marginal zone B-cell lymphoma.[857] A subset of patients with MCL has *p53*/*TP53* mutations/overexpression, which confers poorer prognosis.[858] The *p53* mutations occur more often in MCL with atypical/blastic cytology and p53 mutations precede the development of variant cytology in some patients. Overexpression of p53 protein was observed in 75% of patients with *p53* mutations and in none of the wild-type cases. The median survival of the cases with mutant *p53* was only 1.3 years, whereas the median survival of cases with germline (wild) *p53* was 5.1 years.[858]

Mutations of *p53* are detected in up to 30% of DLBCL and Burkitt lymphoma.[859–862] Overexpression of *p53* has been shown to have a prognostic significance in DLBCL and is associated with chemotherapy refractoriness. The presence of *p53* gene mutations has a negative impact on overall survival in DLBCL. Cox's Regression Model identified that the high-risk International Prognostic Index (IPI) and *p53* gene mutations have an independent negative impact on overall survival in DLBCL.[863] Figure 2.52 presents immunohistochemical detection of *p53* in Burkitt lymphoma.

In patients with MM, *p53* deletions are associated with higher serum calcium and creatinine levels, but there is no association with patient age, gender, β_2-microglobulin, C-reactive protein, hemoglobin level, albumin level, or lytic bone lesions. There is no association of *p53* deletions with 13q deletions or translocations t(11;14) or t(4;14). Patients with *p53* deletions had significantly shorter progression-free (median 7.9 vs 25.7 months) and overall survival (median 14.7 vs 48.1 months) than patients without a *p53* deletion. A multivariate analysis confirmed *p53* deletion as an independent prognostic factor predicting shortened progression-free or overall survival in patients with MM after high-dose chemotherapy and autologous stem cell transplantation.[305]



A B

Figure 2.52 Burkitt's lymphoma (A, H&E section) with strong p53 expression by immunohistochemistry (B)

Neoplastic cells in classic Hodgkin lymphoma (but not NLPHL) frequently express p53. In peripheral T-cell lymphoma, p53 positive cases (by immunohistochemistry) show significantly higher proliferative activity, more frequent expression of bcl-2, and less frequent expression of p21/WAF1 than p53 negative cases.[864] Analysis of the survival curves showed that p53 expression is an independent prognostic variable associated with a significantly poorer clinical outcome, in terms of both overall survival and event-free survival.[864]

The *p53* mutations are present in 15% of AML and 11% of MDS cases.[854] In AML, 33% of mutated cases and 81% of nonmutated cases treated with intensive chemotherapy achieve complete remission. Median survival is shorter in patients with mutated *p53* when compared to unmutated AML (2.5 months vs 15 months). In the MDS patients who received chemotherapy, 8% of mutated cases and 60% of nonmutated cases achieved complete remission or partial remission, and median survival was 2.5 and 13.5 months, respectively.[854] In all MDS cases (treated and untreated), the survival difference between mutated cases and nonmutated cases is also highly significant. The *p53* mutation is a strong prognostic indicator of response to chemotherapy and survival in AML and MDS.[854]

WT1 (Wilms' tumor gene)

- AML
- ALL
- CML

Wilms' tumor gene (*WT1*) is a tumor-suppressor gene located on chromosome 11p13 that encodes a zinc-finger transcription factor that regulates the transcription of a variety of target genes and is involved in post-transcriptional mRNA processing in cell growth and development.[865–868] Apart from the hematopoietic system, *WT1* expression in adult tissues is found in the urogenital tract and CNS. The overexpression of *WT1* in progenitor cells in the bone marrow leads to growth arrest and reduced colony formation.[869,870] *WT1* was initially discovered in a pediatric kidney tumor (Wilms' tumor), but can be overexpressed in several types of solid tumors and in acute leukemias. In the latter, it has been evaluated as a novel prognostic factor, MRD marker, and therapeutic target.

The majority of acute leukemias express the normal, wild type *WT1* gene and only approximately 10–20% of acute leukemias show point mutations and small insertions or deletions, predominantly in biphenotypic leukemia and AML, and rarely in ALL.

In a study by King-Underwood et al, five different point mutations in *WT1* were found.[871] Those alterations were shown to be associated with an unfavorable prognosis in AML.[865–867]

WT1 overexpression has been found in 80–90% of AML and 70–90% of ALL patients.[865,867,872–878] A high expression of *WT1* in acute leukemia at diagnosis correlated in several studies with a poor outcome, especially in AML following standard chemotherapy,[875,877,878] but other studies did not support this observation.[876,879] In CML, *WT1* levels are usually low in the chronic phase and increase as the disease progresses into the blast crisis.[880,881] The *WT1* gene has been shown to interact with the promoter of the multidrug resistance gene (*MDR1*). Galimberti et al found that high levels of *WT1* were significantly correlated with high levels of the *MDR1* gene.[882] Several studies have indicated the prognostic and predictive value of *WT1* as a marker for MRD monitoring.[873,875,877,883,884] Increased levels can be predictive of an impending relapse in AML.

APPLICATION OF CHROMOSOMAL AND MOLECULAR MARKERS IN DIAGNOSIS

Conventional cytogenetic (G-banding), FISH, and molecular methods are commonly applied in the diagnosis of hematologic malignancies and in addition are of increasing practical importance for accurate prognosis and to evaluate for MRD (*see below*).[1,4,5,10,13,15,18,22,33,104,106,107,115,263,309,325,368,632,885–903] Cytogenetic analysis has become routine in the evaluation of patients with myeloid and many lymphoid diseases and, in conjunction with morphology and immunophenotyping, helps to establish definite diagnosis, recognize disease subsets, and is essential in evaluating disease progression. FISH probes are now available for many common chromosomal abnormalities and often provide advantages over classic cytogenetics by detecting chromosomal abnormalities which can be masked or cryptic in conventional G-banding technologies. PCR methods for the determination of clonality by analysis of *IGH* and *TCRγ* gene rearrangements belong to the most widely used group, and involve different *IGH* V framework regions for B-cell analysis and *TCRγ* V and J genes for T-cell analysis. Other commonly used FISH or PCR targets include t(11;14) [*CCND1/IGH*], t(8;14) [*MYC/IGH*], t(14;18) [*IGH/BCL2*], t(15;17) [*PML/RARα*], t(9;22) [*BCR/ABL*], inv(16)/t(16;16), *FLT3*-ITD/mutations, t(2;5) [*NPM/ALK1*], t(11;18) [*API2/MALT1*], t(8;21) [*RUNX1/ETO*], *JAK2* mutations, *IGVH* mutations, and *p53/TP53*. Multiplex PCR assays for the detection of gene rearrangements have been successfully used.[904,905] In a report by Fodinger et al, the combination of the four *IGH* gene primer systems with the multiplex *TCRγ* gene PCR allowed detection of clonality in ~84% of B-cell neoplasms and ~92% of T-cell lymphomas.[905] Meier et al developed a multiplex PCR assay for the simultaneous determination of B- and T-cell clonality and the detection of the chromosomal translocations t(14;18) and t(11;14) in a single reaction with 26 primers [corresponding to the sequences of > 90% of the 69 variables and 6 joining *IGH* genes and 100% of the *TCRγ* V and J genes, the major and the minor breakpoint regions of the t(14;18) and the major breakpoint region of the t(11;14) and the β-globin gene as an internal control] using four color fluorescence and automated high resolution fragment analysis.[906] The European consensus analysis showed that BIOMED-2 multiplex PCR assays [including *IG/TCR* rearrangements, t(14;18) and t(11;14)] were highly reliable in assessment of clonality in B- and T-cell malignancies, particularly when *IGH-IGK* or *TCRγ-TCRβ* strategies (respectively) were combined.[907,908] The fluorescence-based multiplex PCR assay can also be used for the simultaneous detection of gene mutations or fusion transcripts in patients with AMLs.

Molecular or chromosomal aberrations may be detected by PCR in healthy individuals, including the translocations t(9;22) [*BCR/ABL*], t(14;18)

[*IgH/BCL2*], t(2;5) [*NPM/ALK*], and *MLL* duplications, which characterize CML, FL, ALCL, and AML, respectively.[909]

Most common chromosomal aberrations used in diagnosis

Chromosomal and molecular aberrations are frequently found in hematologic malignancies, and their detection is helpful and often necessary for rendering the final diagnosis (Tables 2.1 and 2.2, and Figure 2.53 and 2.54). Nonrandom chromosomal abnormalities such as t(15;17)(q22;q12), inv(16)/ t(16;16), t(8;21) or t(1;19)(q23;p13) have been so closely associated with distinct types of acute leukemias that their recognition can allow diagnosis independent of the other criteria. Identification of t(9;22)$^{BCR/ABL}$ helps to confirm the diagnosis of CML, and allows for targeted therapy with imatinib (Gleevec). APL characterized by t(15;17)$^{PML/RAR\alpha}$ is another malignancy for which cytogenetic/molecular testing is crucial in establishing the definite diagnosis prompting immediate application of targeted therapy (e.g. ATRA). The presence of t(11;18)$^{API2/MALT1}$ by FISH/PCR analysis in gastric MALT lymphoma identifies a subgroup of patients who will not respond to standard *H. pylori* eradication therapy and may need alternative treatment. Demonstration of t(1;22)(p13;q13) seen exclusively in acute megakaryoblastic leukemia in children helps to differentiate this leukemia from nonhematopoietic tumors, such as sarcomas, which

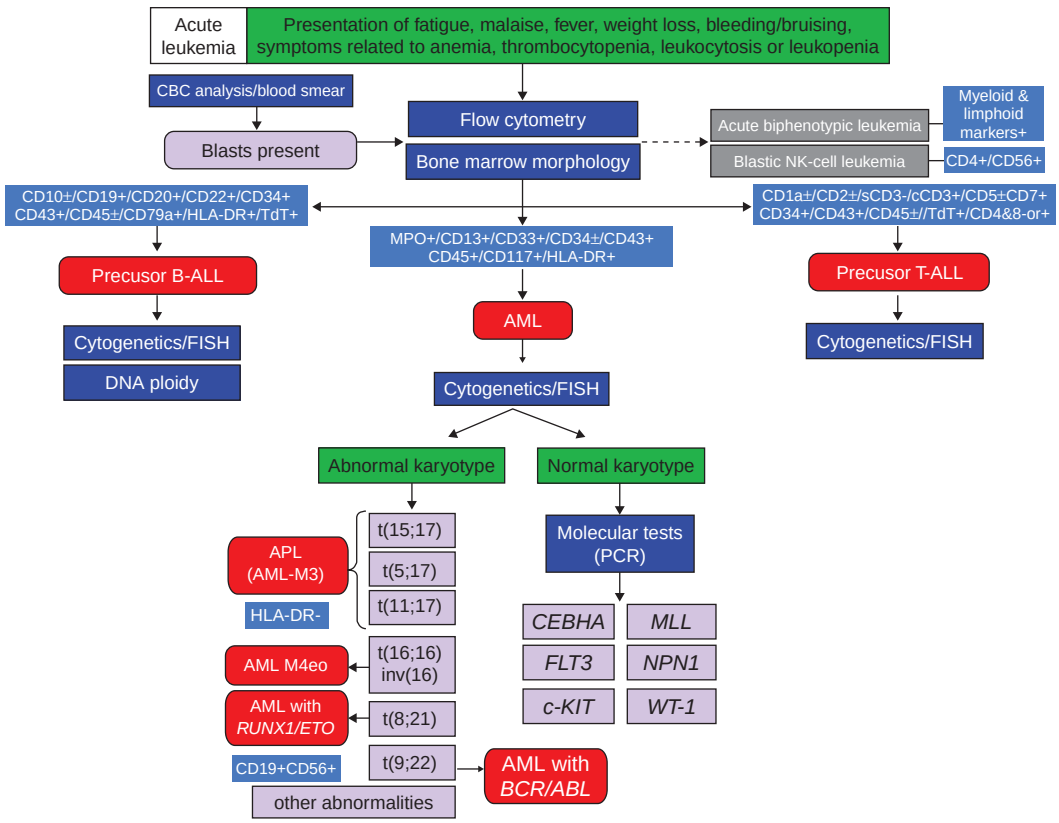


Figure 2.53 Algorithmic approach to the diagnosis of acute leukemia

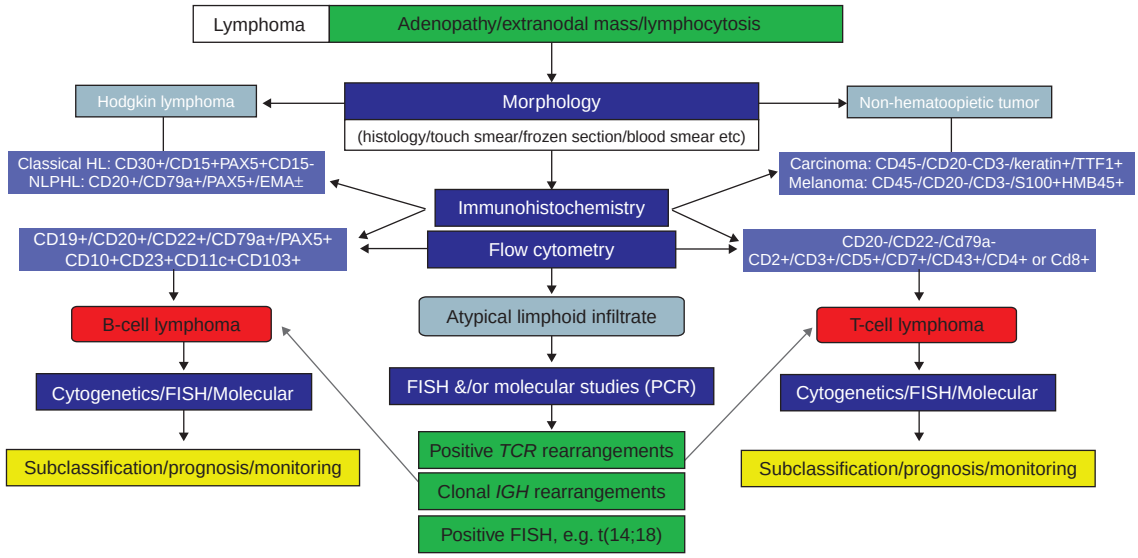


Figure 2.54 Algorithmic approach to the diagnosis of lymphoma

may display similar morphologic features. In the group of hematolymphoid tumors, the application of genetic testing not only helps to diagnose and often subclassify lymphomas, e.g. MCL with t(11;14)*CCND1/IGH*, FL with t(14;18)*IGH/BCL2*, Burkitt lymphoma with t(8;14)*MYC/IGH*, hepatosplenic T-cell lymphoma with isochromosome 7, T-PLL with inversion 14, and ALCL with t(2;5)*NPM/ALK1*, but is also indispensable in differentiating lymphomas from atypical lymphoid hyperplasias (B- and T-cell clonality by PCR, specific translocations/rearrangements). Molecular testing might also help to establish a definite diagnosis in atypical as well as early (emerging) myeloproliferations, e.g. CML versus leukemoid reaction (*BCR/ABL* by FISH and/or PCR) or P. vera versus reactive erythrocytosis (*JAK2* mutation by PCR). The application of cytogenetics and FISH helps to identify therapy-related malignancies, and differentiate them from originally diagnosed tumors. Aberrations frequently seen in therapy-related hematopoietic tumors include 11q23 rearrangements, e.g. t(10;11); t(11;19)] and t(3;8)(q26;q24). Figure 2.55 lists chromosomal changes typical for myeloid versus lymphoid proliferations.

The advantages and disadvantages of each methodology (conventional cytogenetics, FISH, and PCR) are described in Chapter 1. Depending on the type of chromosomal abnormality, one technology may have advantages over the other. Lee et al compared the results of cytogenetic studies and FISH patients with hematologic malignancies and found an overall discrepancy rate of 6.0% at diagnosis and 11.9% at follow-up (with FISH showing an advantage over G-banding), indicating generally greater discrepancy rates at follow-up.[119] The genetic changes with especially large discrepancy rates at diagnosis were del(7q) (20.0%), *PML/RARα* (17.6%), and trisomy 21 (12.5%) and, at follow-up, *BCR/ABL* (28.2%) and *RUNX1/ETO* (24.4).[119] Due to technical problems or cryptic variants, conventional cytogenetics detects t(15;17) in 70–90% of patients with APL. These masked *PML/RARα* fusions can be identified by PCR and/or FISH.

Immunoglobulin gene rearrangement

The majority of lymphomas are of B-cell lineage and consequently analysis of immunoglobulin gene

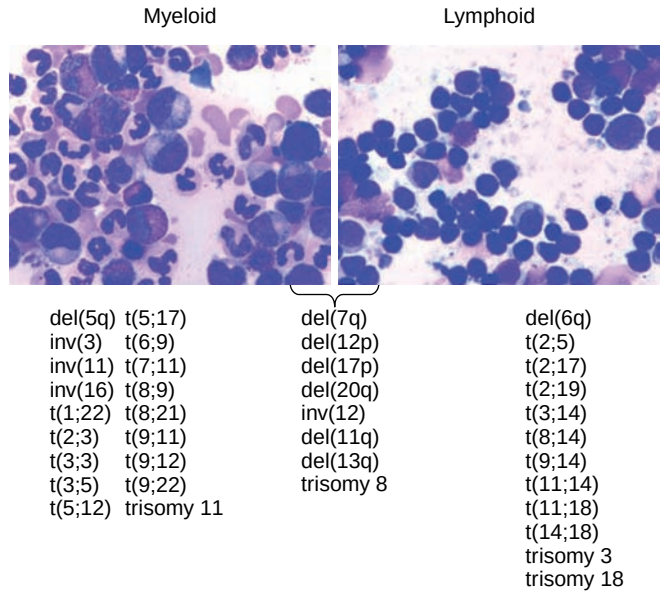


Figure 2.55 Chromosomal changes seen in myeloid versus lymphoid proliferations

rearrangements belongs to most frequently used molecular tests. The distinction between a benign process and a lymphoma is important for diagnosis, prognosis, treatment options, and in evaluation of the response to therapy. The diagnosis of B-cell lymphomas is based most often on histomorphology and immunophenotyping (flow cytometry and/or immunohistochemistry), although many cases require the addition of conventional cytogenetics, FISH, and PCR. Assessment of clonality by immunoglobulin gene rearrangement is crucial in establishing the definite diagnosis in cases with atypical (ambiguous) histology and phenotype, very early (incipient) lymphoproliferations, pleomorphic infiltrate with a paucity of neoplastic cells, low-grade B-cell lymphoproliferations which may be difficult to diagnose based on morphology, or cases with an insufficient amount of tissue for morphologic/phenotypic analysis.[900] Molecular detection of clonality plays an important role in the diagnosis of post-transplant lymphoproliferative disorders.[1,910,911] Among three genes that rearrange (heavy chain gene, kappa and lambda light chain genes), the *IGH* gene rearranges before the light chain genes and is most frequently analyzed.

The immunoglobulin molecule (Figure 2.56) is made up of two heavy chains and two light chains, joined by disulfide bonds. Both heavy and light chains have variable and constant regions corresponding to the *V* and *Cμ* genes. The genes that encode heavy chains are located on chromosome 14 and genes encoding light chains are located on chromosomes 2 (κ;kappa) and 22 (λ;lambda). During lymphocyte maturation, B-cells rearrange their genes producing a fusion gene comprising V (variable), D (diversity), J (joining), and C (constant) segments, which encodes an antigen receptor that is expressed on the surface of B-cells and becomes secreted when B-cells differentiate into plasma cells. The maturing B-cells rearrange their genes differently by splicing out and deleting of a portion of the *IG* gene, in which 1 of 30 D regions is juxtaposed first with 1 of 6 J regions, followed by joining of 1 of ~200 V regions (Figure 2.57). The antibody type (IgA, IgM, IgD, IgE, or IgG) depends on which C region (Cα, Cμ, Cδ, Cε, or Cγ) joins the rearranged VDJ genes. The heavy chain protein (IGH) joins either κ or λ light chain proteins (which are encoded by genes rearranged in a similar manner) to produce antibody. The unique coding sequence for both heavy and light chain genes ensures the diversity

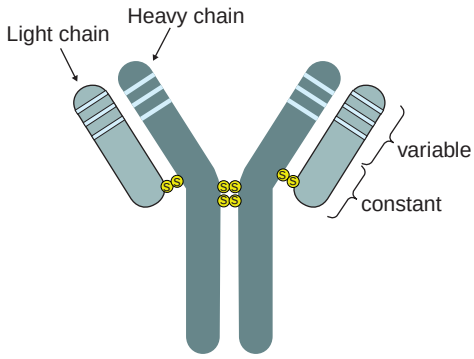


Figure 2.56 The structure of immunoglobulin (Ig)

of antibody production by the plasma cells. The normal B-cell population, therefore, consists of polyclonal *IG* gene rearrangements.

In B-cell neoplasms, the coding sequence characteristic for the B-cell that gave rise to a malignant clone becomes inherited by all malignant cells. This clonal immunoglobulin gene rearrangement can be visualized by Southern blot analysis or by PCR amplification (Figure 2.58).[886,907,910–919] Consensus primers targeting three families of *IGH* variable segments (frameworks 1, 2, and 3) are commonly used for PCR identification of B-cell clonality (FR1-PCR, FR2-PCR, FR3-PCR).[914] About 70% of lymphomas have clonal rearrangements detectable with framework 3 primers, 20% with framework 2, and an additional few cases with framework 1.[920,921] The combined use of primers yields the best results, with the detection of clonality approaching 90%.[913,914,918,922–924] In a multicenter comparison of PCR sensitivity in detecting clonality, a significant difference was observed between FR3 alone as compared with FR3 + FR2 (~57% vs ~74%).[912] Analysis of only a single FR region has several limitations, mostly associated with somatic hypermutations. Somatic hypermutations increase the diversity of the V_H segments encoded in the germline genome and may lead to primer mismatching and consequently false negative results, limiting the use of single FR region-PCR protocols.[925,926]

The main limitation of *IGH*-PCR is false negative results, which vary depending on the methodology used, subtype of lymphoma, and degree of somatic hypermutation of *IGH*.[927] The false negative results due to somatic hypermutation may be seen in B-CLL, but are more typical for lymphomas of germinal center and post-germinal center origin (i.e. FL, a subset of DLBCL, and plasma cell myeloma) since they display a higher rate of somatic mutation. Using Southern blot analysis or PCR targeting, incomplete DJH rearrangements and *BCL2/IGH* fusion may overcome problems associated with somatic hypermutations, and therefore may be more sensitive in confirming clonality.[687,915,921,923,928,929] The detection

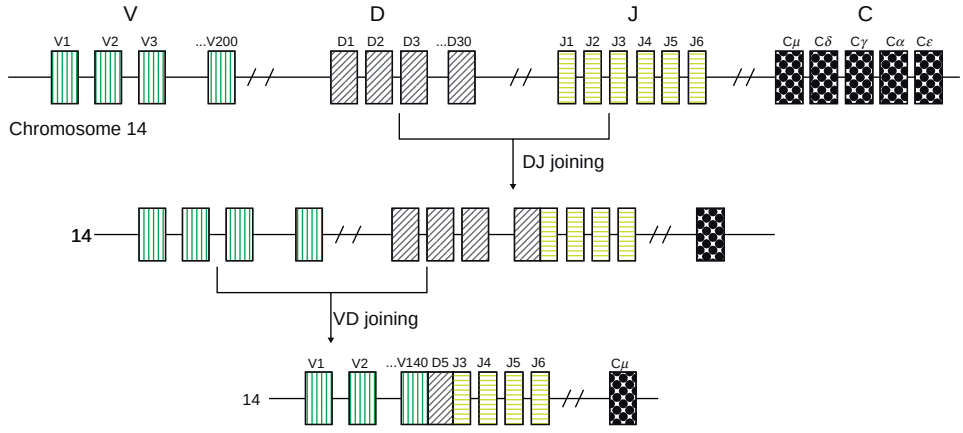


Figure 2.57 The immunoglobulin heavy chain gene rearrangements

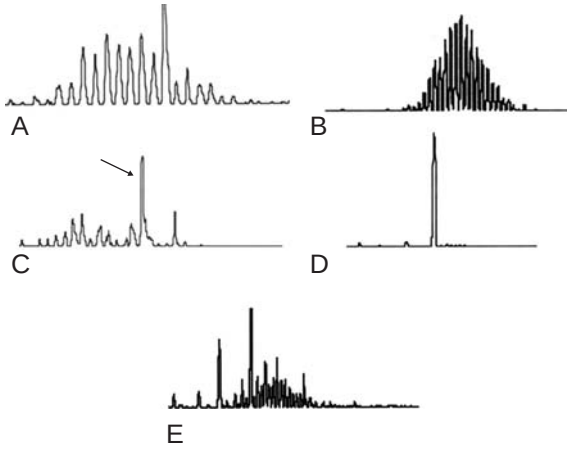


Figure 2.58 PCR analysis for *IGH* rearrangement. A, B, polyclonal sample; C, monoclonal peak in the polytypic background; D, monoclonal peak; E, peak in polyclonal sample (indeterminate results)

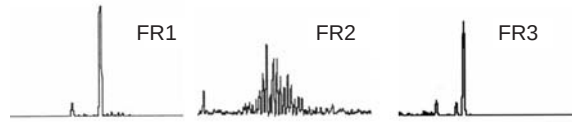


Figure 2.59 PCR analysis of *IGH* rearrangements: the same sample from lymphoma revealed monoclonal peaks with FR1 and FR3, while FR2-PCR showed a polytypic pattern

rate can be further improved when multiplex *IGH* (D_H-J_H) and *IGK* PCR-based strategies are combined,[907,916,930] [*IGL* rearrangements are invariably associated with *IGK* VJ or the κ deleting element (KDE)]. The BIOMED-2 Concerted Action European study group described a very sensitive method with primers targeting different FRs and incomplete D_H-J_H rearrangements (V_H-J_H and D_H-J_H sets of primers), which can detect virtually all monoclonal B-cell proliferations, regardless of high levels of somatic hypermutation.[687,916] In a series reported by Catherwood et al, the addition of BIOMED-2 D_H-J_H rearrangements increased the detection of clonality by 22% in DLBCL.[687] PCR-based techniques have a sensitivity of 1–5%, i.e. PCR can confirm clonality if at least 1% (usually ≥5%) of clonal cells are present in a polyclonal B-cell background.[687,915]

The products of PCR analysis using fluorochrome-labeled primers can be separated by capillary electrophoresis.[919] Polyclonal (random) rearrangement shows a Gaussian distribution with multiple, heterogeneous fragments (peaks) (Figure 2.58 A and B). A monoclonal B-cell population is distinguished form polyclonal cells based on the presence of one dominant peak (the amplified fragment of one size) (Figure 2.58). When the ratio of the major peak to the second largest peak is low (e.g. between 2 and 3), the results are indeterminate (Figure 2.58E). Figure 2.59 shows monoclonal peaks with FR1 and FR3 (FR2-PCR is negative) and Figure 2.60 shows positive results with FR2-PCR, while both FR1 and FR3 display polytypic patterns. Figure 2.61 shows a biclonal B-cell lymphoma (flow cytometry and PCR analysis).

A number of benign (reactive) conditions may yield 'clonal' PCR results, and therefore molecular results should be correlated with clinical and laboratory data, especially morphology and phenotype, before treatment. Table 2.4 lists nonmalignant disorders which may show 'clonal' rearrangements by PCR.

T-cell receptor gene rearrangement

Similarly to B-cells, T-cells also rearrange the genes, but instead of producing different antibodies, the (protein) products of T-cell receptor genes are fixed to the surface of lymphocytes, creating complex receptor molecules and allowing proper interaction with other components of the immune system. The T-cell receptors contain four major protein chains, α, β, γ, and δ, encoded by corresponding *TCR* genes (*TCRA*, *TCRB*, *TCRG*, and *TCRD*). Gene loci encoding α- and δ-chains (*TCRA* and *TCRD*) are clustered on chromosome 14q11, those encoding the β-chain (*TCRB*) and the γ-chain (*TCRG*) are located on 7q34 and 7p15, respectively (Figures 1.9 and 1.16).

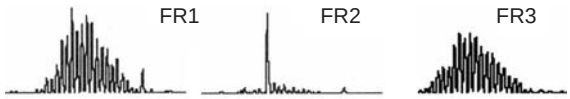


Figure 2.60 PCR analysis of *IGH* rearrangements: the same sample from lymphoma revealed a monoclonal peak with FR2, while both FR1-PCR and FR2-PCR showed a polytypic pattern

Similarly to *IG* genes, *TCR* genes are composed of V and C genes. The V region has three segments (V, J, and D) in the β and δ genes, and only V and J segments in the α and γ genes.

The earliest thymocytes express HLA-DR, CD34, and CD7, have a germ-line configuration of the TCR genes, and are pluripotent (Figure 2.62). The *TCR*δ gene rearranges as soon as the progenitor cell commits to the T-lineage. The earliest definite T-cells (early prothymocytes; pro-T-cells) evolve in the subcapsular region of the thymic cortex and are referred as triple-negative (TN) cells, due to lack of expression of CD4, CD8, and surface CD3 (sCD3$^-$/CD4$^-$/CD8$^-$). They are positive for CD2, CD7 (strong expression), CD34, CD44, TdT (nucleus), and cytoplasmic CD3 (cCD3), and are characterized by a germline configuration of the TCR β chain. In the process of differentiation, TN cells start to express CD1 and CD5, and progressively lose the expression of CD34 and intensity of CD7 expression. Further differentiation occurs in the thymic cortex, through several stages. In the thymic cortex, double negative (DN) T-cells (CD4$^-$/CD8$^-$) start to differentiate into double positive (DP) T-cells (CD4$^+$/CD8$^+$) through several stages. The DN stage is characterized by a continuous rearrangement of *TCRB*, *TCRD*, then *TCRG*, while the *TCRA* rearrangement begins during the DP stage. During the transition from pro-T-cells to DN T-cells, cells rearrange the *TCRB* gene,

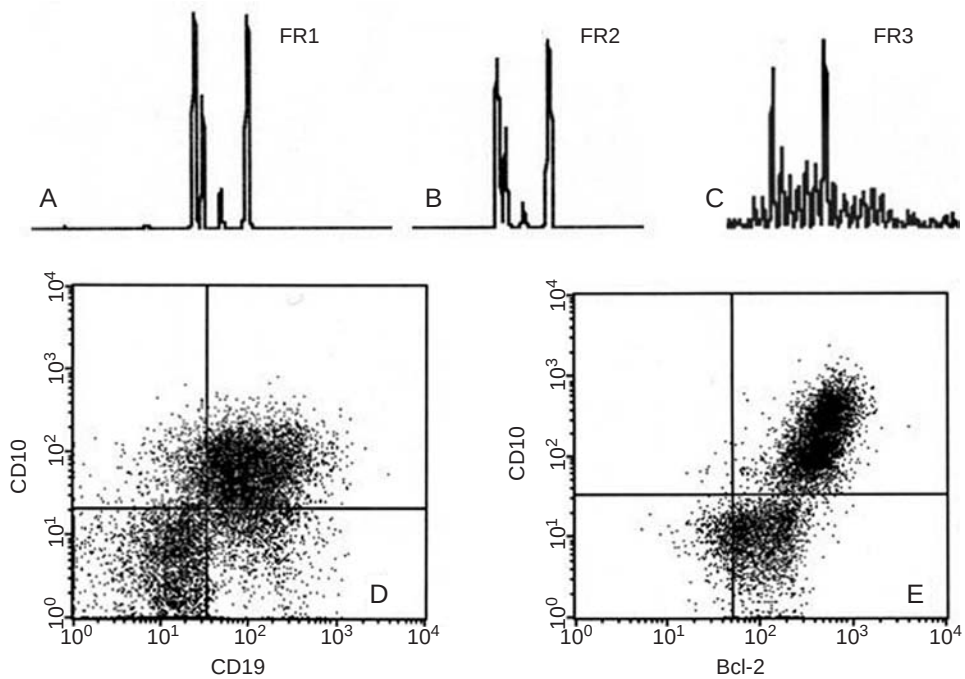


Figure 2.61 Biclonal B-cell lymphoma (PCR and flow cytometry analysis). PCR analysis showed two clonal peaks (A–C), and flow cytometry showed co-expression of CD10 and bcl-2 (D, E)

Table 2.4 Limitations of molecular testing (false positive clonal population)

Disorder	*Lineage*	*Reference*
Large granular lymphocytosis	B	
Castleman's disease	B	
Immunodeficiency-associated disorders	B, T	
Lymphomatoid papulosis	T	
Celiac disease	B	
Pagetoid reticulosis	T	
Viremia	B, T	
Sjögren's syndrome	B	Bahler and Swerdlow[1831]
H. pylori gastritis/healthy tissue adjacent to MALT lymphoma	B	Sa-xena et al[1832]
Hepatitis C	B	Zuckerman et al[505]
T-cells in B-ALL	T	Brumpt et al[1833]
B-cells in AILD lymphoma	B	Lorenzen et al;[1834] Hedges et al[1835]
Small sample with paucity of lymphocytes or presence of one germinal center	B	Elenitoba-Johnson et al[1836]
T-cells adjacent to tumor	T	Lim et al[1837]

a process which is similar to *IGH* gene rearrangement (the Dβ segment joins a Jβ segment, and then the Vβ segment joins the DβJβ complex). At this point cells start the commitment to either TCR$\gamma\delta$ or TCR$\alpha\beta$ lineages. If the *TCR*$\gamma\delta$ rearrangement is successful, cells become T$\gamma\delta^{+}$. In the subset of cells which undergo the rearrangements of the β, α, and γ genes, the β-chain forms a heterodimer with the pre-T-α-chain (surrogate α chain), termed the pre-TCR complex, and starts to differentiate into the $\alpha\beta$ lineage, entering the DP phenotypic stage (Figure 2.63). The pre-TCR forms a complex with CD3 at the thymocyte surface; these cells are characterized by surface expression of pre-TCR and low CD3. The pre-TCR complex blocks further $\gamma\delta$ differentiation (*TCRG* gene rearrangements are

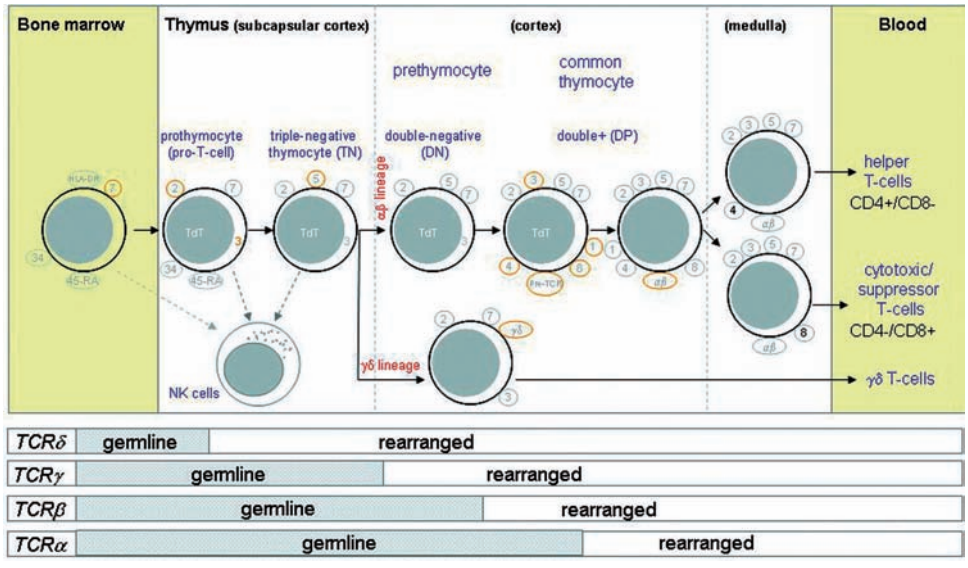


Figure 2.62 T-cell development

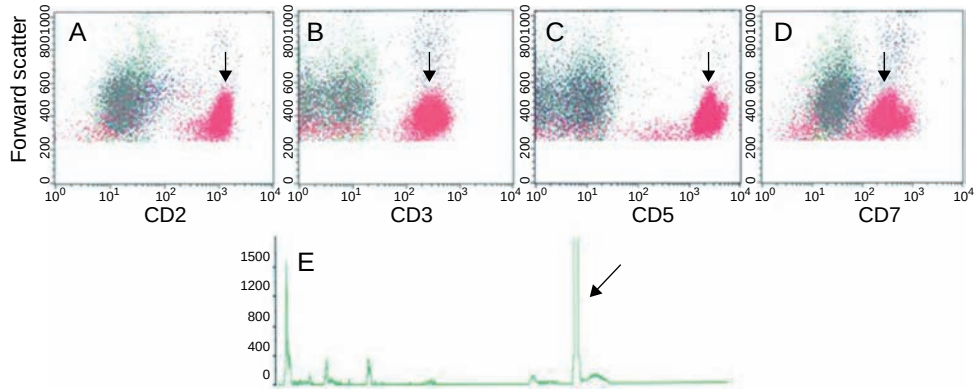


Figure 2.63 T-prolymphocytic leukemia. A-D; flow cytometry (arrows indicate leukemic population expressing pan-T antigens CD2, CD3, CD5 and CD7). E; molecular analysis for TCR gene rearrangements (arrow indicates monoclonal peak)

dysfunctional) and plays a major role in T-cell commitment to the αβ lineage (with subsequent production of $CD4^+/CD8^+$ cells). T-cells committed to the TCRαβ lineage mature into $CD4^+/CD8^+$ cells, which express sCD3 and become negative for TdT. In the thymic medulla, DP cells finish the differentiation process, becoming either $CD4^+$ or $CD8^+$, and then enter the blood as mature T-helper ($CD4^+$) or T-suppressor/cytotoxic ($CD8^+$) cells. Mature T-cells, either *αβ* or *γδ*, demonstrate nongermline patterns of *TCRG* or *TCRD* genes. Mature T-cells are characterized by the membrane expression of the TCR/CD3 complex.

T-cell lymphoproliferative disorders may display rearrangements of one, two, three, or four *TCR* genes. PCR analysis of *TCRB* and *TCRG* genes is most often used to confirm clonality (Figure 2.63), whereas *TCRD* is rarely used. The *TCRA* gene is usually not targeted. Most of the T-cells rearrange one of the 11 variable segments of *TCRG*, 8 of which are homologous to one another and can be targeted by a single consensus primer. Using several consensus primers targeting variable segments and the joining region of *TCRG*, e.g. Vγ1–8, Vγ9, Vγ10, Vγ11, and Jγ1/Jγ2, allows for detection of T-cell monoclonality in up to 95% of cases with sensitivity between 1 and 5%.[931] Multiplex PCR with four TCR loci (*TCRG*, *TCRD*, and *TCRB* including complete [Vβ-Jβ] and incomplete [Dβ-Jβ] rearrangements) offers a sensitive approach to determining clonality early in the diagnostic work-up of T-cell disorders, with *TCRG* being the single most informative locus (clonal rearrangement in 89%), followed by *TCRB* (79%) and *TCRD* (39%).[906,916,932] Multiple primer set PCR methods should obviate a need for the more expensive and time-consuming Southern blot technique and are the preferred diagnostic molecular test for assessing T cell clonality.[932,933] The results can be visualized using capillary or sequencing gel electrophoresis.[934,935] Although capillary gel electrophoresis is superior in assessing T-cell clonality, caution must be exercised when interpreting results, because pseudo-spikes appear to be common in benign tissues with lymphoid populations and are not necessarily indicative of a clonal malignant T-cell population.[936] Using capillary electrophoresis, Luo et al proposed the threshold for identification of a predominant monoclonal population within a polyclonal background – the peak height ratio (Rn) of the peak of interest and the average of the two immediate flanking peaks.[935] After evaluation of monoclonal, reactive, and normal T-cell populations, an Rn of 3.0 or greater was determined to be consistent with a monoclonal population, whereas an Rn between 1.9 and 3.0 was considered an intermediate range. Lee et al defined relative peak height as h1/h2, where h1 represents the peak height of the largest peak above the

normally distributed population, and h2 represents the peak height of the normally distributed curve.[936] Pseudo-spikes were found in almost 20% of histologically benign lymph nodes with a relative peak heights that were more than 0.5 and up to 1.5. The peaks with a relative height of at least 3 represent a true clonal population in diagnostic samples, peaks with relative heights of less than 1.5 may be insignificant, while peaks with relative heights between 1.5 and 3 may require additional testing and close follow-up.[936]

T-cell clonality analysis is an important adjunct in differentiating benign dermatopathic lymphadenitis from early lymph node involvement by cutaneous T-cell lymphoma.[917] Using TCRβ-PCR and TCRγ-PCR, Assaf et al confirmed clonality in 22 of 22 lymph nodes with histologically detectable lymphomatous cells, as well as in 7 of 14 histologically benign lymph nodes with dermatopathic changes (T-cell clonality was not found in any patients with inflammatory skin diseases).[917] In the European study of 188 T-cell disorders (BIOMED-2 Concerted action BHM4 CT98-3936) TCR clonality was detected in 99% (143/145) of all definite cases of T-PLL, T-cell large granular lymphocytic leukemia, peripheral T-cell lymphoma (unspecified), and angioimmunoblastic T-cell lymphoma (AILT), whereas 9 of 43 ALCLs did not show clonal TCR rearrangements.[909] Combined use of *TCRB* and *TCRG* genes revealed two or more clonal signals in 95% of all TCR clonal cases.

The results of molecular tests for T-cell clonality have to be correlated with relevant clinical and laboratory information, including morphology and phenotype. Detection of clonality is not an equivalent of malignancy, since clonal T-cell population can be identified in non-malignant conditions, including large granular lymphocytosis, immunodeficiency-associated disorders, lymphomatoid papulosis, pagetoid reticulosis, autoimmune disorders, and viral infections, and in healthy (elderly) individuals (Table 2.4).[937–946] With advancing age, healthy individuals frequently demonstrate large clonal expansions of $CD8^+$ T-cells in the blood, which persist for long periods of time and appear to be maintained as a population of memory cells.[944–946] Clonal expansion of $CD4^+$ T-cells is less common.[943] Rheumatoid arthritis patients exhibited a significantly increased frequency of T-cell expansion in both the $CD8^+$ and $CD8^-$ subsets.[941] The clonal activation of peripheral T-cells was found to correlate with disease activity in patients with systemic lupus erythematosus.[940] A monoclonal T-cell population is frequently detected in patients with celiac disease-associated ulcerative jejunitis (both in the ulcer and in the intervening mucosa), with the same clone being detected in subsequent enteropathy-associated T-cell lymphoma.[938] Benign cutaneous infiltrates, including pseudolymphomas, *lichen planus,* and *pityriasis lichenoides et varioliformis acuta,* were reported with clonal T-cell populations. For example, Ponti et al reported clonality in 2.3% of cases of benign inflammatory disease in the skin.[947] Clonal expansion of T-LGL cells is often seen in solid organ transplant recipients.[948]

APPLICATION OF CHROMOSOMAL AND MOLECULAR MARKERS IN PROGNOSTIFICATION

Cytogenetics and molecular testing play an important and ever-increasing role in the personalized approach to management of patients with hematologic malignancies. Acquired genomic aberrations have been shown to significantly impact survival in several hematologic malignancies.[6] Chromosomal and molecular aberrations used in the prognostic stratification of the most common hematopoietic tumors are presented in Table 2.5. Chromosomal aberrations as revealed by conventional cytogenetics (G-banding) and/or FISH are widely recognized as the most important prognostic determinants in AML. Although the prognosis for patients with AML is generally poor, the presence of certain chromosomal abnormalities is associated with a better response to therapy and improved survival.

Table 2.5 Most common chromosomal and molecular markers in the prognosis of hematopoietic malignancies.

Marker	*Neoplasm*	*Prognosis*	*Comments*
t(8;21)	AML	good	
inv(16)/t(16;16)	AML	good	
+8	AML	poor	
–7	AML	poor	
+11	AML	poor	
t(1;22)	AML-M7	poor	
FLT3	AML	good	
del(5q)	MDS	good	good prognosis only if sole abnormality 5q– is associated with response to lenalidomide
t(8;14) [*MYC*]	DLBCL	poor	c-*MYC* occurs typically in Burkitt lymphoma subset of DLBCL with *MYC* has poor prognosis
del(11q)	B-CLL	poor	
del(17p)	B-CLL	poor	
del(13q)	B-CLL	good	
t(11;18)	MALT lymphoma	resistance to *H. pylori* eradication therapy	
t(1;14)	MALT lymphoma	resistance to *H. pylori* eradication therapy	
–13/del(13q)	Multiple myeloma	poor	
t(2;5)	ALCL	good	good response to chemotherapy, in contrast to ALCL negative for *ALK*/t(2;5)
p53	Various neoplasms	poor	
Complex karyotypic abnormalities	Various neoplasms	poor	complex karyotypic changes are usually associated with poor prognosis and disease progression

AML patients with t(15;17), t(8;21), or inv(16) comprise the favorable risk group, whereas a complex karyotype predicts an extremely poor prognosis (a normal karyotype and other noncomplex abnormalities comprise the intermediate AML group).

MDS encompass the heterogeneous group of disorders with variable prognosis and risk of progression into acute leukemia. There is no single clinical or biologic parameter that can accurately predict prognosis and often it can be challenging for clinicians to choose the most appropriate treatment for an individual patient. The treatment options depend on whether the patient has a low, intermediate, or high risk and include supportive care measures, including blood transfusions (red cells or platelets), recombinant growth factors (erythropoietin, darbepoetin, filgrastim, pegfilgrastim, sargramostim), allogeneic stem cell transplantation, DNA methyltransferase inhibitors (azacitidine and decitabine), cyclosporin, TLK199/ valproic acid, and immunomodulatory agents (lenalidomide, thalidomide). The presence of specific cytogenetic abnormalities can predict disease manifestations, provide a basis for prognosis, and direct treatment.[103,949] Three risk-based cytogenetic groups (good, intermediate, and poor) can be distinguished. The cytogenetic subgroup with a good outcome includes normal karyotype, –Y alone, del(5q) alone, del(20q) alone; a poor outcome includes complex karyotype (≥3 abnormalities) or chromosome 7 abnormalities; and an intermediate outcome includes all other abnormalities.[13,21,288,950–958] The presence of del(5q), either as the sole karyotypic abnormality or as part of a more complex karyotype, has distinct clinical implications for MDS. Patients with 5q– abnormality display marked erythroid and cytogenetic responses when treated with thalidomide derivative, lenalidomide, often resulting in transfusion independence and cytogenetic remission. Similarly, del(20q) is known to be a favorable

prognostic factor in MDS when it is the sole change. The late appearance of del(20q) at any phase, however, is linked to a significantly unfavorable prognosis, thus indicating the clinical and biologic heterogeneity of del(20q) in MDS.[308] Independent adverse prognostic factors in MDS include complex karyotype, chromosome abnormalities, older age, and prior MDS therapy.

The use of DNA microarrays has defined two molecular subgroups of DLBCL, germinal center B-cell-like (GCB) and activated-B-cell like (ABC), with significantly different mortality rates and responses to conventional therapy. The presence of t(2;5) in ALCL is associated with a good prognosis and chemosensitivity. The molecular or cytogenetic factors associated with prognosis for B-CLL include mutational status of the variable region of the immunoglobulin heavy chain gene (*IGVH* gene), abnormalities of chromosomes 11, 12, 13, and 17, and the status of the *p53/TP53* gene. Deletion of chromosomes 11q and 17p, unmutated *IGVH*, and mutation of *p53/TP53* are associated with aggressive disease and a poor prognosis, whereas the presence of 13q deletion or mutated *IGVH* predicts an indolent form of B-CLL.[240,264,573,576,685,686,700,702,853,896,959–969] In myeloma, the genomic aberrations t(4;14) and del(17p) are important independent predictors of survival.[304] *(See* Chapter 3 for details.)

APPLICATION OF CHROMOSOMAL AND MOLECULAR MARKERS IN DISEASE MONITORING

Cytogenetics/FISH and molecular pathology play an important role in disease monitoring to determine the response to treatment, persistent disease, and early (subclinical) relapse. MRD is defined as a submicroscopic disease detectable in bone marrow and/or blood in patients in complete clinical remission (CR). Detection of MRD has prognostic value in ALL and AML. Cytogenetic/FISH, molecular, and immunologic techniques that are more sensitive than morphology are increasingly being used to assess and quantify MRD.[970–977] Immunologic marker analysis allows the detection of aberrant or unusual immunophenotypes (e.g. $CD45^-$, $CD33^+$ B-cells), while qRT-PCR techniques target fusion regions of chromosome aberrations [e.g. *BCR/ABL*, *IGH/MYC*, *RUNX1/ETV6, NPM/ALK*], chimerism (e.g. short tandem repeats) and clone-specific immunoglobulin and T-cell receptor gene rearrangements.[974,978–981] Analysis of the patient-specific fusion sequences may be more useful for MRD monitoring in certain acute leukemias associated with numerous chromosomal translocations of one gene (e.g. *MLL*).[982] Immunophenotyping by multiparameter 4- or 6-color flow cytometry or allele-specific PCR for the immunoglobulin gene rearrangement allows detection of one leukemic cell in 100 000 normal cells.[983,984] Immunophenotypes that allow detection of one leukemic cell in 10 000 normal cells can be identified in at least 90% of patients with ALL; immunophenotypes that allow detection of one leukemic cell in 1000–10 000 normal cells can be identified in at least 85% of patients with AML.[985] The combination of cell sorting based on leukemia-specific immunophenotype and PCR analysis of short tandem repeats (STRs) has been also successfully used for MRD detection.[978] The rationale underlying MRD studies is to improve the measurement of treatment response (especially early after induction therapy), to provide independent prognostic information, and to optimize therapeutic strategies adapted to the molecular response by identification of low-risk and high-risk patients, who may benefit from treatment reduction or treatment intensification, respectively.

MRD in B-CLL

Although current treatment protocols, especially stem cell transplantation, lead to a CR in most patients with non-Hodgkin lymphoma and some patients with B-CLL, many of the patients relapse. This implies that CR is compatible with the presence of residual malignant cells (MRD). About 25% of

patients with CR (NCI criteria) have detectable MRD by flow cytometry.[977] In patients with B-CLL and NHL, the prognostic significance of MRD is still a matter of debate, as the majority of patients remain MRD^+ after conventional treatment.[986] This is changing, however, with the implementation of new treatment modalities, such as the application of monoclonal antibodies (e.g. Rituxan, Campath), where a significant proportion of patients convert to MRD negativity and experience prolonged remission and longer survival. The identification of new prognostic markers, which have divided CLL into indolent (favorable) and aggressive (unfavorable) subtypes, and the occurrence of CLL in younger patients changed the traditional approach to CLL management, since patients with unfavorable prognostic markers and those of younger age (<55) are at a greater risk of death from progressive CLL.[987] Thanks to the combined use of purine analogs, monoclonal antibodies, and stem cell transplantation, CRs are being obtained in a large proportion of patients with B-CLL.[988–991]

In B-CLL, MRD has been estimated mainly by flow cytometry[977,992–994] or PCR-based techniques, using either consensus PCR or allele-specific PCR (CRD3).[995–998] The allele-specific oligonucleotide (ASO)-PCR assay is one of the most sensitive PCR technologies available (sensitivity up to 1 cell in 10^{-6} normal cells), but it requires the generation of patient-specific primers and is costly and labor in tensive. Consensus PCR is a qualitative method with variable sensitivity (1 cell in 10^2–10^4 normal cells), is less labor intensive, but may be not applicable to CLL patients with the mutations in the *IGVH* gene. Flow cytometry (FC) has a sensitivity of 1 in 10^4 cells. Both FC and PCR yield clinically relevant information in monitoring B-CLL patients, with clonotypic PCR detecting MRD more often than FC.[976,998,999] MRD detection by flow and qRT-PCR were equally suitable to monitor MRD kinetics after allogeneic stem cell transplantation, but the PCR method detected impending relapses after autologous SCT earlier.[998]

Moreno et al compared flow cytometry, PCR (consensus assay), and quantitative PCR (qPCR) in the assessment of MRD in CLL patients.[1000] All MRD^+ samples by consensus PCR (84 of 248 samples) were also found to be MRD^+ by qPCR and FC; among MRD^- samples by consensus PCR, CLL cells were detected by qPCR and FC in 77 (47%) and 39 (23%) of the 164 samples, respectively.[1000] Among samples analyzed simultaneously by FC and qPCR (254 samples from 30 patients), in 49 samples MRD could be detected by qPCR but not by FC (33 of these samples had MRD levels higher than 10^{-4} by qPCR) and only 8 samples (3 patients) were positive by FC and negative by qPCR, demonstrating concordant results between FC and qPCR in the majority of cases. Moreno et al also reported a very good correlation between blood and bone marrow samples.[1000] The persistence of PCR^+/MRD after stem cell transplantation was associated with an increased probability of relapse.[995] Esteve et al reported that the persistence of MRD in B-CLL appeared to have different implications depending on the type of marrow transplantation, autologous or allogeneic.[975] MRD^+ status correlates with clinical relapse.[975,977,994,996,1000] The persistence of MRD after autologous transplantation is highly predictive of clinical progression, but the detection of MRD after allogeneic transplantation does not necessarily predict clinical relapse.[975] The study by Moreno et al and others confirmed a different outcome in patients after autologous versus allogeneic transplantation: allogeneic transplantation results in long-term disease control in some patients, suggesting that they can be clinically cured.[983,1000–1002] It has been suggested that the graft-versus-leukemia effect makes possible the co-existence of some residual leukemic cells with a prolonged CR.[994,1003,1004] It appears that MRD status at the end of therapy is more predictive of duration of remission than conventional response criteria and identifies the patients at risk of early disease progression.[1005] MRD^- remission in CLL is achievable with alemtuzumab, leading to an improved overall and treatment-free survival.[992]

In the series reported by Moreton et al, complete remission, partial remission, and no response to alemtuzumab were observed in 36%, 19%, and 46% of CLL patients, respectively.[992] Among purine analog refractory patients, 50% responded to alemtuzumab.[992] MRD detection within 6 months after autologous transplantation identifies patients with a high relapse risk.[1000] In contrast to patients with a nonprogressive or indolent disease, or elderly patients with a poor performance status and many co-morbid conditions, MRD negativity should be a desired goal of treatment for younger patients with progressive disease or for patients with poor prognostic factors, such as fludarabine-refractory disease, unmutated *IGVH*, ZAP-70 positivity, and/or adverse chromosomal abnormalities such as del(17p) or del(11q).

MRD in B-cell lymphomas

FL is associated with frequent bone marrow involvement at diagnosis, typically with a paratrabecular pattern. Most patients with FL achieve a CR after treatment, but eventually most of them, particularly those with stage IV, relapse due to MRD. The impact of measuring MRD in the bone marrow or blood by real-time quantitative PCR of t(14;18) (*BCL2/IGH* qRT-PCR) on survival is not clear, i.e. some patients have molecular remission without complete clinical remission (most likely due to preferential elimination of tumor cells from blood or bone marrow over nodal sites with new therapy). Molecular negativity in peripheral blood is not associated with a better survival.[1006] Lambrechts et al reported that the presence or absence of $t(14;18)^+$ cells in the circulation in stage III and IV FL treated with conventional remission induction therapy showed no obvious correlation with the clinical remission status and the remission duration.[1007] Also, Mandigers et al did not show any correlation between clinical response and quantitation of circulating $t(14;18)^+$ cells in patients with stage II–IV FL treated with standard chemotherapy and interferon-α; however, the progression-free survival was significantly prolonged in patients with >1 log decrease in circulating $t(14;18)^+$ cells.[1006] Patients who achieve molecular remission after autologous transplant and/or chemotherapy do not necessary have a better disease-free survival.[1008,1009] Serial PCR analysis to determine the molecular response in FL correlates well with outcome, especially when combined with pretreatment β_2-microglobulin level.[1008]

MCL appears to be largely resistant to complete eradication by conventional chemotherapy.[1010] The majority of patients have positive MRD (*BCL1/IGH*-PCR) in bone marrow irrespective of histologic involvement.[1011] A new treatment regimen with rituximab and combination chemotherapy may transiently clear blood or bone marrow of detectable tumor cells, but molecular remission does not translate into prolonged progression-free survival (16.5 months in MRD^+ vs 18.8 months in MRD^-).[1012] Brugger et al showed that one single course of rituximab consolidation given after autologous stem cell transplantation may help to eliminate MRD and may translate into improved event-free survival in both FL and MCL patients.[1013]

MRD in multiple myeloma

FC and patient-specific RT-PCR can be applied to monitor MRD in MM patients. FC immunophenotyping of plasma cells might be useful for detecting MRD in cases with aberrant antigen expression and for selection of therapeutic agents that have specific membrane targets.[1014] Patients with MM in CR after chemotherapy or autologous stem cell transplantation frequently have positive MRD results,[1015,1016] and only 5–15% of patients attain durable PCR negativity.[1016–1018] The subset of MM patients in long term CR after high-dose chemotherapy and allogeneic stem cell transplantation who are PCR/MRD^- is higher and approaches 70%.[1017,1019,1020] The cumulative risk of relapse at 5 years was 0% for PCR/MRD^- patients and 100% for PCR/MRD^+

patients.[1019] MRD/PCR negativity is associated with a very low rate of clinical relapse.[1020,1021] Patient-specific real-time IGH-PCR detects molecular disease prior to the clinical diagnosis of progression or relapse and provides the opportunity for earlier treatment intervention.[1022]

MRD in acute myeloid leukemia

MRD in AML can be analyzed by PCR (e.g. *PML/RARα*, *RUNX1/ETO, FLT3*-ITD/mutations, *NPM1*/ mutations) or by flow cytometry (leukemia-associated aberrant immunophenotype).[970,1023–1028] In multivariate analysis, the MRD level is the most powerful independent prognostic factor in AML patients, followed by cytogenetics and the number of chemotherapy cycles to achieve CR. Based on the level of MRD (number of residual tumor cells determined by FC), San Miguel et al suggested four risk categories for disease-free and overall survival in AML: very low risk ($<10^{-4}$), low risk (10^{-3}–10^{-4}), intermediate risk (10^{-2}–10^{-3}), and high risk ($>10^{-2}$).[1025] The relapse-free survival rates at 3 years for these risk groups were 100%, 85%, 55%, and 25%, respectively. In the series by Venditti et al, an MRD level $\geq 3.5\times10^{-4}$ cells at the end of consolidation therapy strongly predicted relapse and was significantly associated with an *MDR1* phenotype and intermediate or unfavorable cytogenetics.[1028]

The association of long-term clinical remission with molecular disease eradication is well established in AML patients with t(15;17) and inv(16).[1029–1033] MRD positivity by qRT-PCR after consolidation therapy in APL patients or the reappearance of *PML/RARα* transcripts after negative results is highly predictive of relapse.[1034–1037] Serial monitoring of marrow by nested RT-PCR following completion of therapy can be used to reliably predict outcome in APL.[1032,1036] Patients with recurrent PCR positivity (two positive consecutive PCR tests) were found to invariably relapse, whereas relapse was very rare in those with serial negative tests. Liu et al demonstrated that qRT-PCR is potentially superior to regular RT-PCR in the evaluation of the molecular response in APL.[1038] After induction therapy and completion of consolidation, a minor molecular response (3.0–4.9 log-reduction by qRT-PCR) was documented in 35.5 and 96.8% of patients, respectively, which was equivalent to the regular RT-PCR (22.6 and 96.8%), whereas the major molecular response (≥5.0 log-reduction by qRT-PCR) rate was significantly lower (12.9 and 90.3%, respectively).[1038] Loss of the major molecular response was associated with a subsequent loss of the minor molecular response, positive RT-PCR, and then documentation of CNS leukemia or a clinical relapse in 3–6 months.[1038]

Most AML patients with t(8;21) or inv(16) have a good prognosis with current anthracycline- and cytarabine-based protocols. In patients with $t(8;21)^+$ AML molecular remission is a prerequisite, but not a guarantee, for long-term disease-free survival. Long-term complete remission in these patients is associated with persistent molecular disease eradication.[1039] Due to the slow kinetics of *RUNX1/ETO* after consolidation chemotherapy the value of qRT-PCR (nested RT-PCR) to predict early relapse is limited. Additionally, in patients with $t(8;21)^+$ AML, the *RUNX1/ETO* fusion may be present in nonleukemic stem cells, monocytes, and lymphocytes.[1040] In this situation qRT-PCR might help to define the individual relapse risk and improve clinical decision-making.[1039] The large majority of patients with AML associated with t(16;16)/inv(16) in long-term CR were found to be MRD^- by RT-PCR. However, similarly to AML with t(8;21), some long-term survivors remained PCR/MRD^+. In a series reported by Perea et al, the mean amount of MRD detected by flow cytometry in relapsed and nonrelapsed patients was markedly different: 0.3 vs 0.08% at the end of treatment.[1041] The mean number of fusion transcript copies/ABL $\times 10^4$ also differed between relapsed and nonrelapsed patients: 2385 vs 122 after induction, 56 vs 7.6 after intensification, and 75 vs 3.3 at the end of chemotherapy.[1041] Using qRT-PCR, a cutoff level of >10 copies at the end of treatment correlated

with a high risk of relapse: the cumulative incidence of relapse was 75% for patients with qRT-PCR >10 compared to 21% for patients with qRT-PCR levels ≤10.[1041]

Monitoring by qRT-PCR may also have a prognostic impact in AML patients with *NPM1* mutations.[1042] After achieving CR, the mutant copy number was significantly higher in patients with subsequent relapse than in those remaining in continuous CR, and the presence of detectable mutants after treatment predicted relapse if no further chemotherapy was administered.[1042] Furthermore, the patients with any rise of mutant signals during serial follow-up had a 3.2-fold increase of relapse risk compared to those with persistently low or undetectable signals.[1042]

MRD in acute lymphoblastic leukemia

MRD plays a significant role in the measurement of response to treatment in childhood B- and T-cell ALL and in adult B-ALL (the ability to predict relapse based on MRD is weaker in adult T-ALL).[1043–1046] Although MRD levels measured by flow cytometry and PCR correlate well,[1045] these techniques differ in their applicability and sensitivity, and MRD results obtained by one method cannot yet easily be compared with MRD results obtained by another method.[981,1047] The discordant results between FC and qRT-PCR are due to the limited sensitivity of FC analysis within the range 0.01–0.001%, and less often due to the instable or subclonal *IG/TCR* gene rearrangements or a limited quantitative range of the applied qRT-PCR targets.[1047] The complete disappearance of leukemic cells (or their reduction to <1/100 000 cells) may be necessary to achieve a cure of ALL.[1048] Low levels or negative MRD after completion of induction therapy predicts a good outcome in ALL and the risk of relapse is associated with the MRD level.[1049–1053] Combined information on MRD from the first 3 months of treatment (especially at the end of the induction treatment and before the consolidation treatment) distinguishes patients with a good from those with a poor prognosis.[1050] The quantification of residual leukemic cells in serial marrow aspirates during therapy may allow the early detection of relapse. Negative MRD at the end of induction therapy and before consolidation is associated with a 2% relapse rate at 5 years (low risk), whereas patients with intermediate (10^{-3}) or high ($\geq 10^{-2}$) levels of positive MRD at both measurements have a 5-year relapse rate of 80% (high risk).[1050] MRD is highly predictive of relapse in B-ALL patients undergoing stem cell transplantation.[1054] Using multiparameter FC to quantify MRD before transplant, the estimated disease-free survival for MRD^+ and MRD^- patients was 33.3% and 73.5%, respectively.[1055] Also, in patients after bone marrow transplant MRD is predictive of the relapse. Patients who subsequently relapsed were MRD^+ in 88%, and those in long-term complete remission were MRD^+ only in 22% in post-transplant analysis.[1056] MRD in childhood ALL appears to be an independent prognostic factor that allows a precise risk group classification.[986] The finding of TdT^+ cells or cells with an aberrant phenotype in an RBC-free CSF sample with a negative cytomorphology was highly predictive for impending CNS relapse.[1057,1058] Despite clinical and morphologic remission being achieved by over 80% of adult patients with ALL, 5-year survival was limited to 40% of patients, clearly indicating that morphology is insufficient in predicting the outcome. Analysis of disease-free survival rates for MRD^+ and MRD^- patients shows that MRD positivity is associated with increased relapses (being most significant at 3–5 months post-induction and beyond). The association of MRD test results and disease-free survival better predicts the outcome than other standard parameters and is therefore important in determining the management of individual patients.[1049,1059]

MRD in chronic myeloid leukemia

The t(9;22) reciprocal translocation leads to the formation of *BCR/ABL* fusion, with subsequent

activation of tyrosine kinase, responsible for development of CML. (*see* CML, Chapter 3). Imatinib mesylate (Gleevec), which blocks the kinase and became a standard therapy for patients with CML, induces a complete hematologic response in the majority of patients, and complete or nearly complete remission in up to 80% of patients.[50,51,1060,1061] Ninety percent of patients with early stage (chronic phase) CML and 60% of patients with advanced stage CML (blast phase) achieve a hematologic response to imatinib.[1060,1062] The response to treatment is based on hematologic, cytogenetic, and molecular parameters (Table 2.6).[42,50,51,1061,1063,1064] A hematologic response (HR) indicates a return of peripheral blood cell counts and bone marrow morphology to normal. Cytogenetic response (CyR) can be divided into complete, major, minor, and minimal. A complete CyR (CCyR) indicates the disappearance of the Ph, and a major CyR (MCyR) indicates less than 35% Ph^+ cells in the bone marrow (Table 2.6). A major molecular response is defined as a 3-log reduction in the *BCR/ABL* transcript (≤ 0.10 *BCR/ABL* ratio according to the International Scale) and a complete molecular response is synonymous with an undetectable transcript. In patients with a newly diagnosed chronic phase of CML treated with imatinib, the CCyR is greater than 70% and approaches 89% after prolonged treatment.[51,1065,1066] For comparison, patients treated with interferon-α had the CCyR rates of 14–28%.[51,1065]

Because the relapse occurs in a small percentage of patients treated with imatinib, the MRD status by cytogenetics, FISH, and/or PCR is included in the treatment strategies. Patients who had a complete cytogenetic response or in whom levels of BCR/ABL transcripts had fallen by at least 3-log had a significantly lower risk of disease progression than did patients without a CCyR.[1066] The percentage of Ph-positive cells identified by conventional cytogenetics inversely correlates with progression-free survival.[1061,1067] FISH results are more sensitive than standard cytogenetics and correlate well with those by PCR,[1068] allowing for the identification of the *BCR/ABL* fusion gene (both p210 and p190) and enabling the response to treatment to be monitored.[1069] qRT-PCR is the most sensitive

Table 2.6 Definitions of response in CML patients[42,50,51,1062,1063,1072,1073]

	Hematologic response	*Cytogenetic response*	*Molecular response (ratio of BCR/ABL to control gene according to the International Scale)*
Complete	Platelets < 450 × 10^9/l WBC < 10 x 10^9/l Differential count without immature granulocytes and <5% basophils Nonpalpable spleen	Ph^+ cells 0%	Transcripts nondetectable
Major	—	Ph^+ cells 1–35%	≤0.10 (3-log reduction of transcript level)*
Minor	—	Ph^+ cells 36–65%	—
Minimal	—	Ph^+ cells 66–95%	—
None	—	Ph^+ cells >95%	—
Monitoring	Check every 2 weeks until complete response, then every 3 months	Check at least every 6 months until complete response, then every 12 months	Check every 3 months; mutational analysis in case of failure or transcript level increase

* 3-log reduction (or more) in transcript level was considered a major molecular response, but it is recommended to replace the terminology 'log reduction' with a standardized numeric International Scale (IS) expressing the amount of *BCR/ABL* as a percentage of a control gene and anchored to 2 'absolute' values based on validated reference materials of known value.[41,42,1072,1073] The first value will be designated 100% on the proposed IS and the second value will represent a 3 log reduction, i.e. 0.1%

methodology (10^{-5}–10^{-6}) and therefore allows for MRD evaluation in patients in CR after chemotherapy as well as in those undergoing allogeneic or autologous stem cell transplantation.[1070–1073] Failure to achieve a 2-log response at the time of CCyR is an independent predictive marker of subsequent progression-free survival.[1074] In a series reported by Hughes et al, for patients who had a CCyR and a reduction in PCR transcript levels of at least 3 log at 12 months, the probability of remaining progression-free was 100% at 24 months, as compared with 95% for such patients with a reduction of less than 3-log and 85% for patients who were not in CCyR at 12 months.[1075] The achievement of either a 2-log molecular response at the time of CCyR or a 3-log response anytime thereafter is a significant and independent prognostic marker of subsequent progression-free survival.[1074]

Olavarria et al showed that qRT-PCR performed early after stem cell transplantation is useful for predicting outcome, and that the risk of relapse correlates with the number of *BCR/ABL* transcripts.[1076] They classified the results as (a) negative (no *BCR/ABL* transcripts), (b) positive at low level (<100/μg RNA and/or the *BCR-ABL/ABL* ratio <0.02%), or (c) positive at high level (transcript levels exceeded the thresholds defined above).[1076] Three years after stem cell transplantation, the cumulative incidence of relapse was 16.7%, 42.9%, and 86.4%, for negative, positive at low-level and, positive at high-level PCR results, respectively. Using qRT-PCR, Radich et al, showed that the median *BCR/ABL* level of patients who relapsed was significantly greater (40 443 copies/μg RNA) than for those who remained in remission (24 copies/μg RNA).[453] Treatment failure and small increases in the *BCR/ABL* level can identify patients with kinase domain mutations that lead to imatinib resistance, requiring mutational analysis for *BCR/ABL* mutation. Patients with positive PCR results after standard treatment (including an increased dose of imatinib) and/or allogeneic transplantation may benefit from new treatment strategies (dasatinib, nilotinib).

At a consensus conference held in Bethesda, it was proposed to move away from the term 'log reduction' and to introduce a standardized numeric International Scale (IS) expressing the amount of *BCR/ABL* as a percentage of a control gene and anchored to two 'absolute' values based on validated reference material of known value.[42,1072,1073] The first value will be designated 100% on the proposed IS and the second value will represent a 3-log reduction, i.e. 0.1%, and by comparing the value of the 3-log reduction with the value on the internationally agreed scale, each laboratory can derive a conversion factor which can then be used to express the results in any given patient on the IS.[42,1073]

CHAPTER 3

Hematologic malignancies: correlation between morphology, phenotype, and chromosomal/genetic markers

The WHO classification of hematopoietic tumors is presented in Table 3.1. Figures 3.1 and 3.2 present algorithms for the diagnostic approach for hematopoietic tumors.

B-CELL LYMPHOPROLIFERATIONS

Non-Hodgkin's lymphomas are classified into numerous histologic and phenotypic subtypes and generally can be divided into indolent (low grade) and aggressive (high grade). In the B-cell category, low-grade lymphomas include SLL/CLL, FL, marginal zone B-cell lymphoma (MALT lymphoma), and lymphoplasmacytic lymphoma/Waldenström macroglobulinemia.The aggressive group comprises DLBCL and its variants, Burkitt lymphoma, mediastinal (thymic) large B-cell lymphoma, and plasmablastic lymphoma. However, even within a specific category, patients vary considerably with regard to outcome (e.g. BL is more aggressive clinically than DLBCL), and therefore chromosomal and molecular testing is often needed to better characterize the lymphoma in individual patients. Some lymphomas, despite the lack of cytomorphologic features suggesting high grade, have a particularly poor prognosis when treated with multiagent chemotherapy alone (e.g. B-prolymphocytic leukemia and MCL). Figure 3.3 presents an algorithm for the diagnosis of mature B-cell lymphoproliferations. Common chromosomal translocations in B-cell lymphomas are presented in Table 3.2.

B-chronic lymphocytic leukemia

B-CLL is a low-grade lymphoproliferative disorder of mature (peripheral) lymphoid cells (Figure 3.4). B-small lymphocytic lymphoma (B-SLL) is considered a lymphomatous counterpart of B-CLL involving lymph nodes (as well as extranodal sites). Phenotypically, B-CLL is characterized by co-expression of B-cell markers (CD19, CD20, CD22, CD79a, CD79b) with CD5, CD23, CD43, and CD52. B-CLL shows considerable variations in cytomorphologic, immunophenotypic, and prognostic features. Typical B-CLL, which constitutes approximately 80% of cases, can be defined as having more than 90% small lymphocytes with weak expression of surface immunoglobulin and CD20, and

Table 3.1 WHO classification of hematopoietic tumors

Mature B-cell neoplasms
Chronic lymphocytic leukemia/small lymphocytic lymphoma (B-CLL/SLL)
B-cell prolymphocytic leukemia (B-PLL)
Lymphoplasmacytic lymphoma
Marginal zone B-cell lymphoma (MZL)
splenic marginal zone lymphoma
nodal marginal zone lymphoma
extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue
Hairy cell leukemia
Plasma cell myeloma/plasmacytoma
Follicular lymphoma (FL)
Mantle cell lymphoma (MCL)
Diffuse large B-cell lymphoma (DLBCL)
Mediastinal (thymic) large B-cell lymphoma
Intravascular large B-cell lymphoma
Primary effusion lymphoma
Burkitt lymphoma/leukemia
B-cell proliferations of uncertain malignant potential
lymphomatoid granulomatosis
post-transplant lymphoproliferative disorder, polymorphic
Mature T-cell neoplasms
T-cell prolymphocytic leukemia (T-PLL)
T-cell large granular lymphocytic leukemia (T-LGL leukemia)
Aggressive NK-cell leukemia
Adult T-cell leukemia/lymphoma
Extranodal NK/T-cell lymphoma, nasal type
Enteropathy-type T-cell lymphoma
Hepatosplenic T-cell lymphoma
Subcutaneous panniculitis-like T-cell lymphoma
Mycosis fungoides/Sézary's syndrome (MF/SS)
Primary cutaneous anaplastic large cell lymphoma
Peripheral T-cell lymphoma, unspecified
Angioimmunoblastic T-cell lymphoma (AILD lymphoma)
Anaplastic large cell lymphoma (ALCL)
T-cell proliferation of uncertain malignant potential
lymphomatoid papulosis
Hodgkin lymphoma
Nodular lymphocyte predominant Hodgkin's lymphoma (NLPHL)
Classic Hodgkin's lymphoma (HL)
nodular sclerosis Hodgkin's lymphoma
lymphocyte-rich classical Hodgkin's lymphoma
mixed cellularity Hodgkin's lymphoma
lymphocyte-depleted Hodgkin's lymphoma
Chronic myeloproliferative disorders (CMPD)
Chronic myelogenous leukemia (CML)
Chronic neutrophilic leukemia
Chronic eosinophilic leukemia/hypereosinophilic syndrome
Polycythemia vera (PV)
Chronic idiopathic myelofibrosis (CIMF)
Essential thrombocythemia (ET)
Chronic myeloproliferative disease, unclassifiable

Table 3.1 WHO classification of hematopoietic tumors—cont'd

Myelodysplastic/myeloproliferative diseases
Chronic myelomonocytic leukemia (CMML)
Atypical chronic myeloid leukemia
Juvenile myelomonocytic leukemia
Myelodysplastic/myeloproliferative diseases, unclassifiable
Myelodysplastic syndromes
Refractory anemia (RA)
Refractory anemia with ringed sideroblasts (RARS)
Refractory cytopenia with multilineage dysplasia (RCMD)
Refractory anemia with excess blasts (RAEB)
Myelodysplastic syndrome associated with isolated del (5q)
Myelodysplastic syndrome, unclassifiable
Acute myeloid leukemias (AML)
AML with t(8;21), (AML1/ETO)
AML with inv(16)(p13q22) or t(16;16)(p13;q22) (acute myelomonocytic leukemia with eosinophilia; AML-M4Eo)
Acute promyelocytic leukemia [AML with t(15;17)(q22;q12)], APL (AML-M3)
Acute myeloid leukemia with multilineage dysplasia
with prior myelodysplastic syndrome
without prior myelodysplastic syndrome
Acute myeloid leukemia and myelodysplastic syndrome, therapy-related
Acute myeloid leukemia, minimally differentiated (AML-M0)
Acute myeloid leukemia without maturation (AML-M1)
Acute myeloid leukemia with maturation (AML-M2)
Acute myelomonocytic leukemia (AML-M4)
Acute monoblastic leukemia (AML-M5)
Acute erythroid leukemia (AML-M6)
Acute megakaryoblastic leukemia (AML-M7)
Acute basophilic leukemia
Acute panmyelosis with myelofibrosis
Myeloid sarcoma
Precursor neoplasms
Precursor B-cell neoplasms
precursor B lymphoblastic leukemia/lymphoma (B-ALL/LBL)
Precursor T-neoplasms
precursor T-lymphoblastic leukemia/lymphoma (T-ALL/LBL)
blastic NK-cell leukemia/lymphoma (DC2 leukemia)
Histiocytic and dendritic cell tumors
Histiocytic sarcoma
Langerhans cell histiocytosis/Langerhans cell sarcoma
Interdigitating dendritic cell sarcoma
Follicular dendritic cell sarcoma
Mastocytosis
Cutaneous mastocytosis
Indolent systemic mastocytosis
Systemic mastocytosis with associated clonal hematologic disease
Aggressive systemic mastocytosis
Mast cell leukemia
Mast cell sarcoma

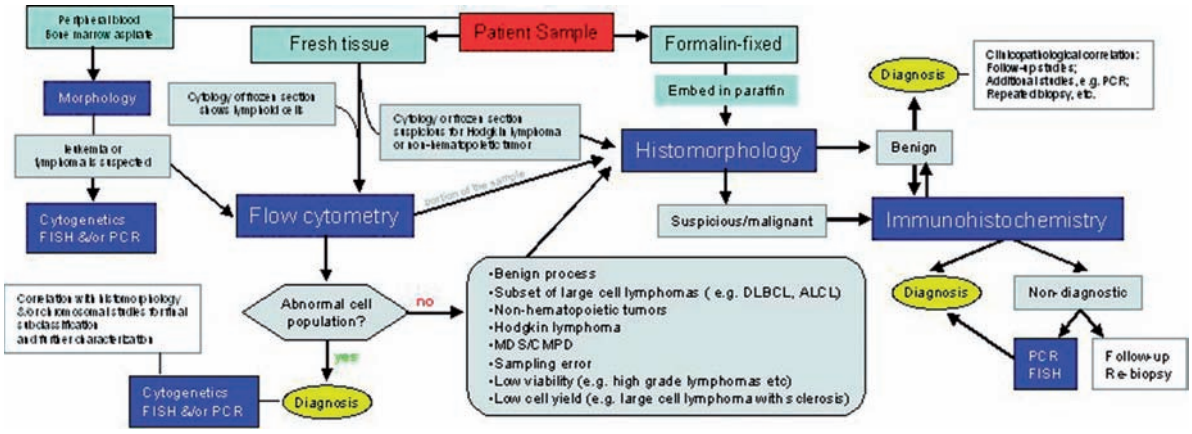


Figure 3.1 Algorithm for lymphoma/leukemia diagnosis

co-expression of CD5 and CD23. Atypical B-CLL shows larger lymphocytes with abundant cytoplasm, prolymphocyte-like or cleaved cells, and aberrant phenotype. The clinical course of B-CLL is variable (Table 3.3) and depends on a number of factors including age, gender, Binet/Rai stage, performance status, laboratory parameters (lymphocyte count, thymidine kinase, soluble CD23, β_2-microglobulin, LDH), atypical cytologic features, pattern and extent of bone marrow infiltration, 17p deletion, *p53/TP53* mutation/loss, deletion of chromosome 11q23, *ATM* status, *IGVH* mutational status, and CD38 and ZAP-70 expression.[264,696,698,1077–1080] Some patients have aggressive disease and require therapy within a relatively short time after diagnosis, whereas others have indolent, asymptomatic disease and are not likely to benefit from palliative chemotherapy. The median survival of patients with B-CLL is 10 years. Patients with poor prognostic factors have a median survival of approximately 3 years. Algorithms for the diagnosis and treatment of B-CLL are presented in Figures 3.5 and 3.6.

Chromosomal aberrations

Genomic aberrations in CLL are important independent predictors of disease progression and survival. Chromosomal abnormalities are detected in the majority of B-CLL patients using molecular cytogenetic technologies, including FISH. Genetic aberrations are found more often by FISH studies when compared to conventional cytogenetics (68.4% vs 36.7%).[968] Mayr et al, used stimulation of CLL cells either with CD40 ligand or with a combination of CpG-oligodeoxynucleotides and IL-2 to increase the frequency of metaphase spreads and revealed translocations in 33 of 96 patients (34%).[967] Although there were no CLL-specific translocations, patients with translocations had significantly shorter median treatment-free survival (24 months vs 106 months) and significantly inferior overall survival (median 94 months vs 346 months).[967]

The most frequent chromosomal abnormalities in B-CLL (Table 3.4) include del(13q), trisomy 12, del(11q)/*ATM*, del(14q), del(6q), and del(17p)/*TP53*.[99,532,685,1081] Patients with a normal karyotype or deletion of 13q14 as the sole genetic abnormality have a better prognosis than those with a complex karyotype or deletion of 11q23 or 17p13. The response rate to chemotherapy is significantly higher in patients with normal karyotypes than in those with abnormal karyotypes, especially with complex changes (Figure 3.7). In series by Dohner et al, the median survival times for patients with 17p deletion, 11q deletion, 12q trisomy, normal karyotype, and 13q deletion as the sole abnormality were

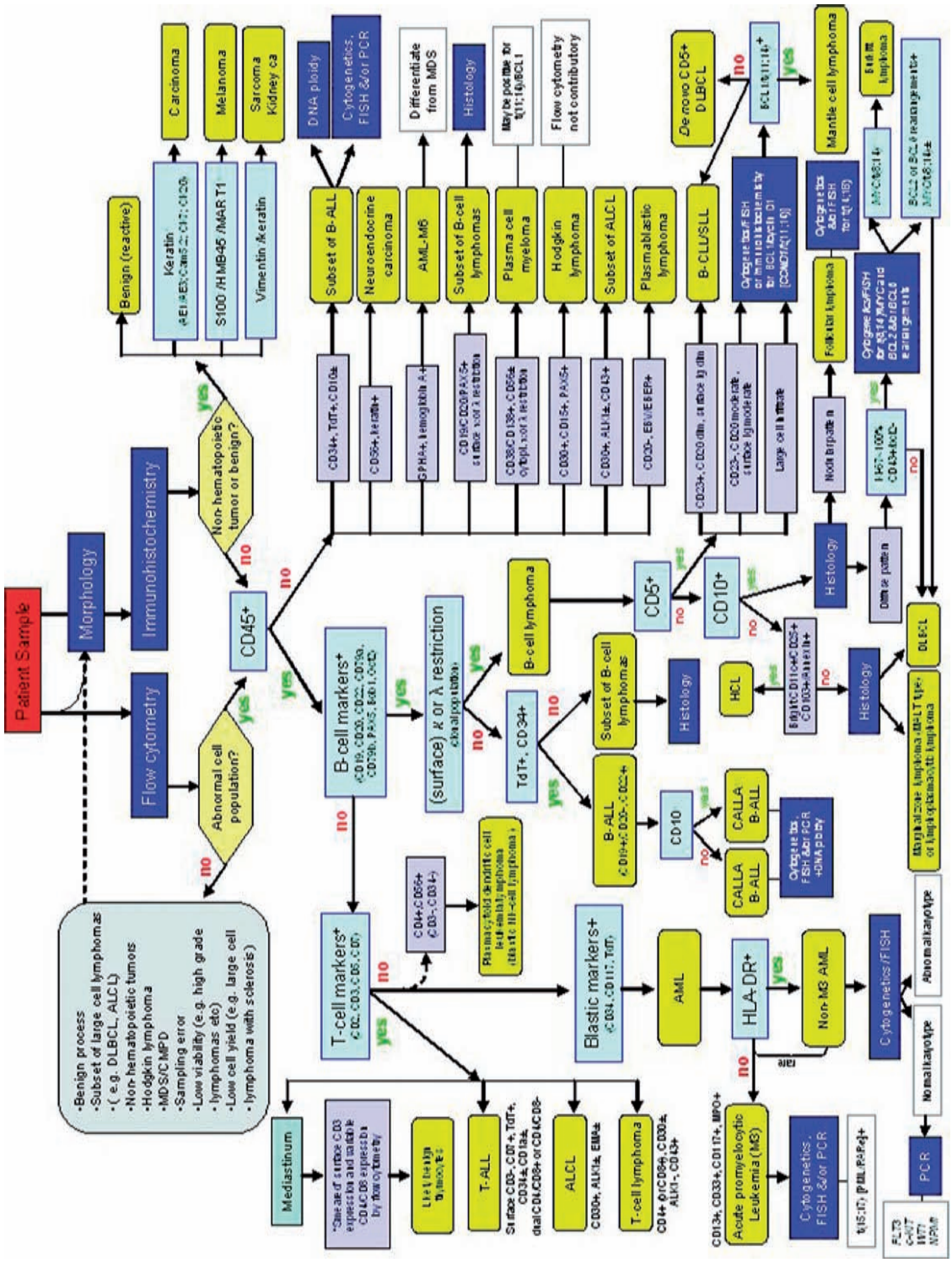


Figure 3.2 Algorithm for the diagnosis of hematopoietic tumors

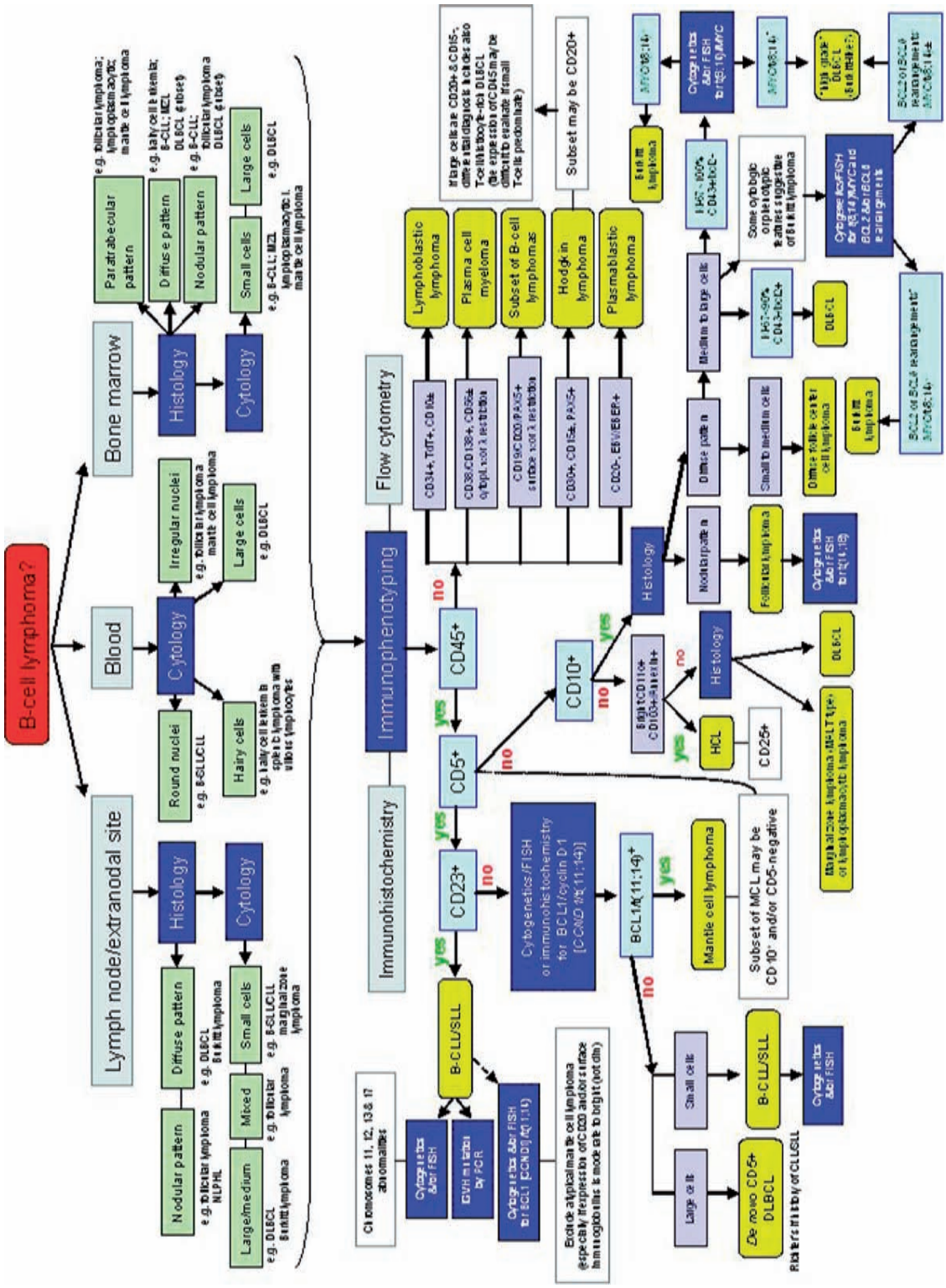


Figure 3.3 Algorithm for the diagnosis of B-cell lymphoma

Table 3.2 Common chromosomal translocations in B-cell lymphomas

Diagnosis	*Translocation*	*Gene(s)*
Burkitt lymphoma	t(14;18)(q24;q32) t(2;8)(p12;q24) t(8;22)(q24;q11.2)	c-*MYC* and *IGH* c-*MYC* and *IGK* c-*MYC* and *IGL*
Diffuse large B-cell lymphoma	t(14;18)(q32;q21) t(3;v)(q27;v)	*IGH* and *BCL2* *BCL6* and various partners
Follicular lymphoma	t(14;18)(q32;q21) t(3;v)(q27;v)	*IGH* and *BCL2* *BCL6* and various partners
Marginal zone B-cell lymphoma (MALT)	t(11;18)(q21;q21) t(14;18)(q32;q21)	*API2* and *MALT1* *IGH* and *MALT1*
Mantle cell lymphoma	t(11;14)(q13;q32)	*CCND1* and *IGH*

32, 79, 114, 111, and 133 months, respectively.[264] Patients with 17p deletions had the shortest median treatment-free interval (9 months), and those with 13q deletions had the longest (92 months).[264] The response to rituximab was noted to vary by cytogenetic group: del(17)(p13.1), 0%; del(11)(q22.3), 66%; del(13)(q14.3), 86%; and +12, 25%.[302]

Deletion 13q14

The deletion 13q14 is the most frequent genetic change in B-CLL, occurring in 40–65% of cases. The presence of either a cryptic deletion of the single locus (q14) or deletion of a larger portion of the chromosome is associated with a favorable prognosis. Acquisition of additional chromosomal changes,

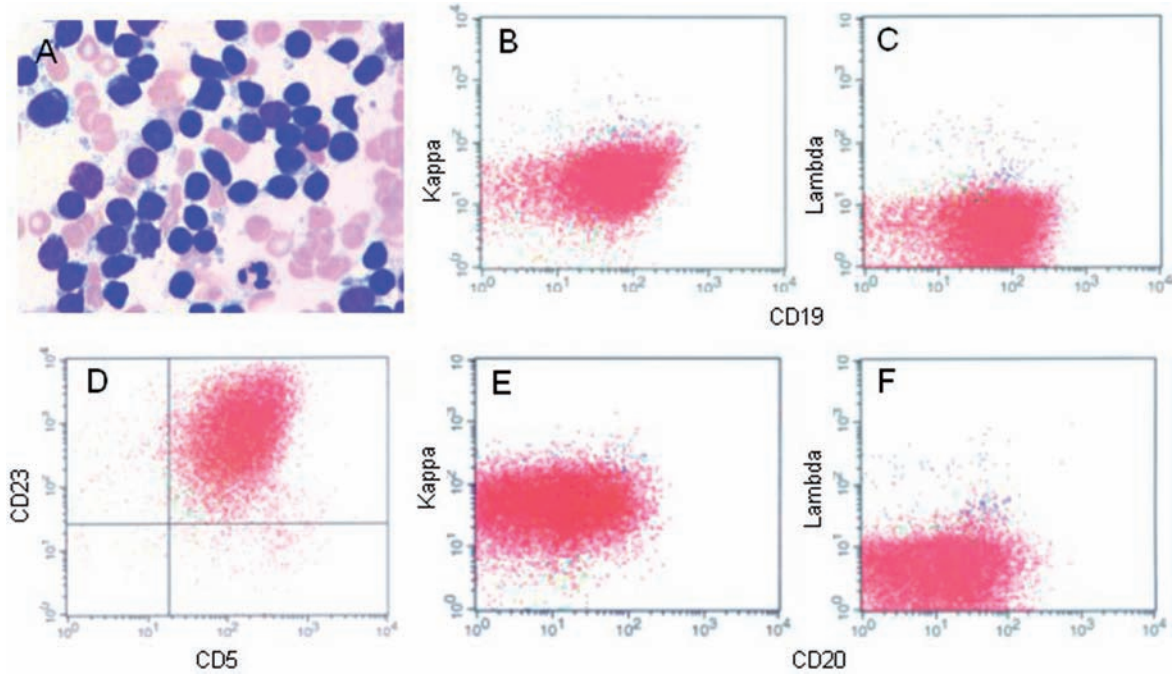


Figure 3.4 B-chronic lymphocytic leukemia (B-CLL). Neoplastic B-cells are small with scanty cytoplasm and condensed chromatin (A); they show moderate expression of CD19 (B and C), dim expression of surface immunoglobulins (kappa in depicted example; B and E), dim CD20 (E and F) and co-expression of CD5 with CD23 (D)

Table 3.3 Chromosomal/genetic changes in B-CLL and their prognostic implication

Factor	*Prognosis*
Normal karyotype	favorable
13q deletion	favorable
Mutated *IGVH*	favorable
Long telomere (Tel-PCR T/S value > 0.3)	favorable
MRD^- status after alemtuzumab or alemtuzumab + fludarabine or SCT	favorable
Trisomy 12	intermediate
Discordant ZAP-70 and CD38 results ($ZAP\text{-}70^+/CD38^-$ or vice versa)	intermediate
CD38 expression (>30%)	poor
ZAP-70 expression	poor
Unmutated *IGVH*	poor
11q deletion	poor
17p deletion or loss or mutation of the *p53/TP53*	very poor

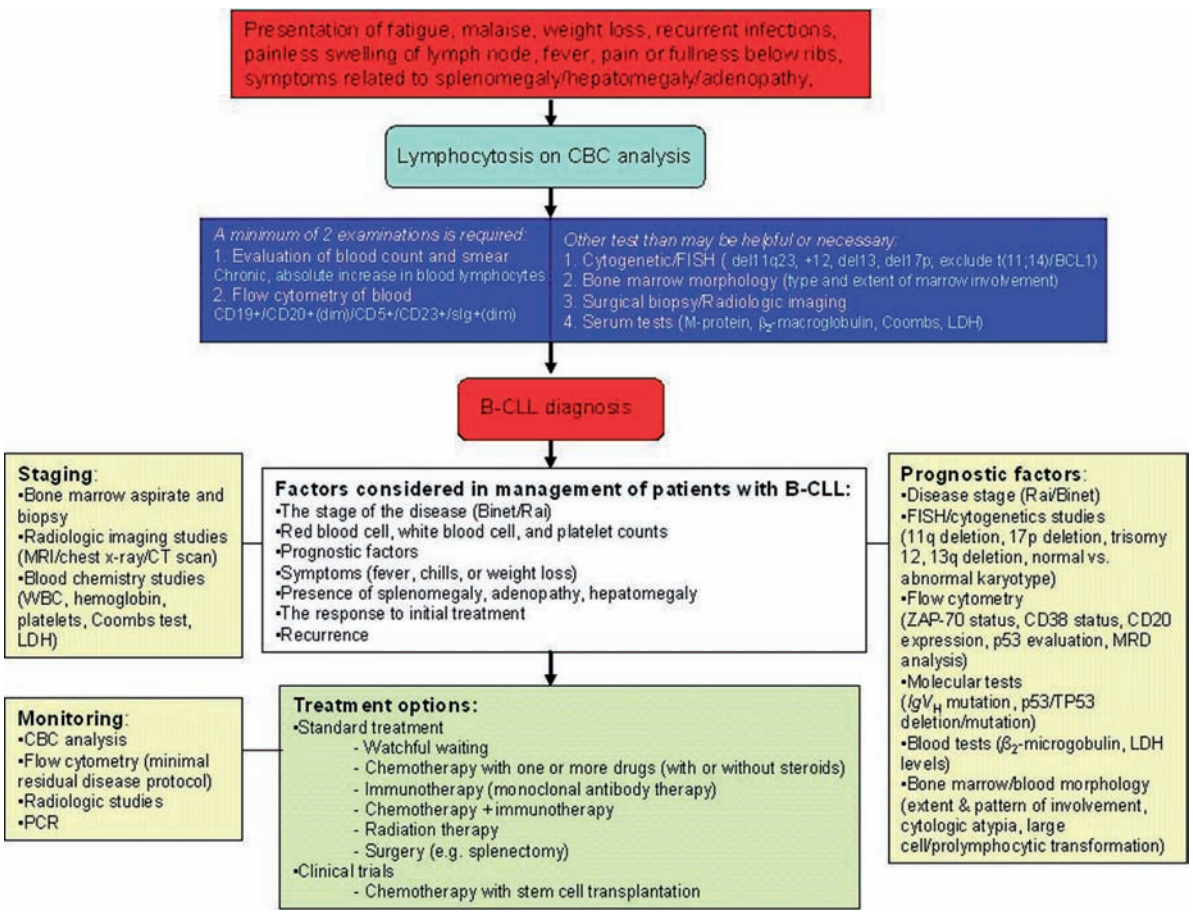


Figure 3.5 Algorithm for the diagnosis of B-CLL

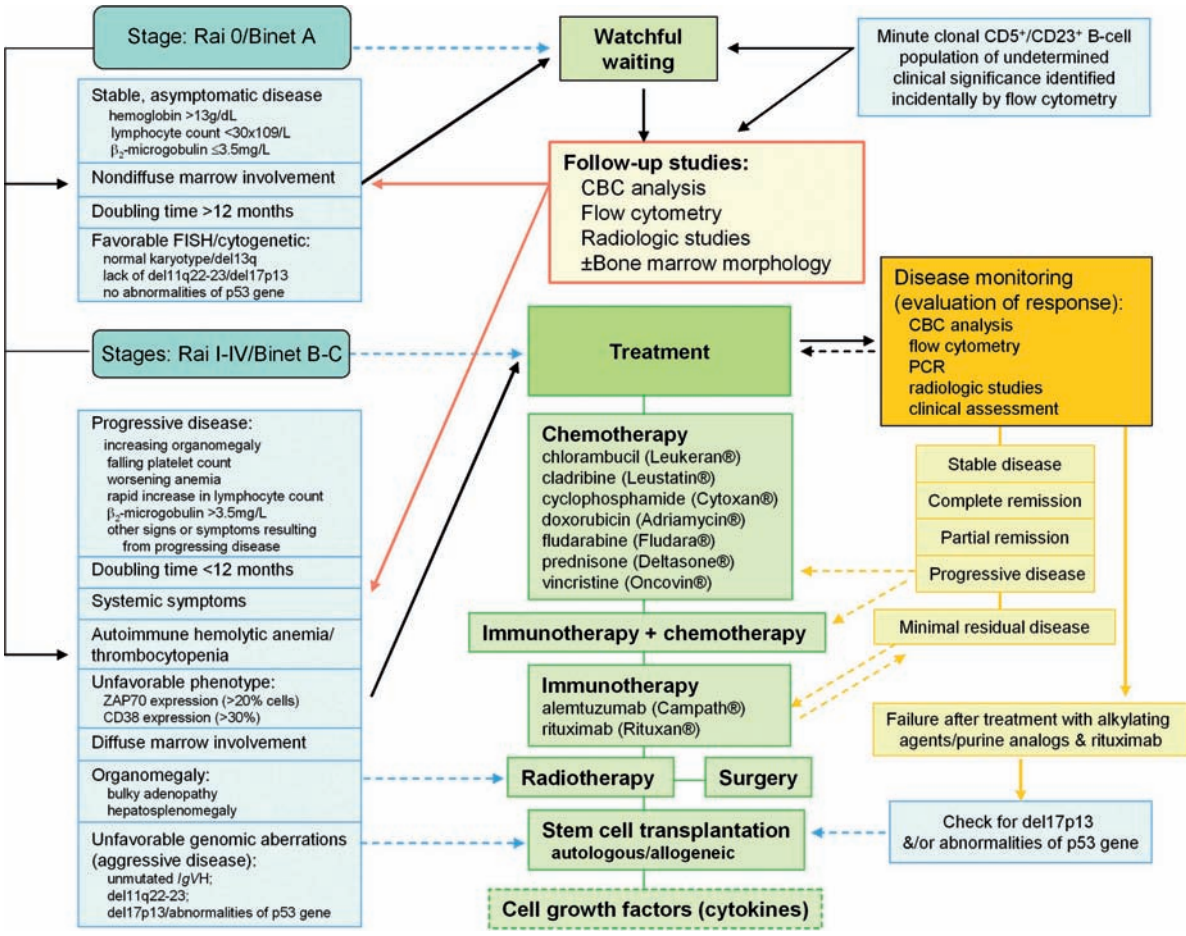


Figure 3.6 Algorithm for the management of B-CLL

especially del(11q), eliminates the favorable outcome of del(13q). Patients with deletion 13q14 (Figure 3.8) as a single aberration have the longest estimated median treatment-free interval and survival time.[240, 264,965] The estimated median survival time of this group was longer as compared to the groups without detectable aberrations and the group with trisomy 12. Deletions of 13q are associated with typical morphologic and phenotypic features.[1082-1085]

Deletions 11q and 17p

Deletions 11q22–23 and 17p13, involving the *ATM* and *p53/TP53* genes, respectively, are independent prognostic markers in multivariate analysis identifying

Table 3.4 Most frequent aberrations in CLL

Reference	*Total (%)*	*del(13q) (%)*	*del(11q) (%)*	*del(17p) (%)*
Dohner et al[896]	82.0	55.0	18.0	7.0
Bacher et al[1831]	74.5	66.2	12.6	10.7
Dewald et al[118]	77.0	64.0	15.0	8.0
Glassman and Hayes[1832]	65.3	40.8	23.4	12.2

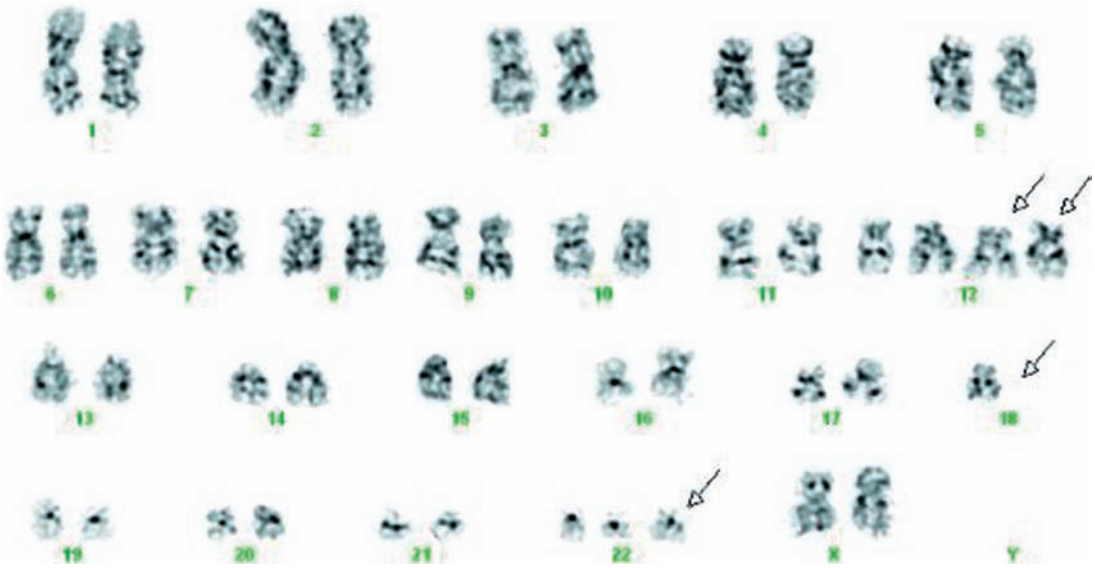


Figure 3.7 B-CLL with clonal evolution. Complex chromosomal abnormalities: 48, XX, +12, +12, –18, +22; cytogenetics

subgroups of patients with rapid disease progression and short survival times.[240,685,969,1086] 11q aberrations which affect the *ATM* gene (ataxia teleangiectasia mutated) identify a subset of B-CLL with extensive adenopathy, rapid disease progression, and inferior survival. The change has been reported in 12–23% of B-CLL patients.[118,896,1087,1088]

The pathogenic role of 17p13 deletions is associated with the tumor suppressor gene *TP53/p53*.

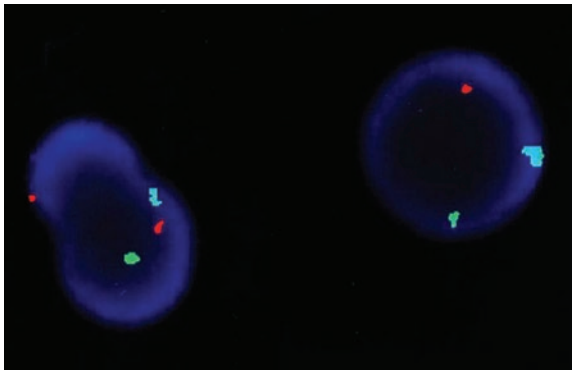


Figure 3.8 B-CLL with deletion 13q; FISH

TP53/p53 abnormalities predict poor survival and resistance to treatment.[736,852] Deletions of *p53* have been reported in B-CLL showing disease progression and atypical morphologic and immunophenotypic features,[851] although in one series, 17p deletions showed an equal distribution between typical and atypical cases.[1083] The presence of the *p53* mutation or del(17q)(p13.1) predicts a poor response to conventional therapy in CLL.[855] Alemtuzumab (Campath) may be an effective initial therapy for patients with *p53* mutations, or del(17q)(p13.1), or both, as opposed to fludarabine, chlorambucil, or rituximab.[855]

Trisomy 12

FISH studies show a similar pattern of chromosomal gains and losses detected in typical and atypical B-CLL,[1083] although trisomy 12 is often associated with atypical variants of B-CLL.[1087,1089–1091] In most studies, patients with trisomy 12q13 have atypical morphology and immunophenotype, a diffuse bone marrow infiltration pattern and/or lymphocyte doubling time below 12 months, elevated serum

β_2-microglobulin levels, and shorter survival times than patients with a normal karyotype or 13q deletions.[264,965,1092] Geisler et al, however, did not show a prognostic significance for an additional copy of chromosome 12 alone or its relationship to atypical morphology.[301] Also, evaluation of the prognostic impact of trisomy 12 by FISH did not reveal a significant difference in survival between patients with or without trisomy 12.[264,301,969,1093,1094] Figure 3.9 shows trisomy 12.

Del(6q)

The 6q27 deletion can be detected in ~20% of patients with CLL.[185] In a study by Cuneo et al, patients with del(6q) showed a higher WBC count, frequent splenomegaly, atypical morphology, CD38 positivity, short time from diagnosis to first treatment, and shorter survival when compared with patients with the favorable cytogenetics.[186] Glassman et al showed more frequent prolymphocytic acceleration in patients with del(6q)$^+$ CLL.[1095]

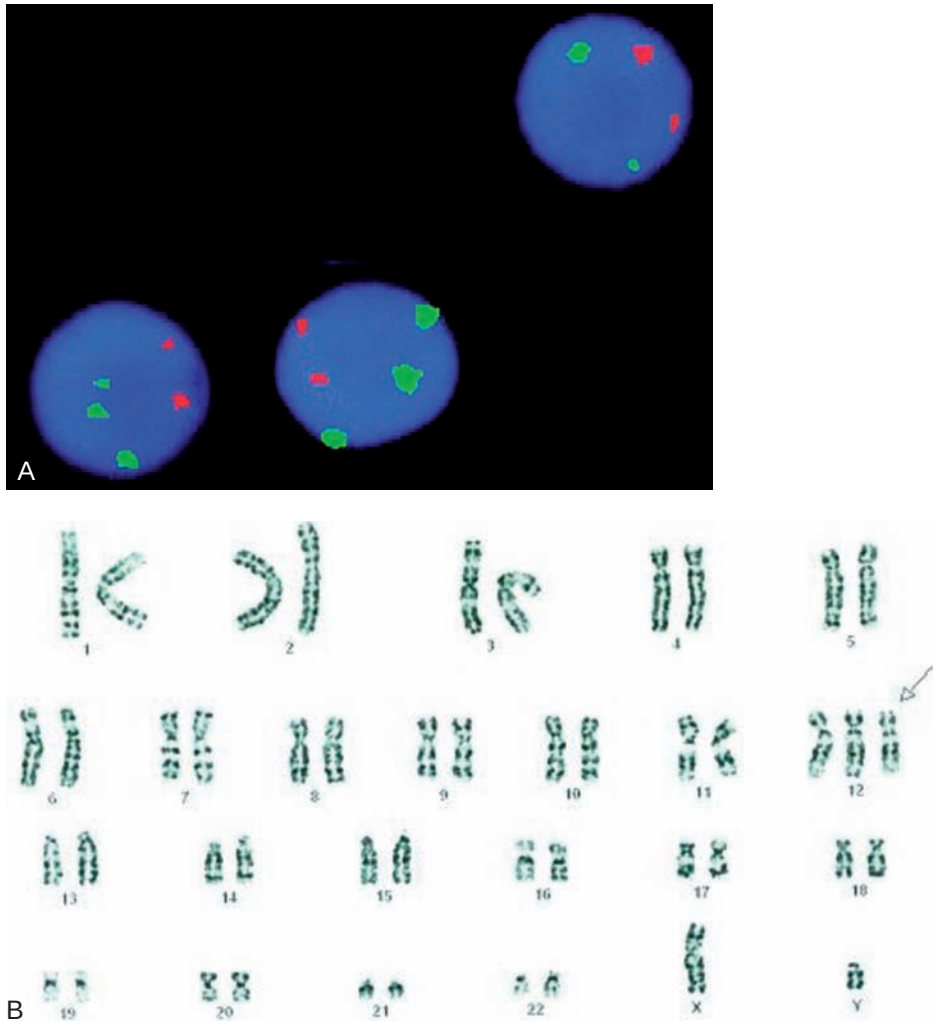


Figure 3.9 B-CLL with trisomy 12; A, FISH; B, cytogenetics

In a series reported by Ripolles et al, all patients with 6q− had other chromosomal abnormalities, which suggests that 6q− occurs as a secondary change.[968]

Trisomy 18

Trisomy 18 is uncommon in B-CLL, occuring more often as a sole abnormality, or in conjunction with trisomies 12 and 19.

Molecular features

IGVH mutation status

During an immune response, a series of point mutations occurs in the immunoglobulin genes of a B-cell. These somatic mutations occur in the germinal centers of the peripheral lymphoid tissue. By sequencing the immunoglobulin heavy-chain variable region (*IGVH*) genes (Figure 3.10), B-CLL can be divided into two types, mutated and unmutated.[694,963] Approximately 60% of B-CLLs can be considered post-germinal center memory B-cells with somatically mutated immunoglobulin variable region genes (mutated *IGVH*), and the remaining cases have V_H genes in the germline configuration (unmutated *IGVH*). In addition, it has been demonstrated that B-CLLs with mutated *IGVH* genes show a relatively high frequency of *BCL6* mutations, further evidence supporting their origin from germinal center related B-cells.[1096] In pivotal studies, Damle et al[694] and

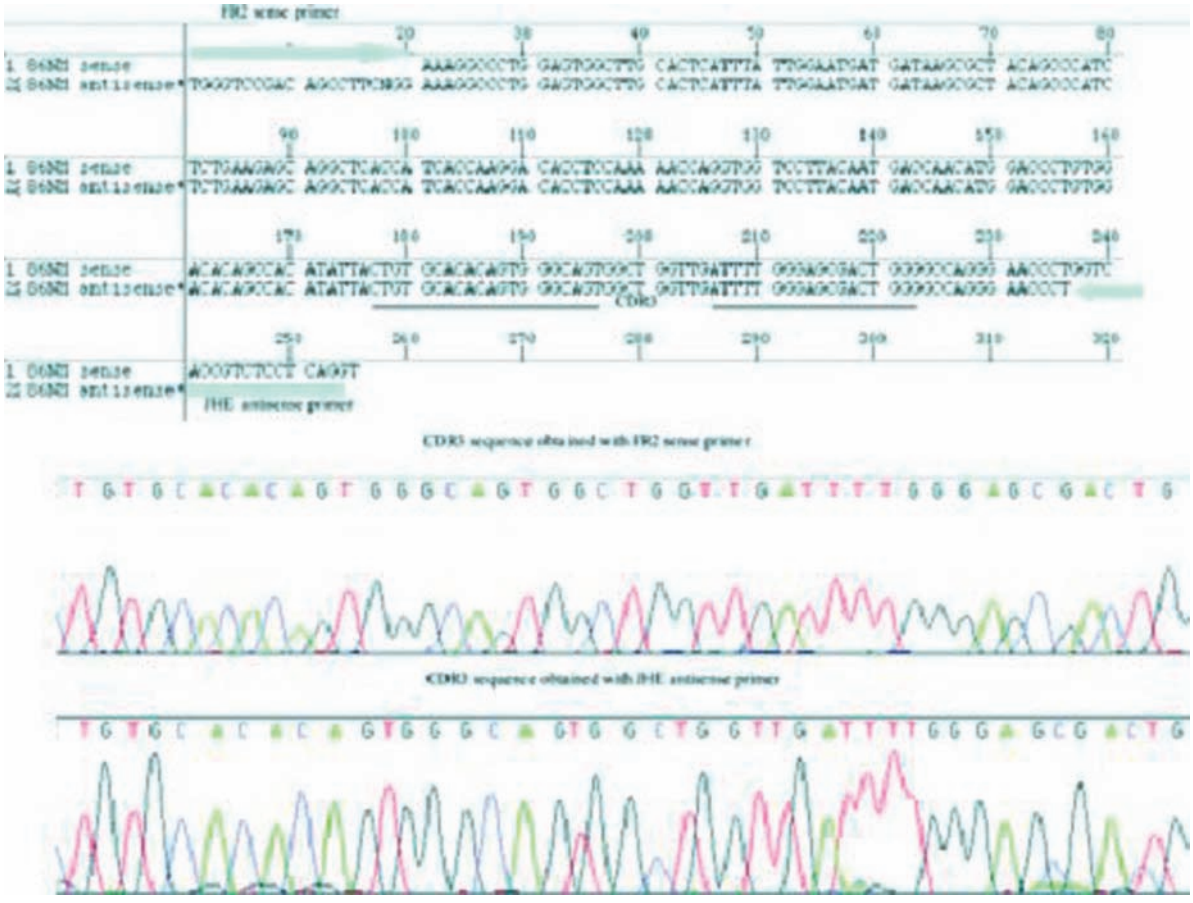


Figure 3.10 *IGVH* mutation; PCR

Hamblin et al[963] have shown that the presence of unmutated V_H genes predicts for an inferior survival in B-CLL. Patients with mutated *IGVH* genes have a relatively benign condition that is stable or only slowly progressive, with an average survival of 25 years. By contrast, patients with unmutated leukemic cells have aggressive disease and an average survival of 8 years. Currently, the unmutated *IGVH* gene is one of the strongest predictor of aggressive disease.[631, 685, 694,700,963,1097,1098] The median survival of patients with mutated *IGVH* genes, unmutated *IGVH* genes, and loss or mutation of the *p53* gene regardless of *IGVH* gene status is 310, 119, and 47 months, respectively.[685]

The unmutated *IGVH* locus is associated with the presence of unfavorable genomic aberrations: the 11q deletion (involving the *ATM* gene) shows a strong correlation.[685,700] whereas the association for the 17p deletion (involving the *p53* gene) varies in different publications from strong[700] to borderline.[685] Abnormalities in 11q and 17p seem to be mutually exclusive in B-CLL cells,[573] and they markedly deteriorate an already bad prognosis for *IGVH*-unmutated patients. High-risk genomic aberrations such as $17p^-$ and $11q^-$ occur almost exclusively in the unmutated subgroup, whereas favorable aberrations such as $13q^-$ are overrepresented in the mutated subgroup.[700]

Comparison between an FC assay for ZAP-70 and the mutational status of *IGVH* genes showed a strong association between the expression of ZAP-70 in B-CLL cells (ZAP-70 level above a defined threshold of 20%) and unmutated *IGVH* genes. However, 23% of B-CLL cases show discordant results (*IGVH*-mutated/ZAP-70^+ or *IGVH*-unmutated/ZAP-70^-).[692]

p53/TP53

Abnormalities of *TP53* (*p53*) in B-CLL (Figure 3.11) correlate with aggressive disease, poor response to standard chemotherapy (e.g. fludarabine), higher rate of transformation, and reduced median survival.[264] Fludarabine may facilitate *p53*-mutant and multidrug resistant CLL clones associated with more

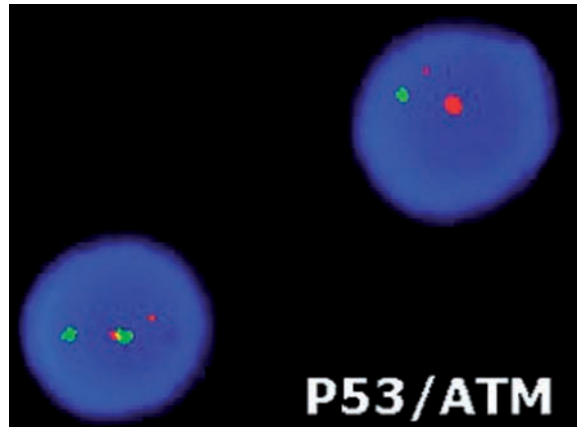


Figure 3.11 B-CLL with deletion of *p53/ATM;* FISH

aggressive disease, and, therefore, patients with *p53* deletions/mutations should be treated preemptively with a targeted therapy earlier in the course of the disease. Osuji et al showed efficacy of alemtuzumab (Campath) in refractory B-CLL irrespective of the *p53* status.[1099] In their series, the overall response rate was 53.6% (complete response, 18%; partial response, 36%) with no significant difference between patients with (overall response rate 50%) and without (complete, 25%; partial, 30%) *p53* deletions. Patients lacking the *p53* deletion, but showing 11q23 deletions had a 20% partial response whereas among those with neither, 61.5% achieved a response (complete, 38.5%; partial, 23%).[1099] The incidence of *p53* abnormalities in the series published by Thornton et al, was 15%, with a significant difference between untreated patients (7%) and the pretreated/refractory group (50%).[1100] The *p53* abnormalities occur more frequently in previously treated than in untreated patients (18.4% vs 9.2%), with a similar trend observed for the *ATM* deletion.[961] Abnormal *p53* predicted for shorter survival, regardless of the method used (FISH or protein expression by FC). The *p53* abnormalities are more common in refractory B-CLL with mutations occuring at the known hot spots. Testing for *p53* (*TP53*) aberrations by FISH and flow cytometry is an effective and

simple way of screening patients who are likely to have aggressive disease.[1100]

Gene expression profile

Gene expression profiling studies show a unique gene expression signature, which differentiates B-CLL cells from benign B-cells as well as other B-cell lymphomas.[1101-1103] Using genomic-scale gene expression profiling, Rosenwald et al showed that B-CLL is characterized by a common gene expression profile irrespective of *IGVH* mutational status, confirming that B-CLL is a single disease with a common mechanism of transformation and/or cell of origin, but with two distinct variants (mutated and unmutated).[1102] DNA microarray of B-CLL showed significant expression differences in 78 genes compared to the reference tonsillar B lymphocytes. A cluster of genes (*LCP1, PARP, BLR1, DEK, NPM, MCL1, SLP76, STAM, HIVEP1, EVI2B, CD25, HTLF, HIVEP2, BCL2, MNDA, PBX3, EB12, TCF1, CGRP, CD14, ILB, GZMK, GPR17*, and *CD79B*) was associated with the unfavorable 11q deletion and also with the unfavorable Binet stages B and C.[1104] The expression of hundreds of other genes correlated with the *IGVH* mutational status, including many genes that are modulated in expression during mitogenic B-cell receptor signaling.[1102] Analysis of 5600 genes in $CD38^+$ vs $CD38^-$ B-CLL showed a common gene expression profile that is largely independent of CD38 expression and only the expression of 14 genes (including genes that are involved in the regulation of cell survival) differed significantly between the two groups.[1105] DNA microarray technology comparing $ZAP\text{-}70^+/CD38^+$ with $ZAP\text{-}70^-/CD38^-$ B-CLL cases showed that the expression of 358 genes differed significantly between the two subgroups, including genes involved in B-cell receptor signaling, angiogenesis, and lymphomagenesis.[690]

The *ATM p53*-dependent DNA damage response pathway plays an important role in the progression of lymphoid tumors, including B-CLL. Deletions affecting chromosome bands 11q22–23 and 17p13 are associated with a reduced expression of the corresponding genes, suh as *ATM* and *p53/TP53*, while trisomy 12 results in the upregulation of genes mapping to chromosome arm 12q.[1106] Using microarray analysis of *ATM*-mutant/*TP53*-mutant, and *ATM/TP53* wild-type B-CLLs, Stankovic et al showed that after exposure to DNA damage the responses are entirely dependent on *ATM* function.[572] Because the *p53* proapoptotic responses comprise only a part of *ATM*-regulated transcription and *ATM* regulates prosurvival responses independently of *p53*, the greater severity of the *TP53*-mutant B-CLLs compared with *ATM*-mutant B-CLLs is consistent with the additive effect of defective apoptotic and elevated survival responses after DNA damage in these tumors.[572] In summary, B-CLL can be split into two variants, one with unmutated immunoglobulin genes which is characterized by aggressive disease with a median survival of ~8 years from diagnosis (affecting mostly men), and the second with mutated immunoglobulin genes which is characterized by an indolent clinical course with a median survival ~290 months (equally distributed between the sexes).

B-prolymphocytic leukemia

B-prolymphocytic leukemia (B-PLL) is an infrequent aggressive disorder of mature B-cells with distinct clinical and pathologic features most often associated with a poor prognosis. B-PLL (Figure 3.12) is characterized by medium sized lymphocytes with nucleoli (prolymphocytes) comprising >55% of lymphocytes in the blood, a very high WBC count, and prominent splenomegaly without significant lymphadenopathy. Immunophenotypically, the leukemic cells express B-cell markers (CD19, CD20, CD22, CD79a, PAX5) but, in contrast to B-CLL, they are usually negative for CD23 and CD5 is expressed only by a subset of cases. The median overall survival time is 5 years and the event-free survival time is 37 months.[1107,1108] The probability of overall survival for 3, 5, and 10 years is 63%, 56%, and 35%, respectively.[1108] As detected by univariate and

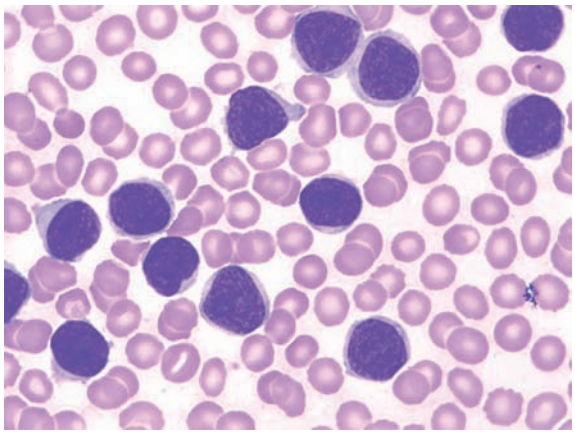


Figure 3.12 B-cell prolymphocytic leukemia (cytomorphology; W-G staining)

multivariate analysis, the advanced age, lymphocytosis (>100 × 10^9/l) and anemia (<11 g/dl) are associated with a poor prognosis and shorter survival. Differential diagnosis includes leukemic phase of MCL [t(11;14)/CCND1], other B-cell lymphoproliferations (e.g. splenic marginal zone B-cell lymphoma or DLBCL in the leukemic phase), and T-PLL.

Patients with *p53* mutations have a worse clinical outcome.[1107] The frequency of *p53* mutation (~50%) in B-PLL is the highest reported in B-cell malignancies and may be responsible for the frequent resistance to therapy.[1109] Apart from *p53*/*TP53* mutations, deletions of 13q14 and 11q23 occur frequently in B-PLL. In a series reported by Lens et al, chromosome 11q23 deletions were found in 39% of cases.[1110] Monoallelic loss of *RB1*, *D13S25*, and *BRCA2* was present in 10/18 (55%), 6/18 (33%), and 3/18 (16%) of the cases, respectively, and, in contrast to CLL, there is a preferential loss of *RB1* with respect to the *D13S25* locus.[1110] Other chromosomal abnormalities seen in B-PLL include t(8;14), t(2;8), +(20q), –X, and +8.[1110-1113]

Hairy cell leukemia

HCL is an indolent chronic B-cell lymphoproliferative disorder characterized by bone marrow involvement, splenomegaly, pancytopenia, and monocytopenia. It affects predominantly elderly men and comprises ~2–3% of all adult leukemias in the United States.[1114] The characteristic 'hairy cells' in the peripheral blood and bone marrow are the hallmark of this leukemia (Figure 3.13). It has a characteristic immunophenotypic profile with bright expression of CD11c, CD19, CD20, CD22, CD25, and CD103.

Cytogenetic studies in HCL are rare. HCLs usually have mutated *IGVH* genes and have no consistent or specific chromosome abnormalities. Clonal chromosomal aberrations are reported in 25–44% of patients and include +1p, +5q13–q31 (~20%), del(7q22–q35) (~6%), +12, chromosome 14 abnormalities, complex abnormalities (including chromosomes 1, 6, 7, 8, and 17), del(17)(q25), and t(11;20)(q13;q11).[1115–1122] Deletions of *p53*/*TP53* have also been reported.[1116]

Follicular lymphoma

FL is the most frequent subtype of low-grade malignant lymphoma in Western countries. Most typical cases show a nodular growth pattern on histologic evaluation (Figure 3.14), t(14;18)(q32;q21) by cytogenetics/FISH (Figure 3.15), and co-expression of CD10, bcl-2, and bcl-6 by FC and/or immunohistochemistry. FL frequently involves the bone marrow with a characteristic paratrabecular distribution (Figure 3.16). FLs are a heterogeneous group of tumors with a variable course, but the majority of cases have an indolent and slowly progressive clinical course with relatively long median survival, good response to initial treatment, and a continuous pattern of relapses, sometimes followed by histologic transformation into high-grade lymphoma.[1123] Based on the absolute number of centroblasts in 10 neoplastic follicles evaluated under a ×40 high power microscopic field (hpf), FL is divided into grade 1 (0–5 centroblasts/hpf), grade 2 (6–15 centroblasts/hpf), and grade 3 (>15 centroblasts/hpf). Based on the presence of centrocytes, grade 3 FL is further subdivided into 3a (centrocytes present) and 3b (no centrocytes).

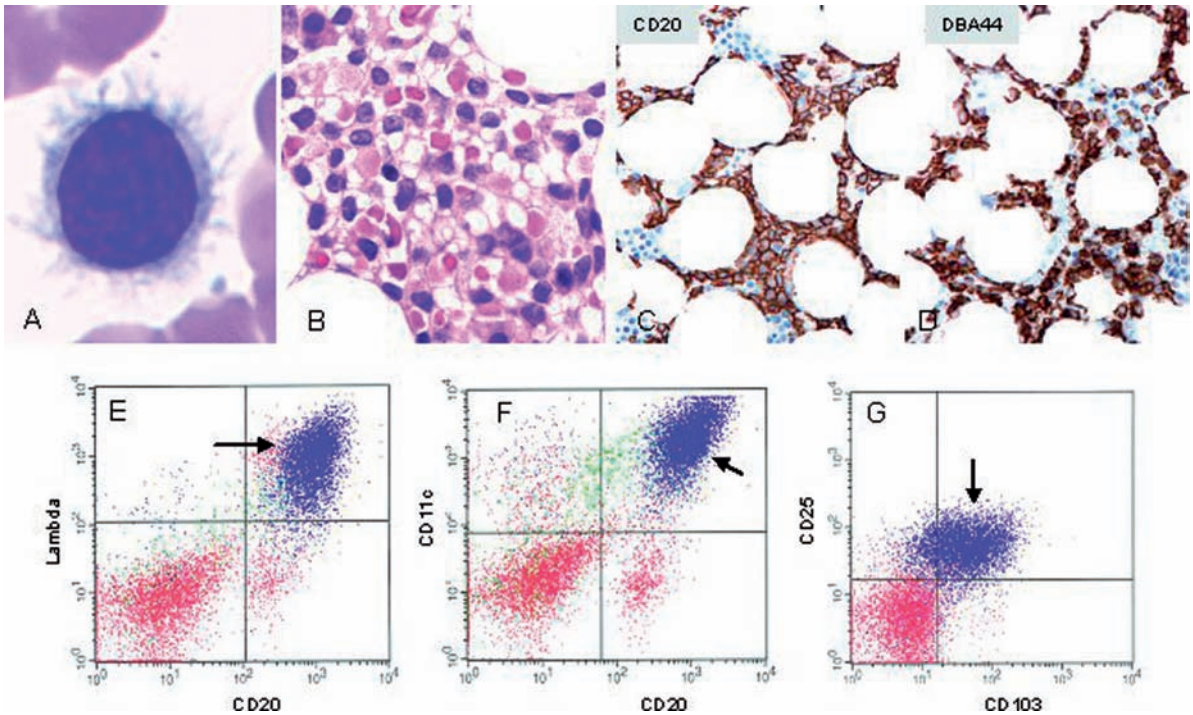


Figure 3.13 Hairy cell leukemia. A, cytology with typical cytoplasmic projections; B, histology (bone marrow core biopsy); C, D, immunohistochemistry; E–G, flow cytometry (FC). Leukemic cells are positive for CD20 and DBA44 (C, D) and by FC analysis show bright expression of CD20 (E, F), surface immunoglobulin (lambda, E), bright expression of CD11c (F), and co-expression of CD25 and CD103 (G)

Transformation and development of resistance to chemotherapy in the course of the disease are the main causes of death in patients with FL.

Chromosomal aberrations

The t(14;18)(q32;q21) is the cytogenetic hallmark of FL (Figure 3.15), which occurs in 80–90% of cases.[100,175] The translocation results in the juxtaposition of the *BCL2* oncogene into the *IGH* heavy chain locus on chromosome 14, leading to its overexpression. Chromosomal breakpoints mainly occur at two different sites on chromosome 18: the major breakpoint region (mbr) and the minor cluster region (mcr), which account for 80 and 10% of translocations, respectively.[1124] Approximately 10% of FL cases lack t(14;18), but show other chromosomal abnormalities.[1125] Follicular lymphomas without t(14;18)(q32;q21) and with *BCL6* rearrangement are usually characterized by prominent nodular architecture, a monocytoid component, and lack of CD10 and bcl-2 expression, but there are no significant differences regarding age, performance status, bone marrow involvement, or overall survival when compared to FL with t(14;18).[1126] There is a general consensus that the t(14;18) translocation status does not bear any prognostic significance.

The presence of an isolated t(14;18) is actually uncommon in FL. The majority of cases display evidence of additional chromosomal changes at initial diagnosis.[1127] Recurrent alterations seen in ≥10% of cases include: +X, +1q21-q44, +7, +12q, +18q, del(1)(p36), del(6q) (Figure 3.17), del(10)(q22-q24), and polyploidy. Changes that correlate with morphologic progression include del(1)(p36), del(6q), del(10)(q22–q24), +7, and polyploidy.[1127] Patients with abnormalities of chromosome region 1p21–22,

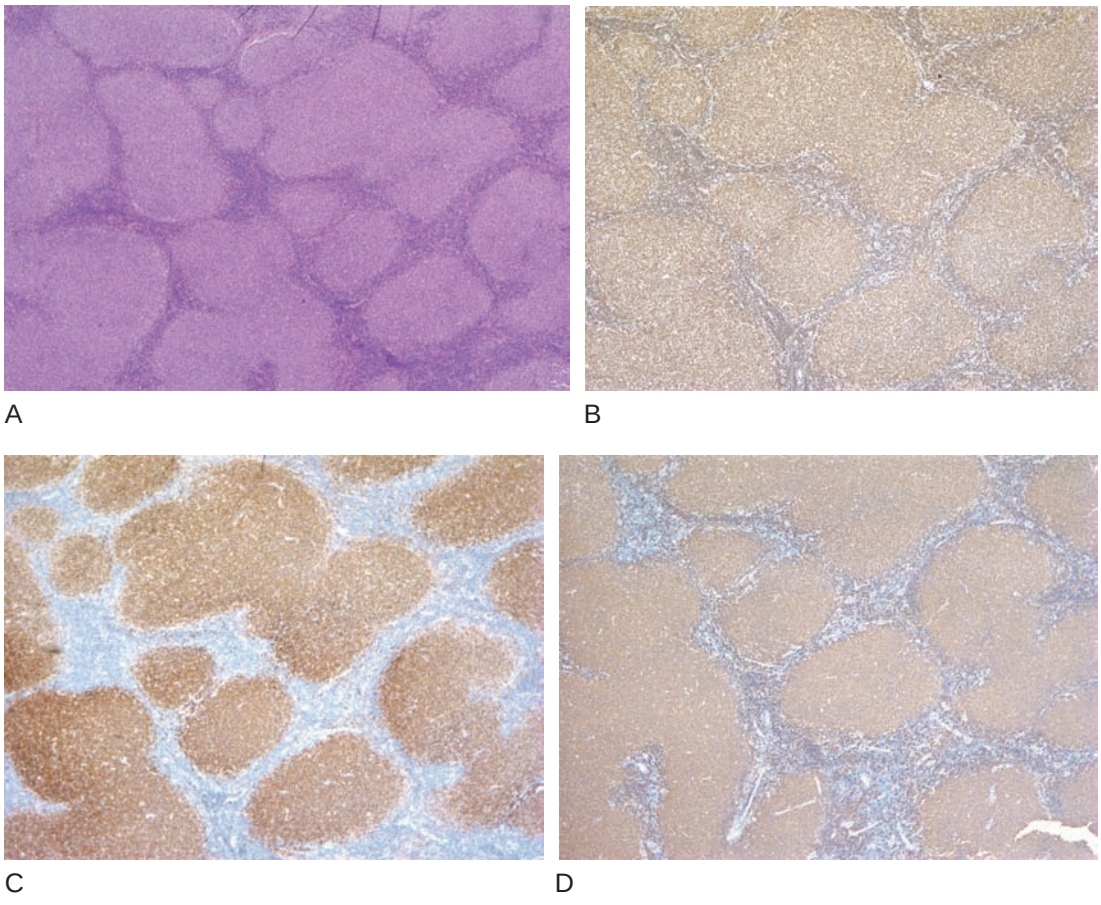


Figure 3.14 Follicular lymphoma, lymph node. A, histology; B–D, immunohistochemistry. Lymph node section shows distinct nodular architecture (A). Lymphomatous cells are positive for CD20 (B), CD10 (C), and bcl-2 (D)

6q23–26, or short arm of chromosome 17 had a significantly shorter survival in univariate analysis.[175] Multivariate analysis identified breaks at 6q23–26 and 17p as independent prognostic factors which increased the risk of transformation into a DLBCL. Unfavorable overall survival is associated also with +12, del(1)(p36), and dup(18q).[293,1128] There is no correlation between numbers of circulating t(14;18)-positive cells and response to first-line treatment in FL.[1006] The cellular proliferation index does not appear to influence the survival rate of patients with FL.[1129]

Gene expression profile

Based on microarray studies, FL is characterized by a typical genetic signature with upregulation of genes involved in cell cycle regulation (*CDK10*, *p120*, *p21CIPI*, and *p16INK4A*), transcription (Pax-5, Id-2), cell-to-cell interactions, TNF, IL-2R, and IL-4R, and downregulation of *MRP8* and *MRP14*, involved in cell adhesion. Based on supervised classification analysis of 81 genes in patients with either an indolent or aggressive clinical course, Glas et al defined a gene expression profile that could predict clinical behavior at the time of diagnosis, with patients from

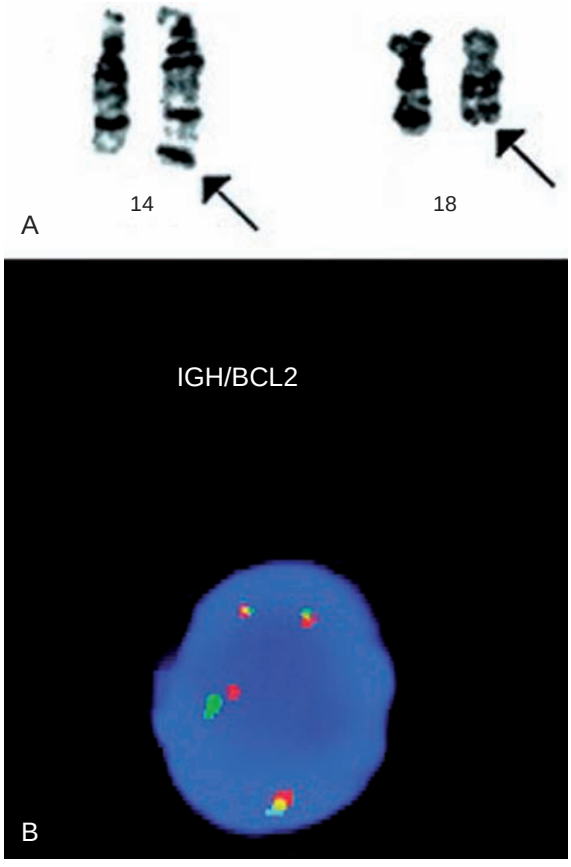


Figure 3.15 Follicular lymphoma with t(14;18) [*IGH/BCL2*]; A, cytogenetics (partial karyotype), B, FISH

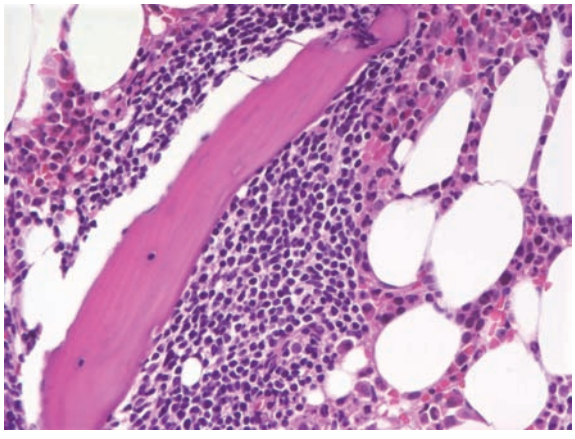


Figure 3.16 Follicular lymphoma involving the bone marrow – typical paratrabecular distribution of neoplastic lymphoid aggregates

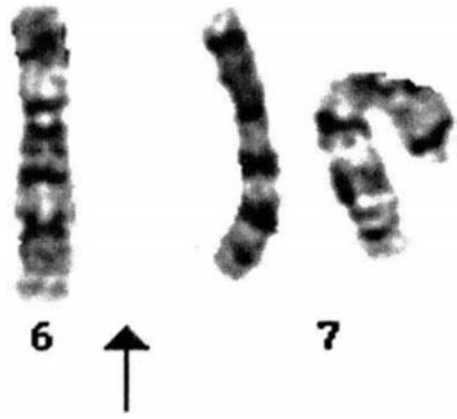


Figure 3.17 Follicular lymphoma with deletion of chromosome 6; cytogenetics (partial karyotype)

the aggressive group characterized by upregulation of genes involved in cell cycle control, DNA synthesis, and metabolism control.[1130] Dave et al demonstrated a correlation between survival and the molecular features of nonmalignant immune cells present in the tumor at diagnosis.[1131] FL with the immune response 1 signature, associated with favorable survival, included genes encoding for T-cell markers (e.g. CD7, CD8B1, ITK, LEF1, and STAT4). The immune response 2 signature, associated with an unfavorable prognosis, included genes known to be preferentially expressed by macrophages, dendritic cells, or both (e.g. TLR5, FCGR1A, SEPT10, LGMN, and C3AR1).[1131]

Transformation to diffuse large B-cell lymphoma

FL often undergoes transformation to more aggressive or high-grade lymphomas. Table 3.5 presents chromosomal and genetic aberrations in FL and their prognostic implications, including risk of transformation into DLBCL. Morphologically, progression of FL is characterized by an increased number of centroblasts (progression from FL grade 1 into FL grade 2 and/ or 3), DLBCL,[1132–1137] or less often blastic transformation in the form of Burkitt lymphoma or precursor B-lymphoblastic lymphoma/leukemia (Figure 3.18) with or without TdT expression.[1138–1141] The transformation

Table 3.5 Chromosomal and genetic aberrations in follicular lymphoma and their prognostic implications including risk of transformation into DLBCL

Chromosome	*Gene*	*Abnormality*	*Reference*
17p13.1	*P53/TP53*	mutations; deletions	Akasaka et al,[635] Sander et al[1149]
9p21	*CDKN2A;*	deletions	Villuendas et al,[599] Pinyol et al,[1178] Elenitoba-Johnson et al[1833]
	CDKN2B	mutations	Akasaka et al,[635] Lossos and Levy[1146]
3q27	*BCL6*	mutations	Matolcsy et al,[1151]
18q21	*BCL2*	rearrangement; mutations	Martinez-Climent et al,[1154] Raghoetier et al[1834]
8q24	*MYC*	rearrangement; mutations; amplifications	Lossos et al[1147]

is commonly associated with accumulation of secondary genetic alterations, which include wide genetic instability,[1142] nonrandom chromosomal anges,[490,1128, 1143,1144] *c-MYC* gene rearrangement,[1138,1145-1147] *p53* tumor suppressor gene mutations,[1148,1149] somatic mutations of the translocated *BCL2* gene,[1150,1151] and somatic mutations of the *BCL6* gene.[1142, 1146, 1152] Abnormalities of chromosome 8q24 may be associated with blastic variants of FL.[1153,1154] *BCL6* mutations accumulate during the transformation process and may deregulate BCL6 mRNA, but an increase in BCL6 mRNA expression is not uniformly required for transformation from FL to DLBCL.[1152] Multiple chromosomal aberrations, including (but not limited to) inv(1)(p36.3q12), t(3;14;18) (p23;q32;q21), t(1;11)(q25;q13), and t(8;22), were observed by Li et al in FL transforming into Burkitt lymphoma, which led to an extremely aggressive clinical course.[1155] A subset of patients with histologic transformation from low-grade FL to intermediate- or high-grade lymphoma enjoyed relatively long-term survival; patients with limited disease and no previous exposure to chemotherapy had a favorable prognosis.[1156] In the majority of patients, however, progression of FL to a higher-grade malignancy is often associated with treatment failure and frequently signals a poor prognosis.[489, 1134,1156,1157] The growth fraction, as assessed by Ki-67 staining, and Trump expression (transferrin-receptor related protein) correlate with histologic grade, but not with recurrence or progression of FL.[1158]

Davies et al, analyzed gene expression profiles of FL transforming into DLBCL.[1159] Based on the expression of CD10, bcl-6, and MUM1/IRF4 by immunohistochemistry, 89% (31/35) of transformed lymphomas had the GCB-like phenotype and the remaining cases were classified as non-GCB-like phenotype ($CD10^-$/$bcl\text{-}6^+$/$MUM1^+$); none of the transformed FLs were of the activated (ABC-like) phenotype.[1159] The majority of genes that were significantly increased upon transformation (14 genes of the 29; 48%) belonged to a set of genes whose expression is associated with cell division (proliferation signature). The transformed DLBCLs characterized by the proliferation signature had recurrent oncogenic abnormalities including mutations of *p53/TP53* (four cases), the c-*REL* oncogene amplification (two patients), and *CDKN2A* (one case). A marked increase in c-*MYC* expression between FL and transformed DLBCL was observed in 70% of pairs, whereas T-cell and follicular dendritic-associated genes predominated among the genes which showed decreased expression upon transformation.[1159]

Mantle cell lymphoma

MCL (Figure 3.19) is an aggressive lymphoma characterized by monomorphic-appearing cells with irregular, indented nuclei, a distinct immunophenotypic profile ($CD5^+$/$CD20^+$/$CD23^-$/$CD43^+$), and overexpression of cyclin D1 (bcl-1) due to juxtaposition of the *BCL1* locus (*CCND1* gene coding for the cyclin D1) to the *IGH* gene as a result of t(11;14)(q13;q32). Figure 3.20 shows typical *CCND1*/*IGH* fusion revealed by FISH analysis.

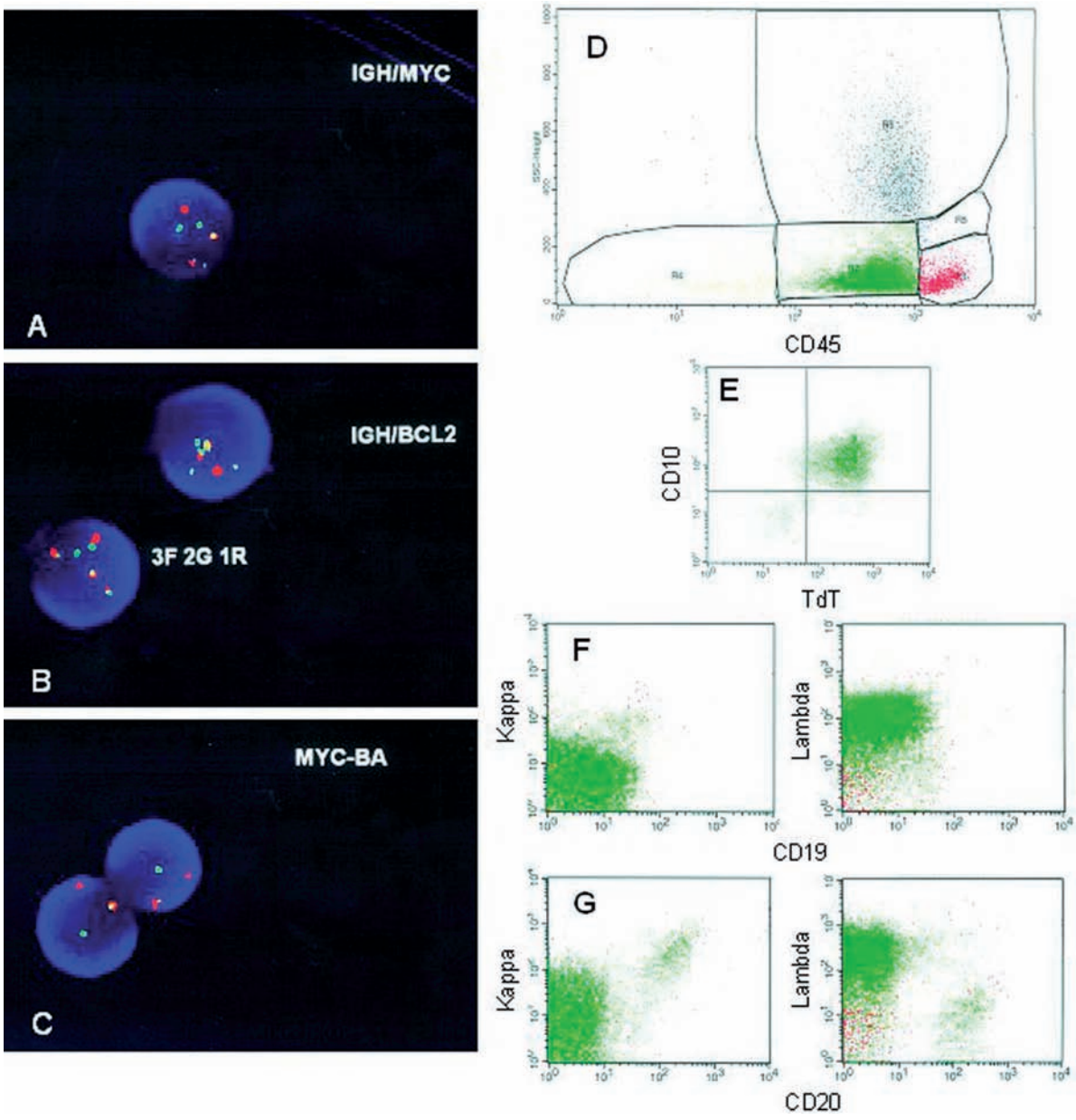


Figure 3.18 Blastic transformation of follicular lymphoma. A–C, FISH analysis showing c-*Myc* translocation (A), *BCL2* translocation (B) and break-apart dual color with split signals (C; red and green split signals; yellow/orange, normal signal). D–G, Flow cytometry analysis shows leukemic cells in blastic region (D) co-expressing CD10 and TdT (E) with clonal lambda expression (F, G), dim CD19 (F), and negative CD20 (G)

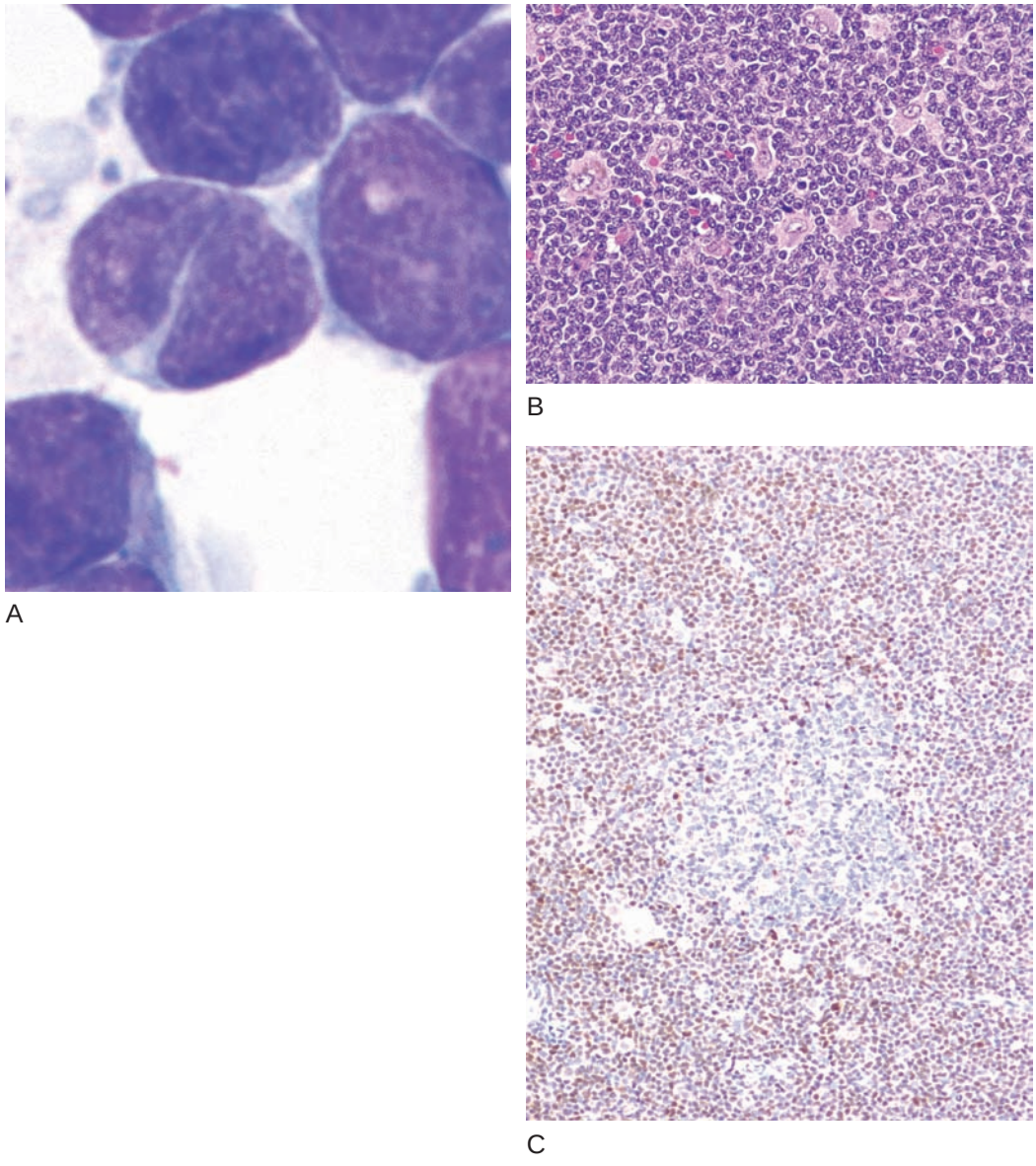


Figure 3.19 Mantle cell lymphoma. Lymphomatous cells have irregular nuclei (A). Lymph node biopsy shows diffuse lymphoid infiltrate (B) composed of small lymphocytes with irregular nuclear contours and scattered histiocytes. Immunohistochemistry shows positive nuclear expression of bcl-1/cyclin D1 (C)

Patients usually present with generalized lymphadenopathy. Bone marrow involvement is observed in 50–80%, hepatosplenomegaly in 30–60%, B-symptoms in 50% and extranodal involvement in 20% of patients.[1137,1160–1162]

Chromosomal aberrations

Many cases of MCL have additional cytogenetic abnormalities and often complex karyotype.[1163–1167] Genome-wide array-based comparative genomic hybridization revealed additional chromosomal

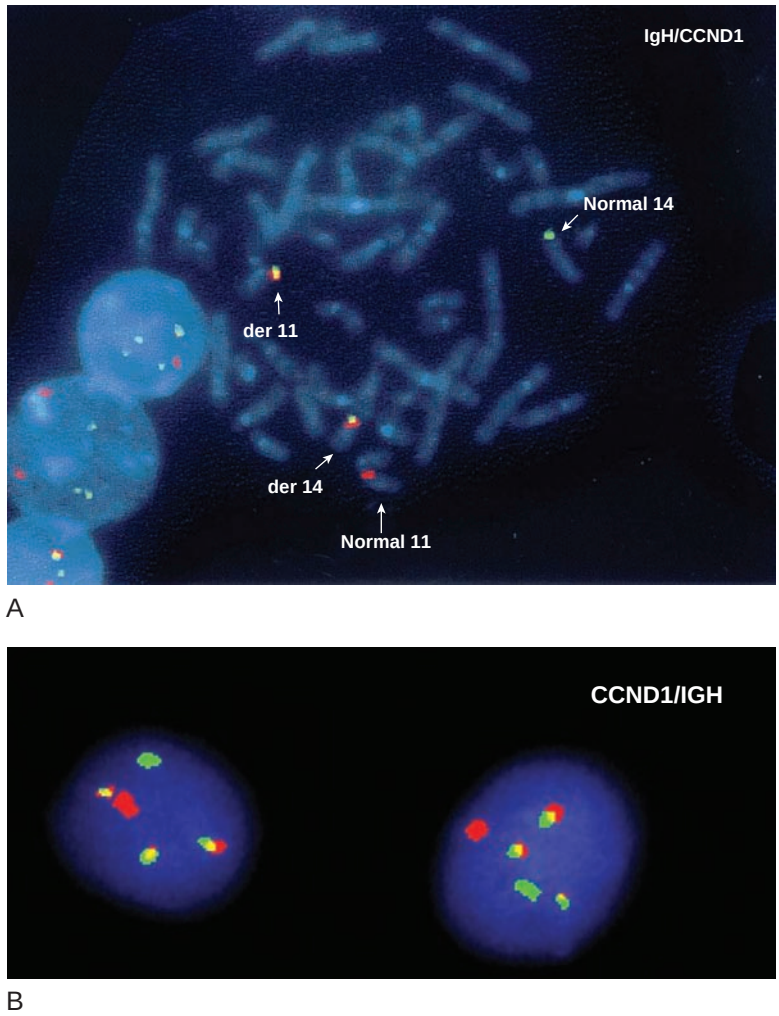


Figure 3.20 Mantle cell lymphoma showing *CCND1/IGH* fusion; FISH

imbalances in MCL (−1p, −5p13p15.3, −6q, −8p −11q, −13q, −20p12.1–12.3, −22q12.1–12.3, +3q, +4p12, +6p, +7q, +8q, +12p, +12q, +17q11q21).[1166,1168] The most common cytogenetic aberrations in MCL include 13q14 deletion, followed by 17p deletion and +12. In a series reported by Parry-Jones et al, 81% of cases had at least one abnormality in addition to t(11;14).[1167] Trisomy 12 and deletions at 6q21 and 13q14 are associated with poorer prognosis.[105,1167] Cases with *TP53* deletion are more likely to have splenomegaly and marked leukocytosis (>30×10^9/l) and less likely to have adenopathy than those without deletion, and cases with deletions at 11q23 and 6q21 are associated with extranodal disease.[1167] Complex karyotype (Figure 3.21) is associated with a poor prognosis.[105] Although both leukemic and nodal MCLs show similar genomic patterns of losses (involving 6q, 11q22-q23, 13q14, and 17p13) and gains (affecting 3q and 8q), genomic loss of chromosome 8p occurs more frequently in patients with leukemic disease (79% vs 11%).[1169] This may indicate the presence of a novel tumor suppressor gene locus on 8p, whose deletion may be associated with leukemic dissemination. In a

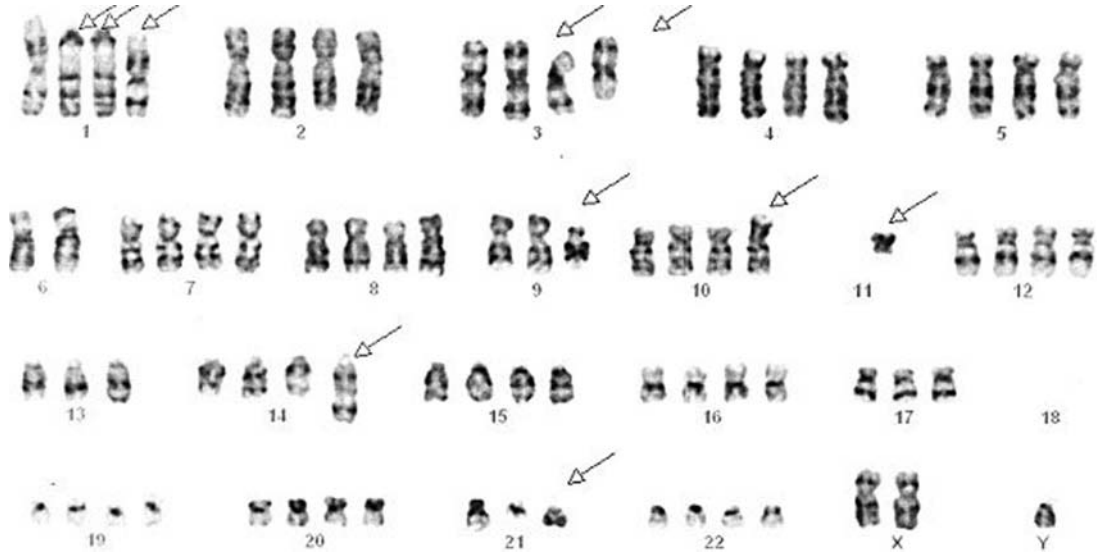


Figure 3.21 Mantle cell lymphoma with complex chromosomal abnormalities (near tetraploid cells with t(11;14), +21, XXY); cytogenetics

series reported by Au et al, common aneuploidies in MCL included −Y, −13, −9, −18, +3, and +12, and common structural changes included +3q, +12q, del(6q), del(1p), del(13q), del(10q), del(11q), del(9p), and del(17p).[226] The commonest breakpoints clusters were 1p21–22, 1p31–32, 1q21, 6q11–q15, 6q23–25, 8q24, 9p21–24, 11q13–23, 13q12–14, and 17p12–13. When analyzed separately as lymph node-based versus blood-based disease, deletions and chromosomal losses were more common in the lymph node group, while gains of chromosome segments 3q and 12q were similar.[226] Among 60 patients with MCL in the leukemic phase reported by Parry-Jones et al, the most common chromosomal abnormalities included −17p13 (46%), −13q14 (43%), −11q23 (25%), −6q21 (12%), and +12 (8%).[1167]

Disease progression

Morphologic progression of MCL is associated with an increase in large blastoid cells, resulting in the so-called blastoid variant.[1170,1171] The blastoid variant of MCL is a very aggressive subtype of lymphoma with a median overall survival of 14.5 months, as compared to 53 months for the patients with a common form of MCL.[1170,1172] A subset of blastoid variants of MCL may have *p53* gene mutations.[1173] Additionally, blastoid MCL subtypes are characterized by distinctly elevated mitotic counts, proliferation indices (53% vs 27% in common MCL), frequent *BCL1* rearrangements at the major translocation cluster locus (59% vs 40%), and overexpression of *p53* (21% vs 6%).[1174] Blastoid MCL subtypes display a tendency for a tetraploid chromosome number chromosome (36% of lymphoblastoid and 80% of pleomorphic types vs 8% of common MCL), a feature clearly separating these neoplasms from other types of B-cell non-Hodgkin lymphoma and possibly being related to cyclin D1 (Bcl-1) overexpression.[1174] Comparative genomic hybridization (CGH) analysis showed an increased number of chromosome imbalances being associated with blastoid variants of MCL (e.g. gains of 3q, 7p, and 12q, and losses of 17p), which may have a prognostic significance.[1175] CGH losses of 17p correlated with *p53* gene deletions and mutations. Leukocytosis, an elevated LDH level, and a high proliferative activity at diagnosis as assessed by the mitotic count and Ki-67

index are associated with an increased risk of blastoid transformation, and elevated serum LDH and blood leukocytosis with a shorter time interval to transformation.[1176] Loss of expression and/or deletions of the *p21Waf1* and *p16INK4a* genes (cyclin-dependent kinase inhibitors suggested as candidates for tumor-suppressor genes) are detected in aggressive MCL, but not in the typical variants. The *p21Waf1* and *p16INK4a* alterations occur in a subset of tumors with a wild-type *p53* gene.[1177] Deletions of the *INK4a/ARF* gene locus are found in up to 30% of MCLs and are associated with a poor prognosis.[1177,1178]

Lymphoplasmacytic lymphoma/Waldenström macroglobulinemia

Lymphoplasmacytic lymphoma is a low-grade lymphoma characterized by a mixture of small lymphocytes, lymphocytes with plasmacytoid features, and plasma cells (Figure 3.22). Occasional cells display intranuclear inclusions (Dutcher bodies). In most patients, adenopathy develops slowly over many years. Extranodal sites may be involved as well, although most of these cases represent marginal zone B-cell lymphoma with plasmacytic differentiation. Waldenström macroglobulinemia (WM) is a B-cell lymphoproliferative disorder characterized by high serum immunoglobulin M paraprotein and the accumulation of clonal B-cells with variable plasmacytoid differentiation (lymphoplasmacytic cells) in the bone marrow. Histologically, WM corresponds to BM involvement by lymphoplasmacytic lymphoma. In advanced disease, patients may acquire organomegaly, anemia, and the hyperviscosity syndrome.

Lymphoplasmacytic lymphoma is characterized by t(9;14)(p13;q32) in 50% of patients. This translocation involves the *PAX5* gene on chromosome 9, which encodes a B-cell specific transcription factor involved in the control of B-cell proliferation and differentiation. Figure 3.23 shows lymphoplasmacytic lymphoma with complex chromosomal aberrations including t(9;14). The presence of abnormal cytogenetic findings [e.g. −8; del(6q)] correlates with a poor prognosis.[1179] Translocations involving the *IGH* locus on chromosome 14q32 are rare in WM.[182,1180] Lack of *IGH* translocations differentiates WM from MM, and many lymphomas including FL, MCL, and DLBCL. WM does not have the t(9;14)(p13;q32) translocation but often has deletions of 6q21.[182] On the other hand, no 6q deletions can be found in IgM MGUS samples and therefore the presence of $6q^-$ helps to distinguish WM from IgM^+ MGUS.[184]

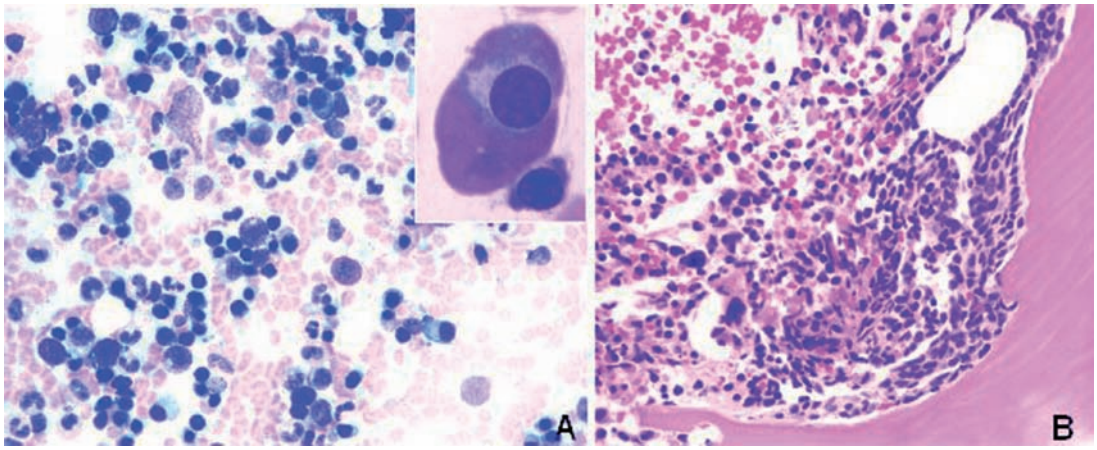


Figure 3.22 Lymphoplasmacytic lymphoma. A, aspirate smear shows lymphocytes, plasmacytoid lymphocytes, and plasma cells (*inset*: flame cell); B, core biopsy shows atypical paratrabecular lymphoid aggregate

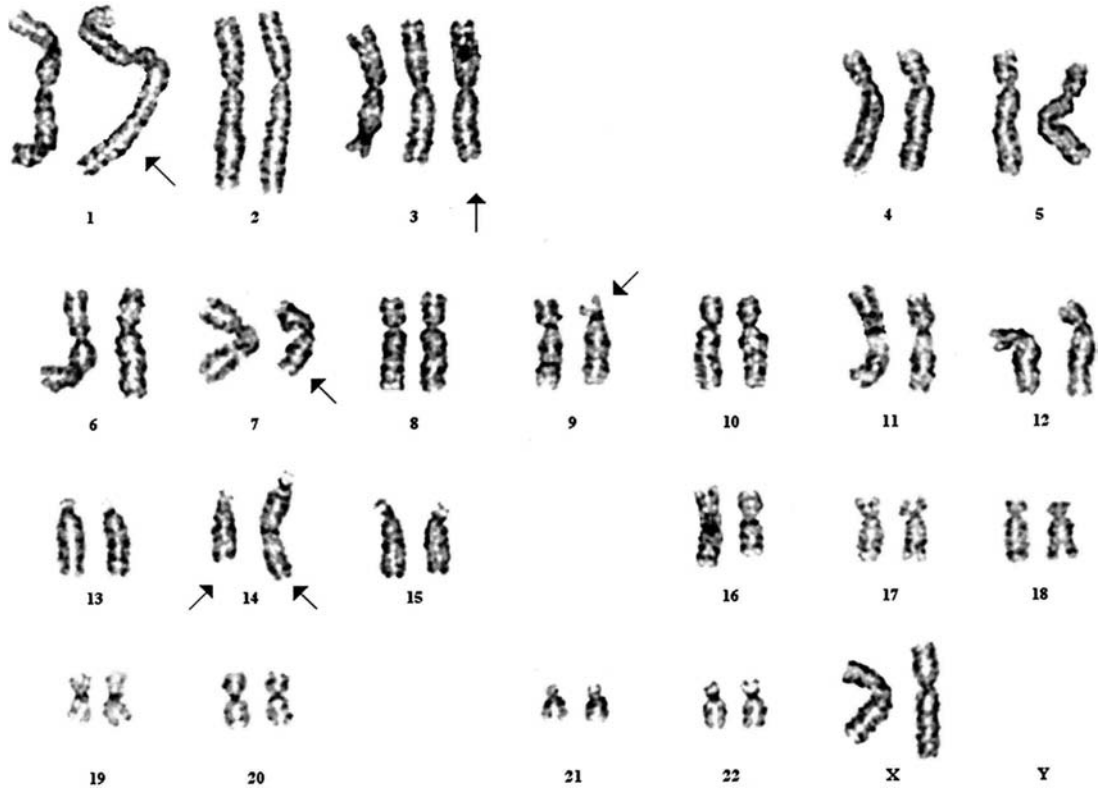


Figure 3.23 Lymphoplasmacytic lymphoma with complex chromosomal aberrations [+3, del(7)(q22q34), del (14)(q32), t(9;14)]; cytogenetics

Gene-expression profiling of WM revealed a homogenous transcription profile, clustered with CLL and normal B-cells on unsupervised clustering, and clearly had a phenotype more similar to CLL cells than MM.[1181] Both WM and B-CLL are low-grade lymphoproliferations derived from memory B-cell populations which usually lack chromosomal translocations involving the *IGH* locus. The *IGH* translocations occur most likely during immunoglobulin VDJ recombination in maturing B-cells or during immunoglobulin hypermutation and isotype switching in mature B cells within the germinal center.[1182] Despite these similarities, different phenotype (CD5 and CD43 expression in B-CLL) and *IGVH* mutation status (almost all WM cases are *IGVH* mutated, compared to ~60% of CLLs) suggest that they may be derived from different memory B-cell populations.

Marginal zone B-cell lymphoma

Marginal zone B-cell lymphoma (MZL) is a low grade B-cell lymphoma, which can involve spleen (splenic MZL), lymph nodes (nodal MZL), and extranodal sites (MALT lymphoma) (Figure 3.24). Extranodal MZLs often arise as a consequence of chronic inflammation and/or infection, e.g. Sjögren's syndrome (salivary gland MALT), *H. pylori* (gastric MALT), *Campylobacter jejuni* (intestinal MALT), *Chlamydia pisttaci* (ocular MALT), and Hashimoto's thyroiditis (thyroid MALT).[1183–1189] MZLs with both bone marrow and nodal involvement are associated with shorter overall survival.[1190]

The chromosomal changes seen in MZL include: +3, +7, +12, +18, t(1;14)(p22;q32), t(3;14)(p14.1; q32), t(11;18)(q21;q21), and t(14;18)(q32;q21).[353, 354,470,472,526,677,1191–1196] The chromosomal

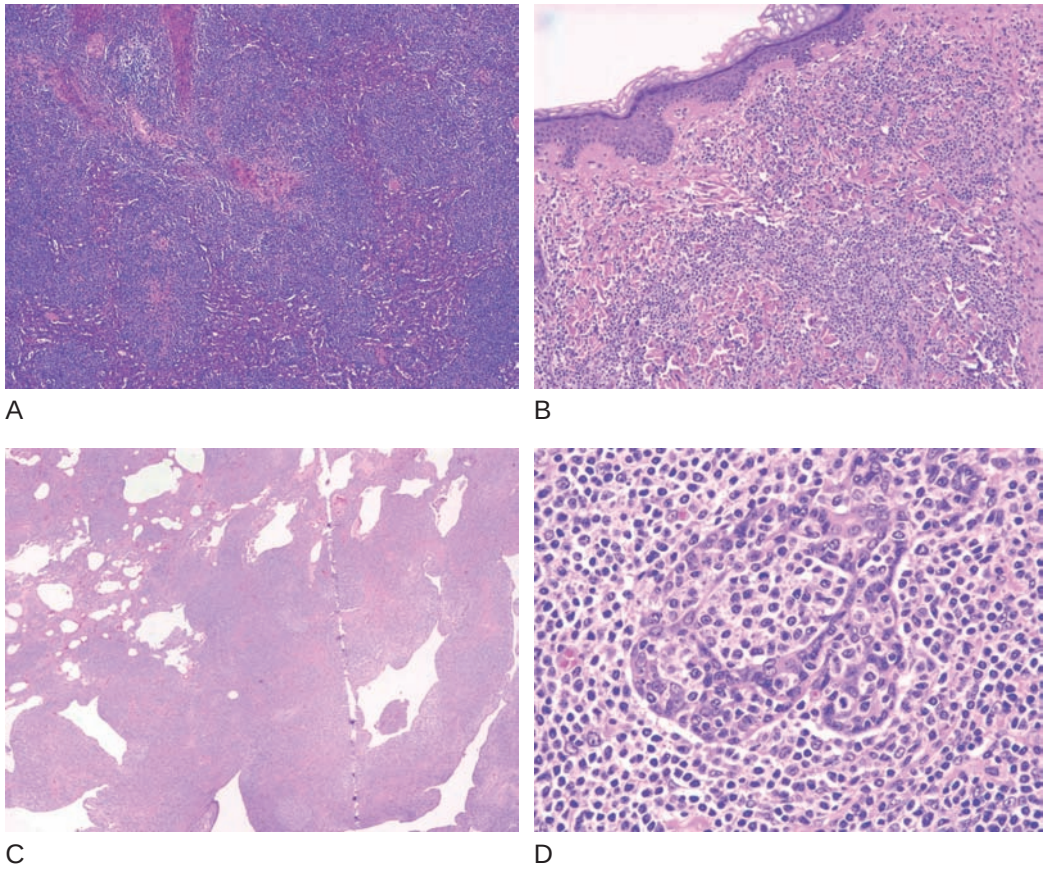


Figure 3.24 Marginal zone B-cell lymphoma: spleen (A), skin (B), lung (C), salivary gland (D), stomach (E, F: high magnification shows lymphoepithelial lesion), lymph node (G, H: high magnification shows cytologic features of monocytoid B-cells)

translocations t(1;14)(p22;q32), t(14;18)(q32;q21), t(11;18)(q21;q21) and t(3;14)(p13;q32) result in rearrangements between *IGH/BCL10*, *IGH/MALT1*, *API2/MALT1*, and *IGH/FOXP1*, respectively.[353,470,678,1196,1197] Translocations in MZL occur with varying frequencies, are mutually exclusive, and can be detected in ~25% of cases (most often *API2/MALT1*, followed by *IGH/MALT1*, *IGH*/unknown translocation partner, and *IGH/BCL10*).[1198] Cytogenetic abnormalities occur with different frequencies at different locations. Trisomy 3 is the most frequent numeric abnormality in gastric, thyroid, and parotid, but not in breast MZL.[1199] The t(11;18) translocation frequently involves MALT lymphomas of the lung and gastrointestinal tract.[23,1198,1200–1203] The translocations t(14;18) involving *IGH/MALT1* and t(1;14) involving *IGH/BCL10* occur in the lung and ocular adnexae, but not in the stomach.[1198] In a series reported by Remstein et al, 23% of translocation-negative primary MALT lymphomas from diverse sites showed complete/partial trisomy 18.[1198]

Splenic marginal zone lymphoma

Splenic marginal zone lymphoma (SMZL) is a disease of the elderly with a median age at presentation of 65 years.[1204] The presence of circulating villous lymphocytes defines splenic lymphoma with villous lymphocytes, which is considered the leukemic

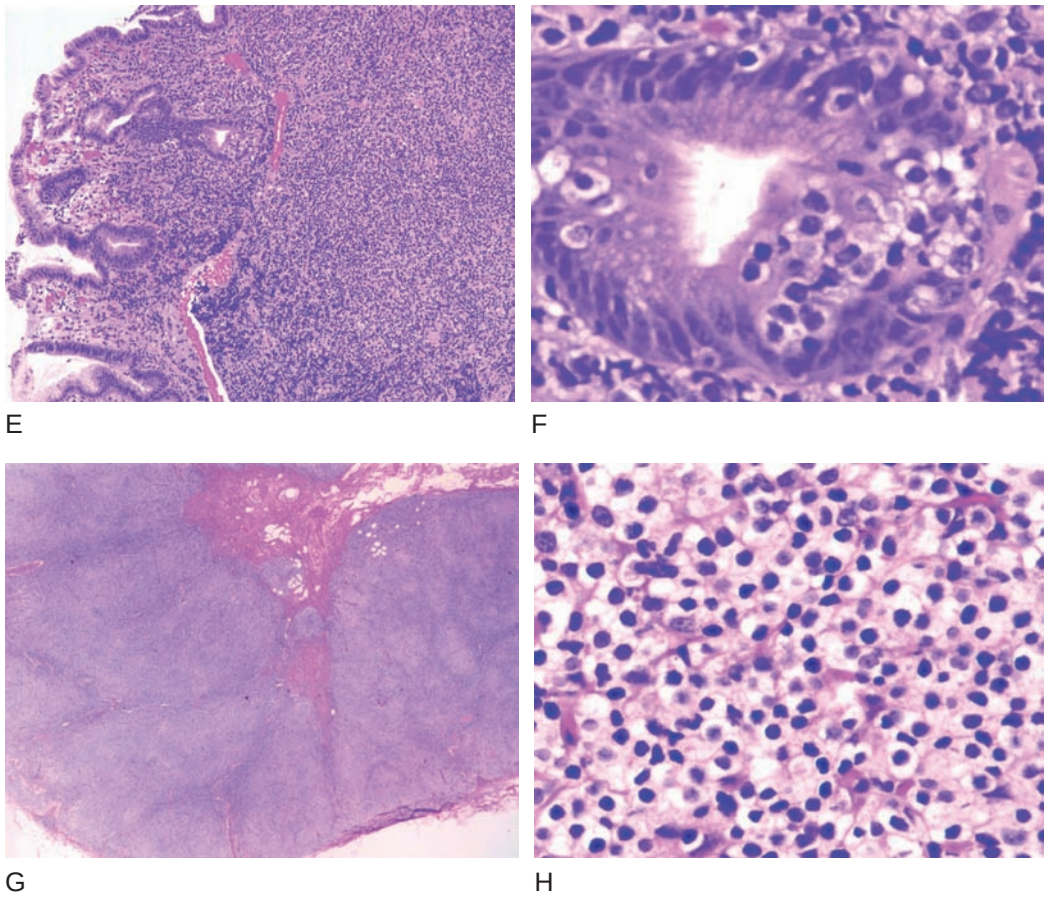


Figure 3.24, cont'd

counterpart of SMZL. SMZL is an indolent extranodal B-cell lymphoma with a the reported median survival of 78% at 5 years and a median overall survival time exceeding 10 years.[1205–1207] In a subset of patients, however, the disease follows an aggressive course.[1208] These patients are more likely to have the 7q31 deletion, lack of *IGVH* somatic mutation, or both.[214] The parameters predictive of shorter cause-specific survival (univariate analysis) include hemoglobin levels <12 g/dl, albumin levels <3.5 g/dl, IPI scores of 2 to 3, LDH levels above normal, age >60 years, platelet counts $<100 \times 10^9$/l, and no splenectomy at diagnosis.[1209] In the same study, values that maintained a negative influence on cause-specific survival in multivariate analysis were hemoglobin levels <12 g/dl, LDH level greater than normal, and albumin levels <3.5 g/dl.[1209]

SMZLs may display del(10)(q22q24), del(7q), del(17p), add(8)(q24), add(3q), add(5q), add(12p), add (9q), t(2;22), t(8;22), or trisomy 3.[214,216,217,1210] In the series reported by Hernandez et al, the most frequent gains involved 3q (31%), 5q (28%), 12q and 20q (24% each), 9q (21%), and 4q (17%), while losses were observed in 7q (14%) and 17p (10%).[220] The loss of 7q loss may play an alternative role in the inactivation of the p53 pathway, thereby favoring tumoral progression.[1211] Loss of an extra copy of 3q and the acquisition of genetic aberrations involving

19p13 and 7p–q22 may play a role in the blastic transformation of SMZL.[1212] A more aggressive clinical course has been related to a high proliferation fraction,[1213] an increased number of large lymphocytes, *p53* mutations (857), and 7q rearrangements.[214–216]

Extranodal marginal zone B-cell lymphoma, MALT type

The gastrointestinal tract is most often involved by MZLs of the MALT type, followed by the lung, salivary gland, thyroid, ocular adnexae, skin, breast, and other locations. Extranodal MZL of MALT is a distinct low-grade lymphoma involving the organs which acquire lymphoid tissue after chronic inflammatory events, such *H. pylori*-associated chronic gastritis, *Chlamydia pisttaci*-induced inflammation (ocular area), or autoimmune disorders like Hashimoto's thyroiditis and myoepithelial sialadenitis/Sjören's syndrome.[1214–1216]

Genetic alterations in MALT lymphomas include: +3, +7, +12, +18, t(11;18)(q21;q21), t(1;14)(p22;q32), t(14;18)(q32;q21), t(3;14)(p14.1;q32), *p53/TP53* mutations, and *p16* deletions with trisomy 3 and t(11;18)(q21;q21) being the most frequent.[109,353,354,472,524–526,677,861,1191,1193,1201,1210,1217–1222] Both chromosomal gains and losses are far more frequent in t(11;18) negative than t(11;18) positive cases.[1223] Recurrent chromosomal gains involving whole or major parts of a chromosome are seen for chromosomes 3, 12, 18, and 22 (23%, 19%, 19%, and 27%, respectively).[1223] Among the recurrent changes, gains at chromosomes 18q and 9q34 may be linked to pathogenesis of lymphoma. MALT lymphomas without translocations, but carrying trisomies of chromosomes 3, 7, 12, or 18, respond to *H. pylori* eradication, but they have a higher risk of large cell transformation. The chromosome translocations occur at markedly variable incidences in MALT lymphoma of different sites, but are always mutually exclusive.[1198,1224] For chromosomal gain at 9q34, Zhou et al suggested that *TRAF2* and *CARD9* may be the target genes.[1223] Both genes have been shown to interact with *BCL10* and activate NF-κB. Partial inactivation of the *p53* gene may play a role in the development of low-grade MALT lymphoma,[1225] whereas complete inactivation may be associated with high-grade transformation (similar to other hematologic malignancies).[1218] Homozygous deletions of *p16* also play a role in large cell transformation.[1218] Transformation of MALT lymphoma to DLBCL occurs in approximately 8% of cases (more frequently in extranodal lymphomas of the MALT type than in nodal and splenic MZL) and is preceded by the emergence of increased numbers of transformed blasts that form sheets or clusters on histologic examination.[1226–1229] Gastric MALT lymphoma usually remains localized for long periods within the tissue of origin. Bone marrow involvement at presentation is uncommon.[1183,1230] Disseminated disease appears to be more common in nongastrointestinal MALT lymphomas.[1229,1231]

t(11;18)

The t(11;18) leads to fusion of the *API2* gene at 11q21 and the *MALT1* gene at 18q21 (Figure 3.25).[1232–1234] The functional *API2/MALT1* fusion product activates NFκB. *API2* is a member of the *IAP* (inhibitor of apoptosis) gene family and is essential for suppression of caspase-dependent apoptosis.[1235] In contrast to trisomy 3, which is

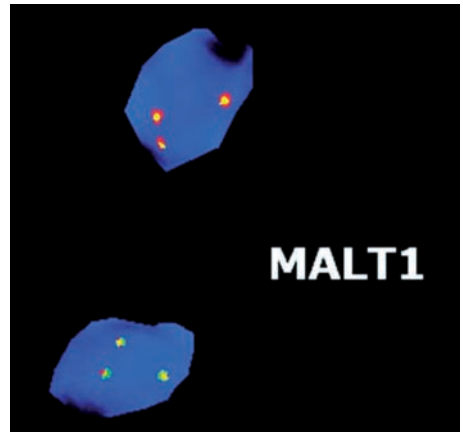


Figure 3.25 Gastric marginal zone B-cell lymphoma (MALT type) with *MALT1* rearrangement; FISH

nearly always accompanied by other numeric and structural chromosomal alterations, t(11;18)(q21;q21) usually occurs as a single abnormality.[472,1236] Although amplifications of *MALT1* are rare, gain of an extra copy of *MALT1* gene is a frequent event in MALT lymphoma.[1222,1223,1237] The t(11;18) occurs most often in lymphomas from lung (~40%) and stomach (24–40%), followed by ocular adnexae (~15%), and orbit (~20%), and is rarely reported in the salivary gland, thyroid, or skin.[23,638,1237] The *API2*/*MALT1* fusion gene is characteristic for MALT lymphomas and is very rare in splenic MZL or DLBCL. In gastric MALT, t(11;18)(q21;q21) is strongly associated with failure to respond to treatment targeted at eradication of *H. pylori.*[477,1238,1239] This translocation, however, seems to be associated with a low risk of both the onset of additional genetic changes and transformation into DLBCL,[1194,1219,1228,1236,1240] but exceptions may occur.[1241] Based on the response to *H. pylori* eradication therapy and the presence of the *API2*/*MALT1* fusion gene, Inagaki et al divided gastric MALT lymphomas into three groups: eradication-responsive and fusion-negative (group A), eradication-nonresponsive and fusion-negative (group B), and eradication-nonresponsive and fusion-positive (group C).[1239] The most common group A tumors are characterized by low clinical stage and superficial gastric involvement, and group C tumors by low *H. pylori* infection rate, low-grade histology, advanced clinical stage, and nuclear bcl-10 expression. Group B tumors have frequent nodal involvement, deep gastric wall involvement, advanced clinical stage, and sometimes an increased large cell component.

t(1;14)

The t(1;14) juxtaposes the *BCL10* gene located on 1p22 to the immunoglobulin gene locus on 14q32, leading to deregulated expression of the oncogene and activation of NF-κB.[353,354,470] The t(1;14)(p22;q32) and its variant t(1;2)(p22;p12) occur in ~3% of MALT lymphomas. MALT lymphomas with t(1;14)(p22;q14) and t(11;18) (q21;q21) show strong nuclear (as well as cytoplasmic) expression of bcl-10 protein, although the value of aberrant nuclear bcl-10 immunohistochemical staining as a surrogate marker for the *API2*/*MALT*1 gene fusion is uncertain.[23,355,357,358] Tumors without both translocations do not display nuclear staining for bcl-10 protein. No association could be demonstrated between the latter and the presence of *BCL10* mutations.[357] The frequency of both t(11;18)(q21;q21) and bcl-10 expression is significantly higher in tumors that have disseminated beyond the stomach than those confined to the stomach.[358] MALT lymphomas with t(1;14) tend to be at a more advanced stage.

t(14;18)

The t(14;18)(q32;q21) which involves the *IGH* gene on 14q32 and the *MALT1* gene on 18q21 (not the *BCL2* gene located on the same chromosome seen in the majority of FLs) can be identified by *IGH/MALT1* D-FISH probe. It occurs in 5–18% of MALT lymphomas, most often involving the lung, liver, skin, ocular adnexae, and salivary gland, but not the spleen, stomach, or GI tract.[470,1198] All of the MALT lymphomas featuring the t(14;18)(q32;q21) are negative for t(11;18)(q21;q21) by RT-PCR, but trisomies 3 and/or 18 are identified in one third of cases.[470]

t(3;14)

The t(3;14)(p14;q32) fuses the *FOXP1* gene at 3p14 with the *IGH* gene.[677] In a series reported by Streubel et al, 10% of MALT lymphomas harbored t(3;14)(p14.1;q32) comprising tumors of the thyroid (3/6), ocular adnexae (4/20), and skin (2/20), whereas tumors of the stomach, spleen, and lung were negative.[677] Most t(3;14)(p14.1;q32)$^+$ MALT lymphomas harbored additional genetic abnormalities, such as trisomy 3.[677]

Nodal marginal zone B-cell lymphoma

Nodal MZL occurs most commonly in the cervical lymph nodes, is more aggressive than extranodal

MZL (MALT lymphoma), and presents with an advanced stage III/IV disease.[593,1190,1242–1244] Patients with nodal MZL have a lower 5-year overall survival and failure-free survival than patients with MALT lymphoma. Traverse-Glehen et al found peripheral blood involvement in 23%, anemia in 24%, thrombocytopenia in 10%, and the presence of serum M component in 33% of cases.[1243] MZL frequently shows plasmacytoid or plasmacytic differentiation (61%) and an increased number of large cells with a high mitotic rate (57%).[1243] In a series of 47 patients with primary nodal MZL reported by Arcaini et al, 45% had stage IV disease and 24% had positive hepatitis C virus serology.[1242] Based on the Follicular Lymphoma International Prognostic Index (FLIPI), 33% were classified as low risk, 34% as intermediate risk, and 33% as high risk with a 5-year overall survival of 69%. In univariate analysis, a worse overall survival was associated with high risk FLIPI, age >60 years, and raised LDH (lactate dehydrogenase), but in a multivariate analysis, only FLIPI predicted a worse overall survival.[1242]

Abnormalities identified in more than 15% of patients with non-MALT MZLs included +3/+3q (37%), 7q deletions (31%), +18/+18q (28%), 6q deletions (19%), +12/+12q (15%), and 8p deletions (15%).[1245] Trisomy 3/3q, 7q deletions, +18, and +12 were seen in different combinations in more than 30% of patients in comparison to 2% in small lymphocytic lymphomas/chronic lymphocytic leukemias, 1% in MCLs, and 7% in follicular lymphomas.[1245]

Cutaneous marginal zone B-cell lymphoma

Primary cutaneous MZL is an indolent lymphoma composed of small B-cells, including marginal zone (centrocyte-like) cells, lymphoplasmacytoid cells, and plasma cells. It occurs most commonly on the trunk or extremities, especially the arms. In contrast to primary follicle center lymphoma, presentation with multifocal skin lesions is frequent. Cutaneous MZLs have a tendency to recur in the skin, but dissemination to extracutaneous sites is uncommon. The prognosis of cutaneous MZL is excellent with a 5-year survival close to 100%. In spite of its indolent course, some patients develop extracutaneous spread and eventually die of lymphoma.[1188,1246,1247] Chromosomal studies may reveal t(14;18)(q32;q21) involving the *IGH* gene on chromosome 14 and the *MALT1* gene on chromosome 18, and t(3;14)(p14;q32) involving the *IGH* and *FOXP1* genes. Other translocations observed in gastric MALT lymphomas, such as t(11;18)(q21;q21) and t(1;14)(p22;q32), have not been found in cutaneous MZL. Gallardo et al observed the presence of aberrant nuclear Bcl-10 expression in a significant number of primary cutaneous MZL cases (36%, 15/42).[1248] This aberrant expression was significantly related to the development of extracutaneous disease. In contrast, neither the t(11;18)(q21;q21) translocation nor other *MALT1* gene translocations could be demonstrated and therefore t(11;18)(q21;q21), strongly linked to extracutaneous MALT lymphomas, does not seem to play a role in primary cutaneous MZL.

Diffuse large B-cell lymphoma

DLBCL is the most common type of lymphoma, with an annual US incidence of >25 000 cases (it accounts for approximately one-third of the total number of adult non-Hodgkin lymphoma patients). DLBCL may occur in nodal and extranodal sites and is characterized by a marked degree of morphologic, genetic, and clinical heterogeneity. It was initially grouped into an intermediate prognostic grade by the International Working Formulation. The WHO classification divides DLBCL into several morphologic and clinical variants (Figure 3.26): centroblastic, immunoblastic, anaplastic, T-cell-rich/histiocyte-rich, thymic (primary mediastinal), intravascular, ALK$^+$, and plasmablastic,[1,438] with the centroblastic variant being the most frequent type (>80%).The majority of DLBCL are positive for CD45 and B-cell markers (CD19, CD20, CD22, CD79a, and Pax-5). Rare cases may be CD20 negative. Patients with DLBCL have a highly variable

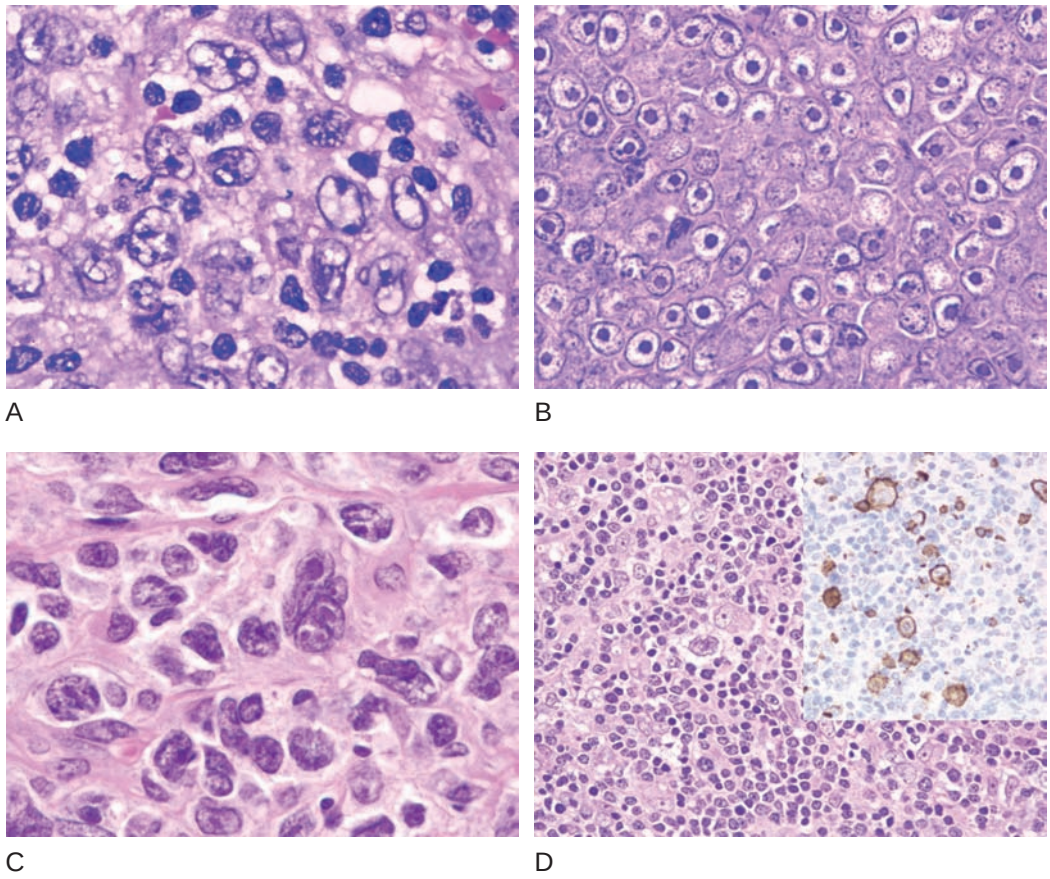


Figure 3.26 Diffuse large B-cell lymphoma: centroblastic (A), immunoblastic (B), anaplastic (C), and T-cell rich (D; *inset*: CD20 immunostaining showing scattered large B-cells)

course, response to treatment, and prognosis, which depends on many clinical, morphologic, phenotypic, and genetic parameters. A significant proportion of DLBCL seems to occur from a transformation of an unknown indolent lymphoma.[1249,1250] Current treatment of DLBCL is based on the combination of chemotherapy including cyclophosphamide, adriamycin, vincristine, prednisone (CHOP) or a dose-intense CHOP-like regimen, and rituximab (R-CHOP). Approximately 60% of patients with DLBCL relapse after conventional anthracycline-based combination therapy.[1249,1250]

Based on the immunophenotype, DLBCL can be classified into three expression patterns: a germinal center pattern (pattern A), expressing CD10 and/or bcl-6 but not activation markers (Figure 3.27); an activated germinal center pattern (pattern B) expressing at least one germinal center marker and one activation marker; and an activated non-germinal center B-cell pattern (pattern C) expressing *MUM1*/*IRF4* and/or CD138, but not germinal center B-cell markers.[1251] Patients with pattern A have a much better overall survival than those with the other two patterns. Frequent chromosomal/genetic abnormalities in DLBCL include t(14;18), t(8;14), and *BCL6* rearrangements. The 18q gains or amplifications and 17p losses are associated with aggressive clinical behavior.[296]

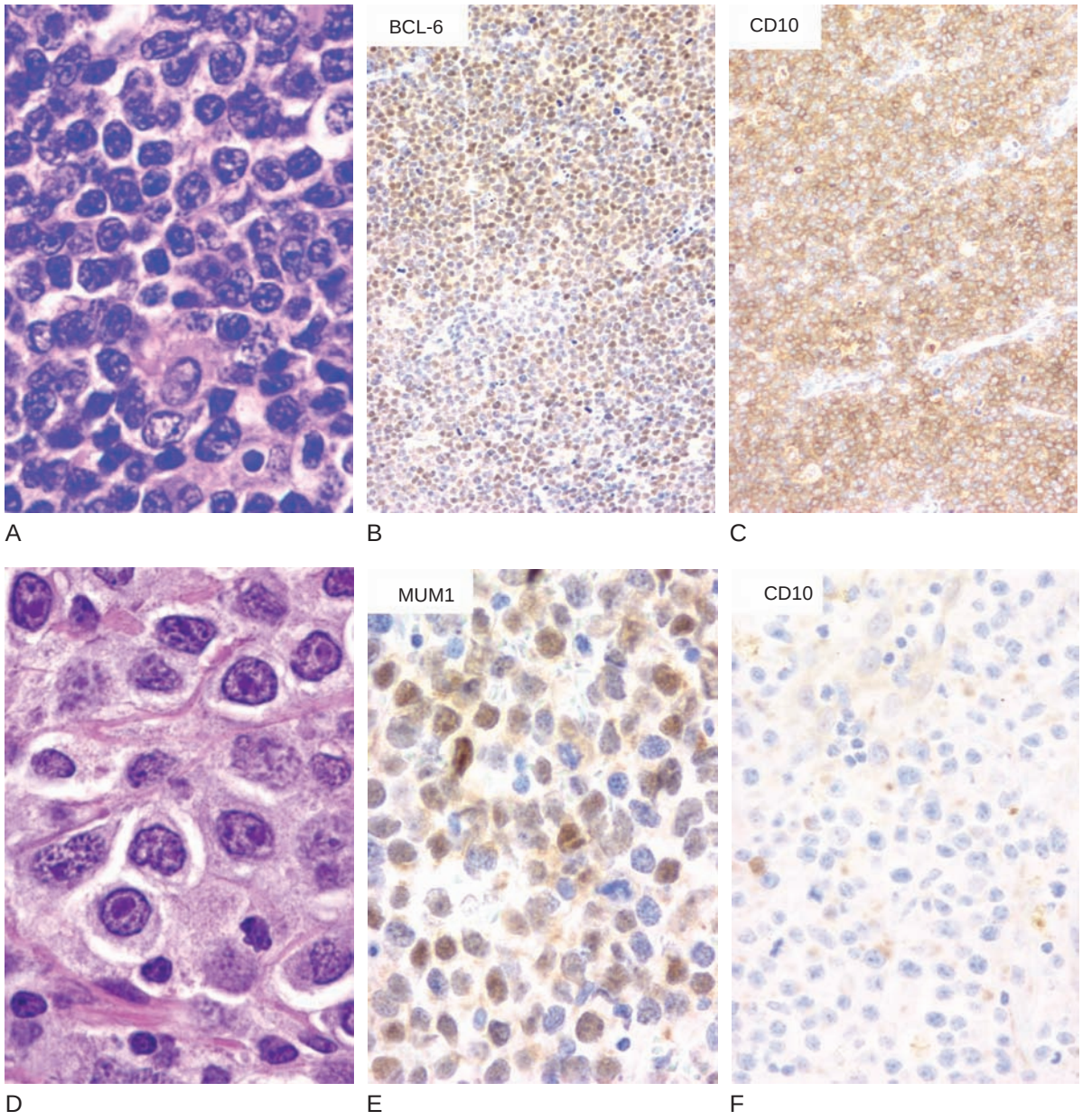


Figure 3.27 Diffuse large B-cell lymphoma; A–C, germinal center B-cell-like phenotype with positive BCL-6 (B) and CD10 (C); D–F, activated type with positive MUM1 (E) and negative CD10 (F)

Chromosomal/molecular aberrations

Chromosomal aberrations in DLBCL are common and often complex. The most frequent chromosomal change involves the *BCL6* gene at 3q27; other translocations involve c-*MYC* and *BCL2* genes.

BCL6

In DLBCL, the chromosomal translocations of 3q27 involving the locus of the *BCL6* gene are the most characteristic and common genetic abnormalities occuring in 30 to 40%. The partners of the *BCL6*

chromosomal translocations most often include the *IG* genes on chromosomes 14q32 (*IGH*), 2p12 (*IGK*), and 22q11 (*IGL*), but more than 20 other partners have been reported. In addition to *BCL6* translocations, small deletions and somatic point mutations occuring in the *BCL6* regulatory region are reported in 70% of DLBCLs. The presence of mutations is independent of translocation-generated rearrangements, with some mutations significantly deregulating *BCL6* expression. Patients with *BCL6* rearrangement have a less favorable outcome. Rearrangement of *BCL6* is found more often in patients with extranodal (36%) and advanced (39%) presentation versus primary nodal disease (28%).[762,1252]

t(14;18)

The t(14;18)$^{IGH/BCL2}$ translocation typical for FL is seen in 15% of nodal DLBCL. The expression of bcl-2 protein by immunohistochemistry, which does not correlate with t(14;18), is associated with a worse prognosis.

t(8;14)

The t(8;14) translocation occurs in 5–10% of DLBCL. It involves c-*MYC* at 8q24. The proliferation index of nearly 100% (Ki-67), monotonous proliferation of medium-sized cells, $CD10^+$, $Bcl\text{-}2^-$, and low frequency of mutation of the *IGVH* gene are helpful for the histologic distinction of BL from MYC^+ DLBCL.

TP53 (p53)

TP53 is a tumor-suppressor gene that acts as a multifunctional transcription factor involved in cell cycle arrest, apoptosis, cell differentiation, replication, DNA repair, and the maintenance of genomic stability. Mutations in the *p53* gene leading to a functionally defective protein have been detected in about 20% of DLBCLs and are associated with drug resistance, shorter disease-free survival, shorter survival, and generally a more aggressive clinical course and poor outcome.[597,861,862,1253,1254] *TP53* mutations occur almost exclusively in DLBCL tumors lacking *BCL6* translocations and, vice versa, *TP53* mutations are rarely confirmed in cases with constitutive expression of *BCL6* due to chromosomal translocation. This suggests that *BCL6* inactivates *TP53* during DLBCL lymphagenesis, thus bypassing the need for *TP53* mutations. Immunohistochemically, *p53* is detected in DLBCL in 30–40% of cases.[597] The clinical significance of *p53* expression is controversial, with some studies showing an adverse effect on outcome,[593,1255] whereas others have shown no effect.[589,599,600] The *p53* mutations accompanied by bcl-2 expression were shown to be associated with a very poor outcome;[600] however, the negative prognostic impact of overexpressed bcl-2 and p53 protein appears to be of no concern for patients older than 65 years of age.[602]

Genetic profiling

Recent studies using gene expression profiling have identified patterns of gene expression, as well as individual genes that appear to have important prognostic significance, related to underlying tumor biology. The presence of somatic mutations in the V region of *IG* genes is commonly used as a marker of germinal center (GC) transit since normal pregerminal center lymphocytes harbor unmutated *IG* genes.

Gene expression profiling using microarray technologies provides new insights into the biology and genetics of DLBCL, which can be used to predict outcome and risk stratification.[631,1256] Evaluation of DLBCL gene expression profiles by cDNA microarray techniques and oligonucleotide microarrays has identified molecularly distinct forms of DLBCL: GC B-cell-like DLBCL (GCB), characterized by the expression of genes normally expressed by GC B-cells, and non-GC like DLBCL. Non-GC-like DLBCL consists of activated B-cells (ABC)-like DLBCL, and 'type 3' subtypes.[632] The ABC-like DLBCL is characterized by the expression of genes involved in activation of B-cells as well as some genes normally expressed by plasma cells, thus suggesting their post-GC origin.[39,631] Type 3 is a heterogeneous DLBCL subtype that does not express high levels of either the GC or ABC set of genes.

The GCB group is characterized by a better prognosis in comparison with ABC or type 3 groups. The GCB group shows t(14;18) translocation, CD10 expression, c-*REL* amplification, and evidence of ongoing somatic hypermutation in the *IGVH* genes, and is characterized by a better overall survival.[632,1257,1258] The t(14;18)(q32;q21) translocation involving the *BCL2* gene and the amplification of the c-*REL* locus on chromosome 2p have been detected almost exclusively in GC-like DLBCL. The t(14;18) is present in 45% of GCB-DLBCLs and only in 8% of ABC-DLBCL.[1259] Other reports confirmed that t(14;18) translocations are prevalent, but not exclusive to GCB lesions.[1258,1260] Similarly, immunohistochemical markers such as CD10 and bcl-6 that have been reported to be expressed predominantly in the GCB type were also seen among non-GCB samples in the study reported by Poulsen et al.[1259] However, in that study, three samples that were classified as ABC using microarrays were GCB by immunophenotyping, indicating that phenotyping and expression profiling produce similar, but not identical classifications of the DLBCL. High expression of NF-κB target genes has been observed in ABC-like DLBCL, but not in GC-like DLBCL.

Patients with high *BCL6* gene expression (mRNA analysis by RT-PCR) had a better prognosis, with a median overall survival of 171 months, whereas patients with low *BCL6* gene expression had an overall survival of 24 months.[629] High bcl-6 mRNA expression appears to be a favorable prognostic factor in DLBCL.[629] Colomo et al, however, did not find a relationship between bcl-6 expression and prognosis.[23] The biologic consequences of bcl-6 expression in DLBCL might vary according to the presence or absence and the nature of the underlying alteration of the *BCL6* gene.[627] Genes which correlate most with outcome in DLBCL include *nor1*, *pde4b*, *pkc-β2*,[631] and *LMO2*, *BCL6*, *FN1*, *CCND2*, *SCYA3*, and *BCL2*.[1261] The expression of *LMO2, FN1,* and *BCL6* correlated with prolonged survival, and the expression of *CCND2*, *SCYA3*, and *BCL2* correlated with short survival.[1261]

FOXP1 expression has been demonstrated in a subset of DLBCL and is more common in the non-GC activated B-cell type. Uniform high *FOXP1* expression was demonstrated in ~18% of patients with DLBCL. The high level of expression was almost exclusively confined to patients who lacked the GC phenotype, expressed *MUM1* and *BCL2* in the absence of t(14; 18), and were identified as a subgroup of patients with particularly poor outcomes in a group with already poor prognoses. Positive *FOXP1* expression is an independent poor prognostic factor in DLBCL.[680,681] *FOXP1* and *MUM1* are the best predictor genes defining the ABC type of DLBCL.[632] T-cell leukemia/lymphoma-1A (TCL1A) mRNA overexpression in DNA microarray and tissue microarray analysis was shown to correlate with relapse rate in DLBCL patients.[1262] Table 3.6 shows molecular, pathogenetic, and clinical features distinguishing GC-like and ABC-like DLBCL.

Table 3.6 Molecular, pathogenetic, and clinical features distinguishing GC-like and ABC-like DLBCL

	Germinal center B-cell-like (GCB)	*Activated B-cell-like (ABC)*
Cell of origin	Germinal center B-cell	Postgerminal center B-cell (?)
Ongoing Ig mutation	Yes	No
Phenotype	CD10+/– Bcl-6+	MUM1+ CD138+/–
Oncogenic mechanisms	BCL2 translocation Chromosome 2p amplification of c-*REL* locus	Constitutive activation of NF-κB
Karyotypic changes	t(14;18) Gain 12q12	Trisomy 3 Gain 3q, Gain 18q21–q22 Del(6q21–q22)
Clinical outcome	Favorable (60% 5-year survival)	Poor (35% 5-year survival)

Specific clinical variants of diffuse large B-cell lymphoma

Nodal and extranodal DLBCLs differ in clinical presentation, behavior, and prognosis.[1263] Extranodal DLBCL is associated with older age and poorer performance status, but also lower tumor burden. In extranodal DLBCL, ~50% of the cases are stage I and ~35% are stage IV, whereas in nodal DLBCL the patients are relatively equally distributed between the four stages. For stage I patients, extranodal DLBCL was independently associated with poor survival. In contrast, among stage IV patients those with extranodal DLBCL survived longer.[1263]

Intravascular large B-cell lymphoma

Intravascular large B-cell lymphoma (IVLBCL; angiotropic lymphoma; Figure 3.28) is a specific subtype of DLBCL characterized by proliferation of large lymphomatous cells within the lumina of small to medium sized vessels.[1,1264] The IVLBCL is an aggressive and usually disseminated disease (Ann Arbor stage IV in 68% of cases) that predominantly affects elderly patients (median age 67–70 years, range: 34–90 years) with B symptoms (55–76%), anemia/thrombocytopenia (63–84%), hepatosplenomegaly (77%), bone marrow involvement (75%), high serum lactate dehydrogenase level (86%), and hemophagocytosis (61%).[1264,1265] The involved sites include: bone marrow (32–66%), brain (40%), skin (40%), liver (17–26%), spleen (16–26%), kidney (21%), endocrine glands (16%), lung (16%), prostate (16%), heart (11%), lymph nodes (11%), GI tract (8%), uterus (8%), gallbladder (3%), and sporadically other organs.[1264–1266] Patients with disease limited to the skin ('cutaneous variant'; 26% of cases) were invariably females with a normal platelet count, and they exhibited a significantly better outcome than the remaining patients.[1265] Most patients present with systemic symptoms (fever, weight loss), cutaneous lesions, neurologic symptoms, and/or pain. Laboratory abnormalities include anemia (63%), thrombocytopenia (29%), leukopenia (24%), high LDH (86%), high β_2-microglobulin, and hypoalbuminemia (18%).[1266]

The tumor cells express CD20 and may display a GC B-cell-like phenotype (20%) with CD10/bcl-6 immunoreactivity. There is aberrant CD5 expression in a significant proportion of cases. Murase et al reported CD5 expression in 38% and showed a higher prevalence of marrow/blood involvement for this group.[1264]

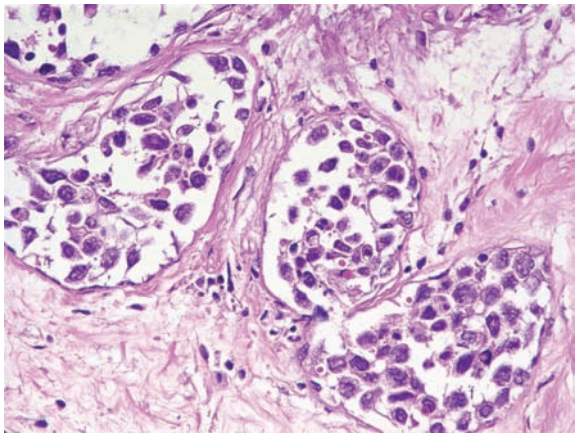


Figure 3.28 Intravascular large B-cell lymphoma

Cutaneous diffuse large B-cell lymphoma

The term primary cutaneous B-cell lymphoma was introduced in the early 1990s to identify a heterogeneous group of lymphoproliferative disorders with distinctive clinical features characterized by clonal proliferation of B-lymphocytes primarily involving the skin.[1267–1270] The WHO-EORTC (European Organization for Research and Treatment of Cancer) recognizes four main types of B-cell lymphomas in the skin: (a) primary cutaneous MZL, (b) primary cutaneous follicle center cell lymphoma (PCFCCL), (c) primary cutaneous DLBCL, leg type, and (d) primary cutaneous DLBCL, other. Primary cutaneous B-cell lymphomas are generally associated with a favorable prognosis, with good response rates to radiotherapy and chemotherapy. However, the relapse rate varies between 25% and 68% according to different studies.[1271–1275] A large series of primary

cutaneous B-cell lymphoma published by Zinzani et al, confirmed the good overall prognosis of these tumors, with a 10-year overall survival rate of 85%, a 92% complete response rate after the first treatment, and a low tendency to extracutaneous spread.[1276] However, nearly half of the patients experienced relapse after the first treatment (46.7%) and 23.8% experienced two or more relapses during the follow-up.[1276] Both cutaneous MZL and PCFCCL differ significantly in prognosis from DLBCL localized in the lower limb. Willemze et al reported a uniquely poor prognosis associated with DLBCL, leg type.[1269] In the case of primary cutaneous DLBCL, location on the leg seems to be the main independent prognostic factor for determining poorer outcome;[1277] the presence of multiple lesions, involvement of more than one body site, and phenotype had no effect on survival.[1277]

Testicular diffuse large B-cell lymphoma

Primary testicular DLBCL has a poor prognosis despite localized presentation and doxorubicin-based chemotherapy. Touroutoglou et al reported that the failure-free survival rate at 153 months was 16% for all patients or 32% and 0% for those with IPI scores ≤1 and >1, respectively.[1278] The CNS or contralateral testis were involved in all patients who failed to respond to primary therapy, and in 50% of those who relapsed from complete remission.[1278] In a series reported by Darby et al, 40% of patients with testicular lymphoma relapsed following a complete response and the median time to relapse was 9 months.[1279] Only one of these relapses was in the CNS, one was in bone, one was in skin, and two were in the contralateral testis. The majority of relapses (58%) occurred in the lymph nodes.[1279] The study by Bosga-Bouwer et al indicates that 6q deletions play a major pathogenetic role in DLBCL of the testis and that many of these deletions are part of unbalanced translocations.[190] Cytogenetic and FISH (chromosome 6 painting probe) analysis show structural abnormality of the long arm of chromosome 6, with deletion or addition of material of unknown origin in the majority of testicular DLBCLs (72%).[190]

Diffuse large B-cell lymphoma of bone

Primary non-Hodgkin's lymphoma of bone is rare, comprising ~5% of extranodal lymphomas.[1280–1285] Most primary bone lymphomas are classified as DLBCL (followed in frequency by ALCL, T-cell type). DLBCL of bone can occur in children and adults.[1280–1285] All cases reported by Huebner-Chan et al were negative for *IGH/BCL2* rearrangement.[1284] The majority of DLBCLs of bone express p53.[1284]

Central nervous system lymphoma

Primary CNS lymphoma (PCNSL) comprise 5–7% of primary brain tumors. PCNSL occurs in immunosuppressed patients, especially those with AIDS or those who have received solid organ transplants. It is uncommon in immunocompetent patients. Most primary CNS lymphomas in immunocompetent patients are DLBCLs characterized by poor prognosis, compared with systemic forms. Unlike HIV-associated primary CNS lymphomas, these lymphomas developing in immunocompetent patients are not associated with EBV and occur usually over the age of 50 years. Concerning this histogenesis, primary CNS DLBCLs are thought to be of GC cell origin, based on their bcl-6 expression[1286] and ongoing mutational activity.[1287,1288] The prognosis of primary CNS DLBCL is poor, with a 5-year overall survival rate of approximately 30%.[1289,1290] In patients with primary DLBCL of the CNS, expression of *p53* or c-*MYC* is associated with a poorer overall survival than in those without. Expression of bcl-6 is significantly associated with a longer overall survival (median survival 101 months vs 14.7 months for bcl-6$^-$ tumors),[1291] although the study by Camilleri-Broet et al did not confirm the prognostic significance of bcl-6 expression.[1292] Since traditional prognostic markers in non-CNS DLBCL, such as staging and IPI scores, are not applicable to primary CNS DLBCL, evaluation of *p53*, c-*MYC*,

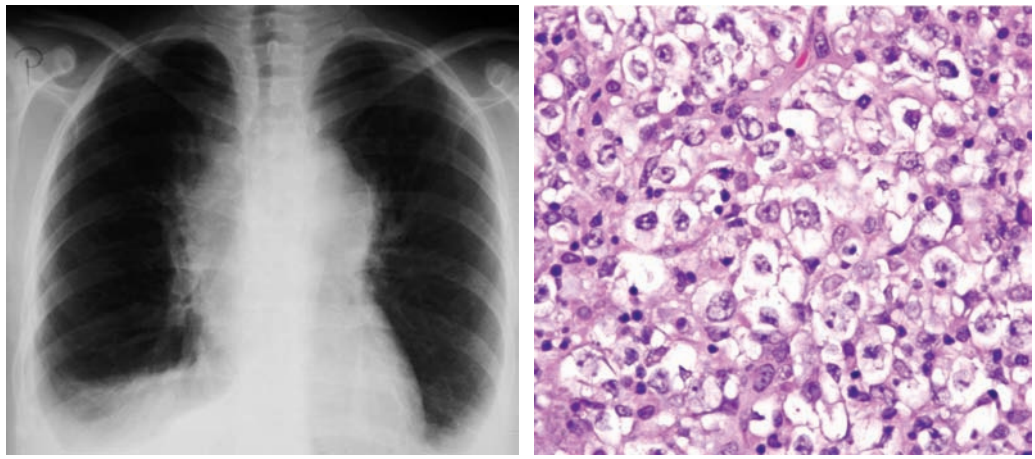
and bcl-6 by immunohistochemistry may be warranted as part of the prognostic evaluation in immunocompetent patients with primary CNS DLBCL.[1293] While DLBCL can arise at any time – from early GC to late post-GC, primary CNS DLBCL antigens correspond to those mainly expressed from late GC to early post-GC (low CD10 expression, the predominant bcl-6$^+$/MUM1$^+$ phenotype, and the CD138 negativity).

Mediastinal diffuse large B-cell lymphoma

Mediastinal (thymic) DLBCL encompasses cases of otherwise typical nodal DLBCL and primary mediastinal (thymic) large B-cell lymphoma (Figure 3.29). Primary mediastinal (thymic) DLBCL accounts for approximately 5% of aggressive lymphomas and has a predilection for young women, bulky disease, high LDH levels, and frequent intrathoracic extension to adjacent organs such as pleura, pericardium, and lung.[1294] Extrathoracic dissemination and bone marrow involvement are uncommon. Clinically, primary mediastinal large B-cell lymphoma resembles classic Hodgkin lymphoma, but on histology it shows diffuse large cell infiltrate with characteristic clear cytoplasm and prominent fibrosis. Studies with gene expression profiling showed that mediastinal DLBCLs have a distinct biology and should be considered separate from nodal DLBCL.[1295,1296] More than one-third of the genes that were more highly expressed in primary mediastinal lymphoma were also characteristically expressed in Hodgkin lymphoma cells.[1295,1296] Malpeli et al, showed that 54% of primary mediastinal B-cell lymphomas possessed mutations in the intronic region of *BCL6* prone to hypermutations and the mutational spectrum differed from that in FL or nodal DLBCL.[1297] Primary mediastinal large B-cell lymphoma exhibits characteristic genetic abnormalities including frequent gains of 9p and distinct high-level amplifications with a defined consensus region on 9p24, where *JAK2* genes are located. In contrast, *BCL2* and *BCL6* rearrangements, frequently observed in DLBCL, appear to be rare. Primary mediastinal large B-cell lymphoma has a relatively favorable clinical outcome, with a 5-year survival rate of 64% compared with 46% for other DLBCL patients.[1296]

De novo *CD5$^+$ diffuse large B-cell lymphoma*

De novo CD5$^+$ DLBCL is known to have phenotypically and genotypically different characteristics



A B

Figure 3.29 Primary mediastinal (thymic) large B-cell lymphoma. A, radiology; B, histology

from CD5$^-$ DLBCL. CD5$^+$ DLBCL is characterized by a survival curve that is significantly inferior to that for patients without CD5 expression.[231,1298–1300] CD5$^+$ DLBCL shows a higher incidence of bone marrow and spleen involvement.[1299–1301] Based on comparative genomic hybridization (CGH) analysis regions of genomic aberrations observed in >20% of cases of both the CD5$^+$ and CD5$^-$ groups of DLBCL were gains of 1q21–q31, 1q32, 3p25–q29, 5p13, 6p21–p25, 7p22–q31, 8q24, 11q23–q24, 12q13–q21, 16p13, 18, and X and losses of 1p36, 3p14, 6q14-q25, 6q27, 9p21, and 17p11–p13.[1302] Although both groups showed similar genomic patterns of gains and losses, gains of 10p14-p15 and 19q13 and losses of 1q43-q44 and 8p23 were found to be characteristic of CD5$^+$ DLBCL.[231,1302] A comparison with results reported for MCL, B-CLL, and Richter's syndrome demonstrated that the CGH pattern of CD5$^+$ DLBCL was markedly different.[231] Characteristic cytogenetic abnormalities in *de novo* CD5$^+$ DLBCL were also reported by Yoshioka et al, with a high incidence of chromosomal aberrations affecting 8p21 and 11q13.[1303] Major chromosomal breakpoints were concentrated at 8p21, 11q13, and 3q27. Patients with 8p21 aberrations showed aggressive clinical features, including advanced stage of disease, elevated serum LDH level, poor performance status, and an inferior survival curve compared with patients who had 11q13 changes. The conflicting results of previous studies on prognosis in *de novo* CD5$^+$ DLBCL may be explained in part by the differences in chromosomal changes.[1303]

Large B-cell lymphoma expressing the ALK kinase

Large B-cell lymphoma expressing the ALK kinase is a rare variant of large B-cell lymphoma with immunoblastic or plasmablastic morphology and cytoplasmic expression of ALK-1.[370–372] In most cases it lacks the t(2;5)$^{ALK/NPM}$ translocation characteristic for (T-cell) ALCLs. Apart from granular cytoplasmic ALK positivity, tumor cells may express CD45, CD56, CD138, CD43, CD10, IgA, and lambda. They lack CD20 and CD79a. Most patients present with advanced disease. The clinical course is aggressive despite polychemotherapy.[370] The majority of cases are associated with t(2;17)(p23;q23), which results in fusion between *CLTC* (clathrin gene) on 17q23 and *ALK* on 2p23.[372] Only rare cases of ALK$^+$ DLBCL with *ALK/NPM* fusion due to t(2;5) have been reported.[568,569] Stachurski et al, described ALK$^+$ DLBCL with complex karyotype and cryptic *ALK* gene insertion to chromosome 4q22–24.[373]

Diffuse large B-cell lymphoma with Burkitt-like features (Burkitt lymphoma vs DLBCL)

Translocations between the c-*MYC* and immunoglobulin genes, t(8;14)(q24;q32), t(2;8)(p13;q24), and t(8;22)(q24;q11), are observed in almost all BLs. The c-*MYC* can be occasionally seen in other B-cell neoplasms, like DLBCL, lymphoblastic lymphoma, FL and plasma cell myeloma.[178,266,760,1304] A distinction between Burkitt lymphoma and DLBCL has important clinical implications; however, this distinction can be difficult and is not reliable based on morphology and immunophenotype alone. BL and DLBCL are both aggressive lymphomas and may have overlapping histologic, phenotypic, and molecular features (gray zone Burkitt lymphoma/DLBCL or Burkitt-like lymphoma). Currently, based on WHO classification, Burkitt-like lymphoma is considered synonymous with 'atypical Burkitt lymphoma' and comprises cases which demonstrate a high growth fraction (Ki-67 index exceeding 99%), phenotype of Burkitt lymphoma, and cytogenetic evidence of a c-*MYC* rearrangement with atypical cytomorphologic features. When compared to Burkitt lymphoma, DLBCL is the more heterogeneous disease at the molecular and cytogenetic level, with translocations, including *BCL6* breakpoints (20–35%), *BCL2* breakpoints (15–20%), and c-*MYC* breakpoints (5–15%), and often complex karyotype. In c-*MYC*$^+$ DLBCL the Ki-67 index varies from 48 to 89.7%.[1305] DLBCLs are often bcl-2$^+$ and CD43$^-$ and only a subset of DLBCL

expresses CD10 (Burkitt lymphomas are bcl-2$^-$, CD43$^+$, and CD10$^+$).

In a series reported by Haralambieva et al, 35% of Burkitt lymphomas were diagnosed as DLBCL based on histologic criteria alone.[1306] Their exclusion from Burkitt lymphoma was mainly based on the nuclear pleomorphism with the presence of centroblasts and/or immunoblasts among smaller, more classic Burkitt lymphoma. Vice versa, 29% of lymphomas categorized as Burkitt lymphoma by histology belonged to the DLBCL category based on phenotypic and cytogenetic (FISH) parameters (double c-*MYC* and *BCL2* or *BCL6* breakpoint, lack of detectable c-*MYC* breakpoint, and/or immunophenotype incompatible with Burkitt lymphoma).[1306] The reported frequencies of the c-*MYC* breakpoint in DLBCL vary between 4 and 15% depending on the method used (cytogenetics, FISH, Southern blot, or long distance PCR), and therefore this marker should not be used alone but only in combination with other meaningful markers. Lymphomas with a combination of c-*MYC* and *BCL2* should be distinguished from true Burkitt lymphoma with a sole break in c-*MYC* as they often represent a very aggressive disease with a poor clinical outcome. Dual translocation of c-*MYC* and *BCL2* is characterized by a rapid clinical course and extremely poor outcome, and some cases with dual translocation of c-*MYC* and *BCL2* are regarded as instances of transformation of FL. Tumors with a concomitant c-*MYC* and *BCL6* are less common. Hummel et al analyzed genomic profiling in 220 mature aggressive B-cell lymphomas using Affymetrix U133A GeneChips.[887] The distinctive molecular signature for Burkitt lymphoma consisted of 58 genes, including several target genes of the NF-κB pathway (e.g. *BCL2A1*, *FLIP*, *CD44*, *NFKB1A*, *BCL3*, and *STAT3*) that distinguishes activated B-cell-like or germinal-center B-cell-like lymphomas.[887] By correlating the gene expression profile with chromosomal aberrations, these authors distinguished three main cytogenetic groups within aggressive B-cell lymphomas: c-*MYC*-simple (lymphomas with *IG/MYC* fusion and a low chromosomal complexity without *IGH/BCL2* fusions and *BCL6* breakpoints), c-*MYC*-complex (lymphomas with non*IG/MYC* fusions or lymphomas with *IG/MYC*, a high chromosomal complexity score, and *IGH/BCL2* or *BCL6* breakpoints) and c-*MYC*-negative (*MYC*-negative lymphomas).[887] Of the 176 lymphomas without the molecular signature for Burkitt lymphoma, 155 were DLBCL. Of these 155 cases, 21% had a chromosomal breakpoint at the c-*MYC* locus associated with complex chromosomal changes and an unfavorable clinical course.[887] Also in the study reported by Dave et al, BL and DLBCL differed with respect to the signature of the c-*MYC* target genes as well as other gene expression signatures including a subgroup of germinal-center B-cell genes.[888] NF-κB target genes are expressed at lower levels in Burkitt lymphoma than in DLBCL.[887,888]

Bcl-2 expression by IHC may be a surrogate marker for *BCL2* and *BCL6* breakpoints. Immunohistochemistry for Ki-67, CD10, and bcl-2 in combination with FISH analysis for c-*MYC* breakpoints could be a minimum panel to be used for the diagnosis of BL in adult patients. Additional FISH analysis for *BCL2* and *BCL6* breakpoints might be advisable as well, especially when cytomorphologic and immunophenotypic features are equivocal. An algorithm for diagnosing Burkitt lymphoma versus DLBCL is presented in Figure 3.30.

Diffuse large B-cell lymphoma in children

Lymphomas account for approximately 6% of pediatric malignancies, and represent primarily high-grade lymphomas, the most prevalent entities being Burkitt lymphoma (43%), precursor B-lymphoblastic lymphoma (7%), and DLBCL (13%). Childhood DLBCL responds well to therapy and has an event-free survival of about 90%.[1307–1309] A large series by Oschlies et al showed a predominance of pediatric DLBCLs with germinal center B-cell-phenotype and lack of t(14;18) by standard cytogenetics and FISH.[1310]

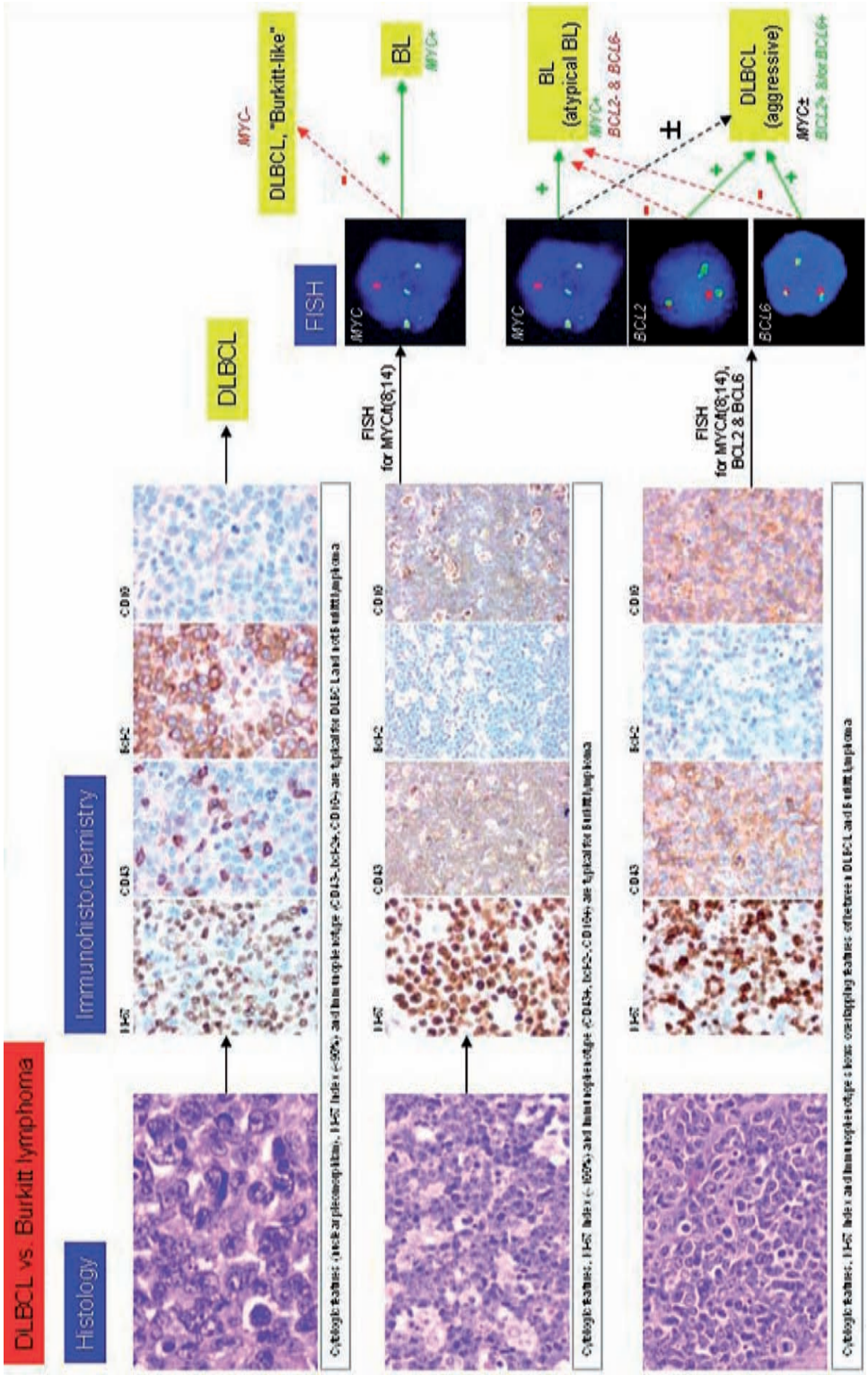


Figure 3.30 Algorithm for differential diagnosis of Burkitt lymphoma versus DLBCL

Plasmablastic lymphoma

Plasmablastic lymphomas are a heterogeneous group of tumors.[296] The most common type, plasmablastic lymphoma of the oral mucosa (Figure 3.31), is characterized by immunoblastic morphology, plasma cell phenotype, a high rate of association with HIV infection, and an unfavorable outcome.[296] The second subtype, plasmablastic lymphoma with plasmacytic differentiation, presents in both nodal and extranodal sites, HIV infection in a subset of patients, EBV positivity, and a very aggressive clinical course.[296] Plasmablastic lymphomas display morphologic features of DLBCLs, but differ by their phenotype, which resembles a plasma cell tumor rather than DLBCL. Monoallelic *TP53* deletions are common.[1311] Plasmablastic lymphomas are resistant to standard chemotherapy and autologous stem cell transplantation and have a median overall survival of 14 months.[1311]

Burkitt lymphoma

Burkitt lymphoma (Figure 3.32) is an aggressive B-cell lymphoma of mature B-cell phenotype characterized by a bulky disease, common extranodal location, a high degree of proliferation (Ki-67 index approaching 100%), and the deregulation of the c-*MYC* gene, most often due to t(8;14)(q24.1;q32). It can present as lymphoma or leukemia. Three variants of Burkitt lymphoma are recognized: endemic, sporadic (spontaneous), and associated with immunodeficiency (HIV). Adult patients with sporadic or immunodeficiency-associated Burkitt lymphoma typically present with extranodal disease. The endemic variant is most commonly observed in children aged 4 to 7 with frequent involvement of the mandible and maxilla, and abdominal organs, especially the kidneys. The sporadic forms occur mainly in young adults, and present as abdominal disease. A significant subset of BL is associated with EBV infection (endemic and HIV-associated forms). With the current chemotherapy regimens, the overall survival rate is approximately 90% in children and 50–70% in adults.[1307,1312–1314]

The diagnosis of Burkitt lymphoma is based on morphology, immunophenotyping, and cytogenetics/FISH studies. Morphologically, Burkitt lymphoma is composed of a monotonous infiltrate of medium-sized cells with numerous mitoses, apoptotic cells, and scattered macrophages with engulfed apoptotic bodies, creating a 'starry-sky' appearance (Figure 3.32).

Chromosomal/molecular markers

The majority of Burkitt lymphoma cases have t(8;14)(q24;q32), resulting in the juxtaposition of the c-*MYC* oncogene (on chromosome 8q24) with the *IGH* heavy chain locus (on chromosome 14) (Figure 3.33). Remaining cases have either t(2;8)(p11;q24) or t(8;22)(q24;q11.2), that juxtapose *MYC* to the immunoglobulin light chain genes, kappa (*IGK*) on 2p11 and lambda (*IGL*) on 22q11, respectively.[758]

Approximately 60–70% of sporadic Burkitt lymphomas in adults have additional chromosomal abnormalities.[81,1315,1316] Barth et al compared endemic Burkitt lymphoma with sporadic Burkitt lymphoma and concluded that the former has a homogeneous immunohistology and few secondary genomic aberrations, whereas the c-*MYC*-rearranged sporadic form is more heterogeneous with regard to immunophenotype and often displays complex chromosomal abnormalities.[1317] In a series of Burkitt lymphoma in 33 children and 37 adults reported by Onciu et al, all patients had either the t(8;14) translocation or one of the variants, t(8;22) (3 children and 6 adults) or t(2;8) (2 adult patients).[1318] In the same series, additional abnormalities were present in 54 of 70 (77%) patients, including 27 children (81%) and 27 adults (73%). Approximately half of the tumors had a complex karyotype, with a similar incidence in children (17/33 patients; 51%) and adults (19/37 patients; 51%).[1318] The most frequent additional abnormalities in Burkitt lymphoma affect chromosomes 1 [30–44%; addition or duplication of 1q,

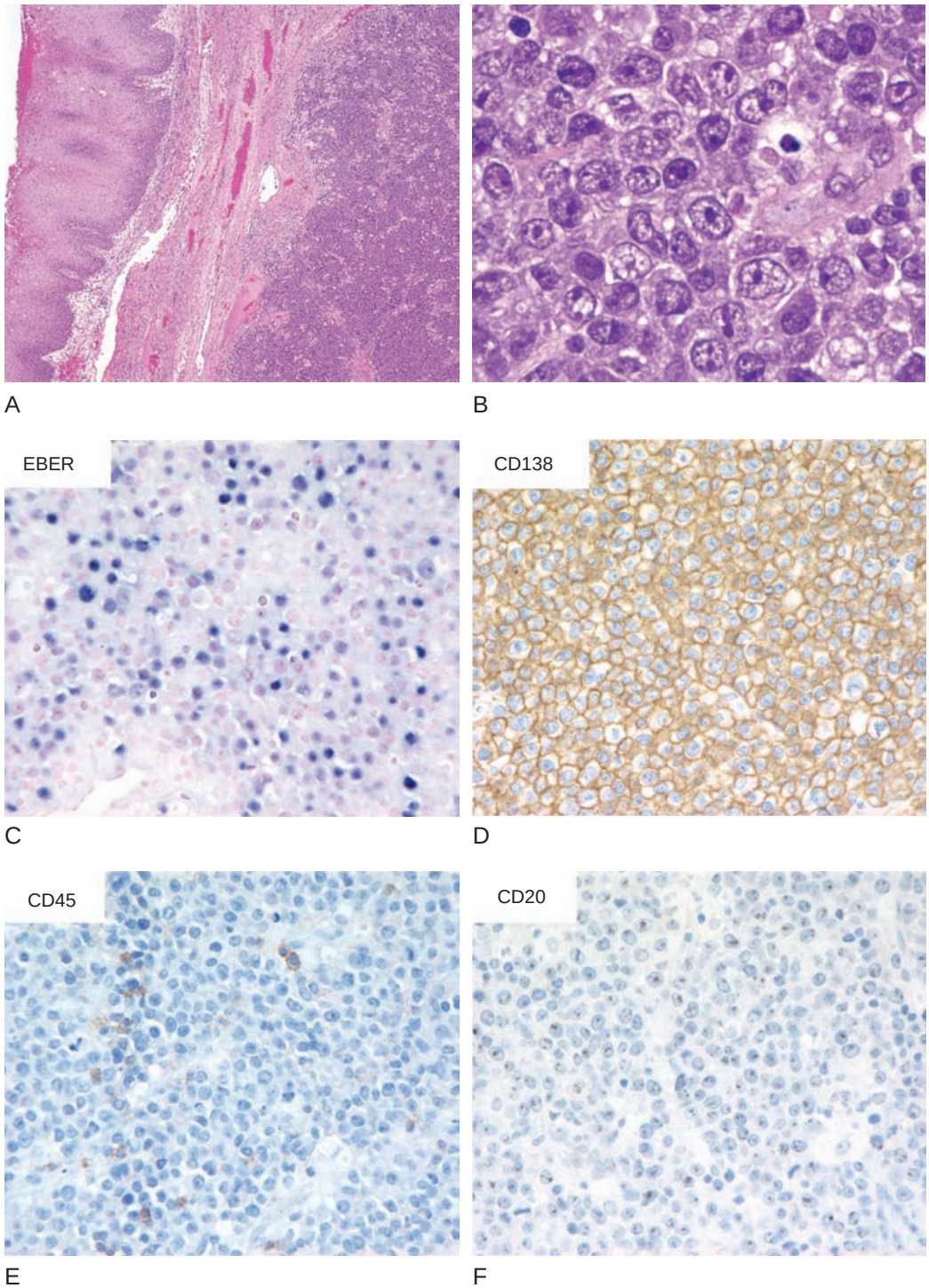


Figure 3.31 Plasmablastic lymphoma, oral mucosa. A–B, histology (A, low power; B, high power); C–F, immunohistochemistry: lymphomatous cells are positive for EBER (C) and CD138 (D), and negative for CD45 (E) and CD20 (F)

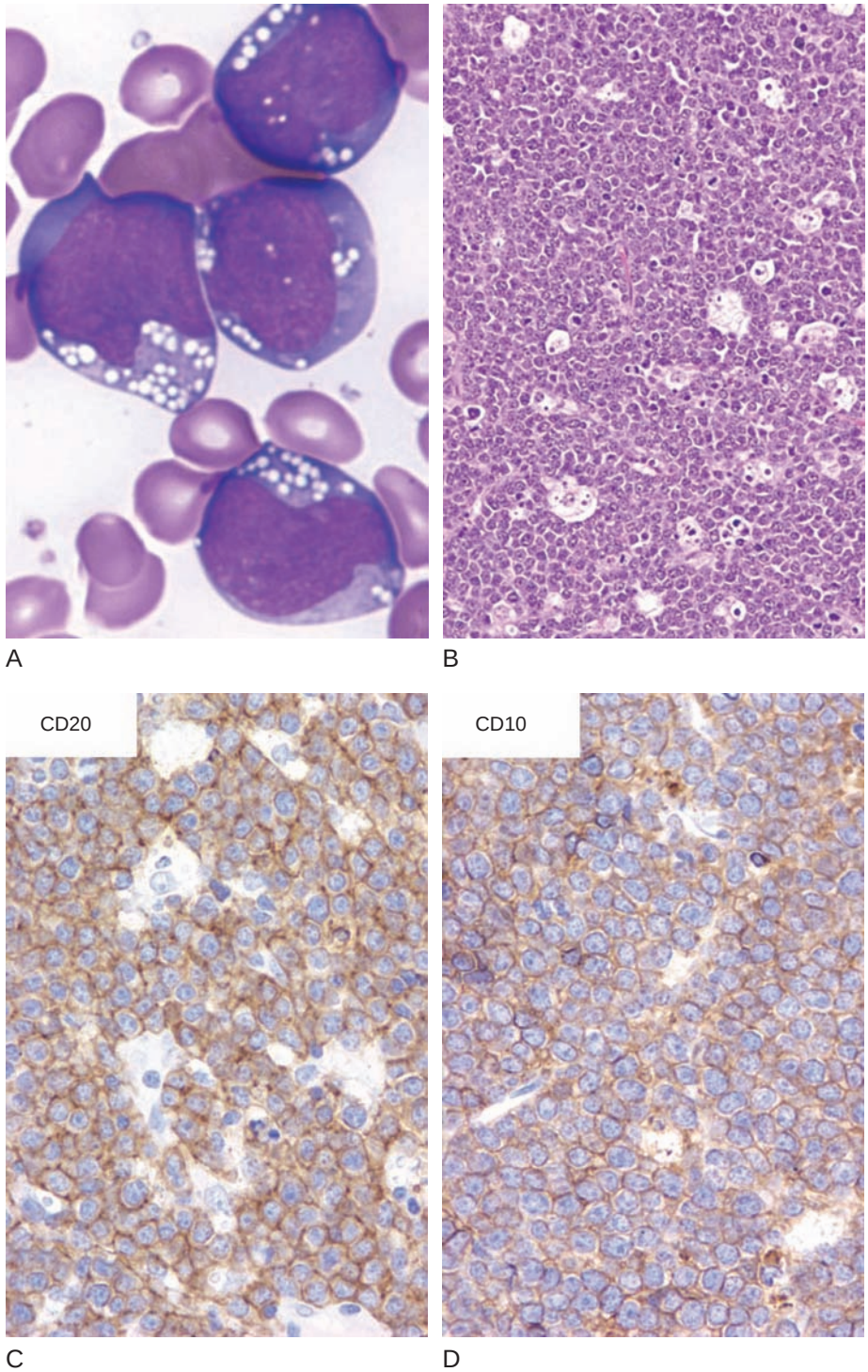
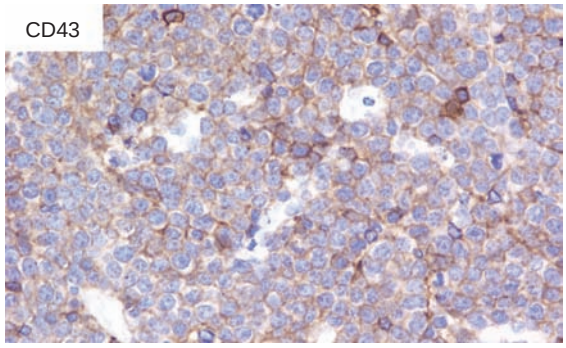
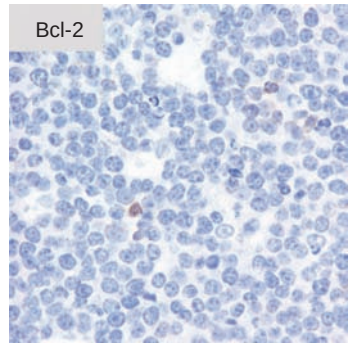


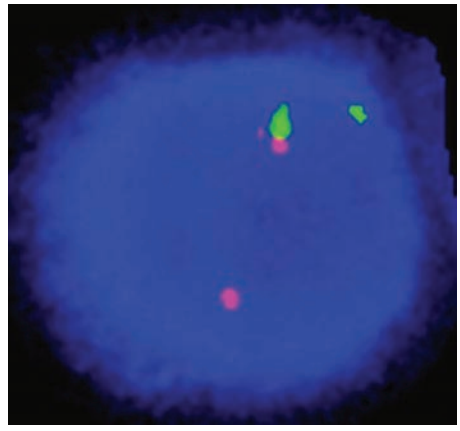
Figure 3.32 Burkitt lymphoma. Burkitt cells are medium-sized with increased nuclear–cytoplasmic ratio and vacuolated cytoplasm (A). Tissue section shows diffuse lymphoid infiltrate with 'starry-sky' pattern (B). Lymphomatous cells are positive for CD20 (C), CD10 (D), CD43 (E) and are negative for bcl-2 (F). FISH shows *c-MYC* (G)



E



F



G

Figure 3.32, cont'd

balanced translocations], 6 [17%; terminal or interstitial deletions or additions, i(6), trisomy 6, and t(1;6)], 13 [11–25%; 13q rearrangement], 17 [17%], and 22 [21%].[221,429,1315, 1318–1320] Chromosome 13q abnormalities are found more frequently in the pediatric population and are associated with a worse prognosis in children. Chromosome 17 abnormalities are present with similar frequency in pediatric and adult Burkitt lymphoma, and are associated with poor prognosis in adults. Valnet-Rabier et al reported that the expression of c-*FLIP* in Burkitt lymphyoma was associated with a poor prognosis, with a high death rate within the first year of diagnosis, adult onset, and chemoresistance.[1321] The 2-year overall survival with c-*FLIP* expression was 24% compared with 93% in the absence of this marker. The presence of abnormalities on 1q and imbalances on 7q were associated with a short survival.[221]

The c-*MYC* deregulation [t(8;14)] is not specific for Burkitt lymphoma, and can also be detected in approximately 15% of DLBCLs as well as in secondary precursor B-lymphoblastic leukemia/lymphoma, aggressive lymphoma transforming from FL, and in some cases of MM.[760,762,11401304–1306,1317,1322,1323] The differential diagnosis between Burkitt lymphoma and DLBCL is very important from the clinical point of view, since the treatment and prognosis of two disease differs (*see above*: DLBCL, specific clinical variants).

Multiple myeloma (plasma cell myeloma)

MM also termed plasma cell myeloma (Figure 3.34), is a clonal disorder of B-cells at the last stage of differentiation characterized by bone marrow infiltration

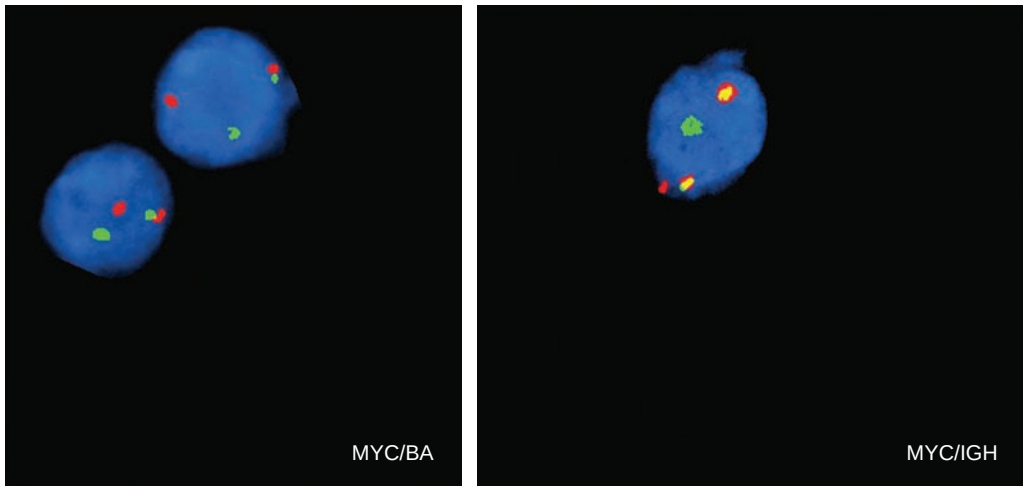


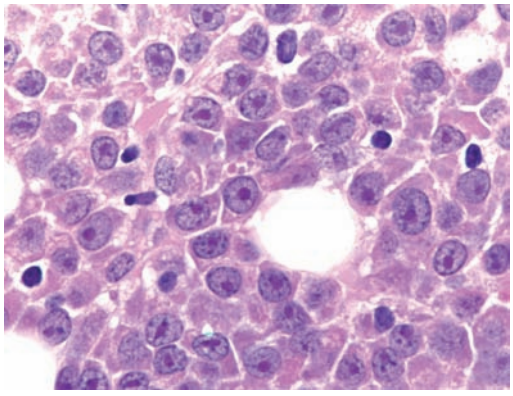
Figure 3.33 Burkitt lymphoma. A, FISH analysis for *MYC* (break-apart probe); B, FISH analysis showing *MYC/IGH* fusion

by plasma cells, production of a monoclonal immunoglobulin with serum monoclonal protein (M-protein), and lytic bone lesions. WHO criteria require >10% plasma cells in the marrow for the diagnosis of MM. The diagnosis and subclassification of the various plasma cell dyscrasias depend on the correlation of radiologic imaging data (bone lesions) and laboratory data (M-protein type and amount) with morphologic, cytogenetic, and phenotypic findings. On immunophenotyping, MMs are usually positive for CD138, MUM1, light and heavy chain immunoglobulins, OCT-2 and BOB-1; more than 50% of tumors express CD43, CD117, and/or CD56, and approximately one-third of cases are positive for bcl-1, Pax-5, CD20, and/or EMA. Patients with <10% plasma cells in the bone marrow usually represent MGUS, which is believed to be a precursor of MM.

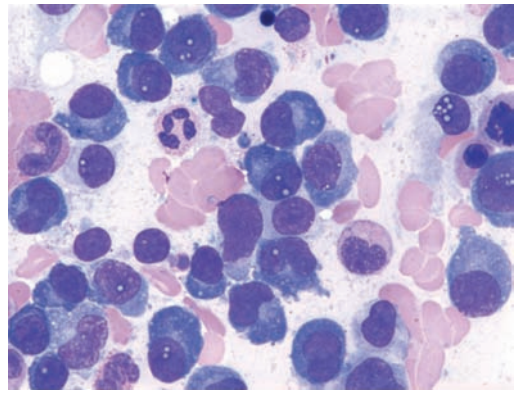
Clinical outcomes for MM are highly heterogeneous. The genomic aberrations t(4;14), t(14;16), t(14;20), and del(17p), together with the β_2-microglobulin level, are important independent predictors of survival.[304] In univariate statistical analyses del(13), t(4;14), nonhyperdiploidy, and del(17p) negatively impact both the event-free survival and the overall survival, whereas t(11;14) and c-*MYC* translocations do not influence the prognosis.[304] High-risk disease is associated with t(4;14), t(14;16), t(14;20), deletion 17q13, aneuploidy or deletion chromosome 13 by conventional cytogenetics, or a plasma cell labeling index >3.0.[1324]

Chromosomal aberrations

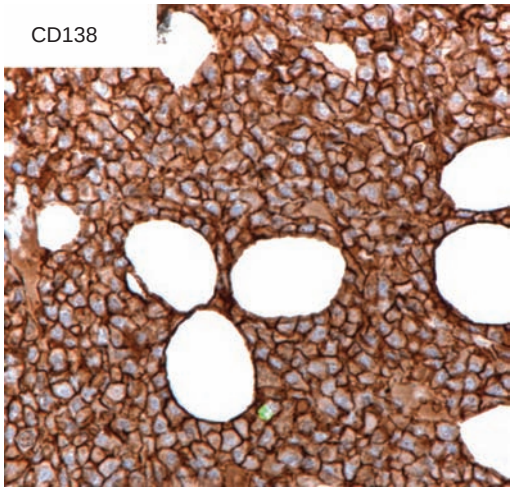
Chromosomal abnormalities occur in up to 90% of patients with MM; they are most often complex and can be identified either by conventional cytogenetics or more frequently by FISH.[112,266,267,304,1325–1329] Among numeric changes, gains predominantly involve chromosomes 3, 5, 7, 9, 11, 15, 19, and 21, and losses, chromosomes 8, 13, 14, 17, Y, and X. The frequencies of monosomies and trisomies for chromosomes 15 and 18 are similar.[267] MM can be subdivided into hyperdiploid and nonhyperdiploid variants.[189,1330–1332] Hyperdiploidy is present in ~60% of cases, and is often associated with trisomies of chromosomes 3, 5, 9, 11, 19 and 21.[14] In a series of 513 patients reported by Avet-Loiseau et al, with a median follow-up of 41 months, univariate statistical analyses revealed that del(13), t(4;14), nonhyperdiploidy, and del(17p) negatively impacted both



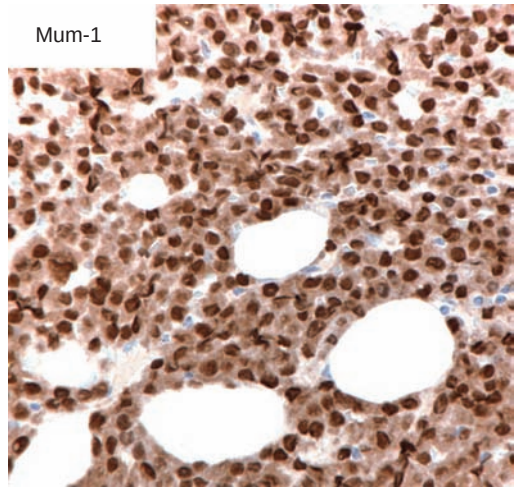
A



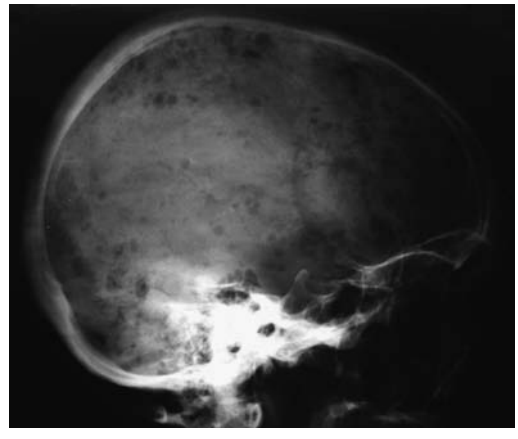
B



C



D



E

Figure 3.34 Multiple myeloma. Bone marrow is replaced by atypical plasma cells with nucleoli (A, histology; B, aspirate smear). Neoplastic plasma cells are positive for CD138 (C) and Mum-1 (D). Radiologic imaging shows typical bone lesions (E)

the event-free survival and the overall survival, whereas t(11;14) and c-*MYC* translocations did not influence the prognosis.[304] Multivariate analyses on 513 patients annotated for all the parameters showed that only t(4;14) and del(17p) retained a prognostic value for both the event-free and overall survivals.[304]

MGUS patients display a high incidence of numeric alterations. The most common single chromosome abnormalities detected in MGUS were gains of chromosomes 9 (23%) and/or 6 (21%), and loss of chromosomes 13 (21%) and/or 17 (17%).[1333] Compared with MM, MGUS patients were found to have both a lower incidence of gains of chromosome 9 (23% vs 54%) and monosomy 13/del(13q) (21% vs 38%). One of the few differential genetic lesions between myeloma and MGUS is the presence of *Ras* mutations in the latter.

Chromosome 1

Rearrangements involving the long arm of chromosome 1 are common in MM and have been associated with tumor progression.[1334,1335] Amplification of chromosome band 1q21 (Amp1q21; FISH analysis) was reported as 0% in MGUS, 45% in smoldering MM, 43% in newly diagnosed MM, and 72% in relapsed MM.[1336] Patients with newly diagnosed MM with Amp1q21 had an inferior 5-year event-free and overall survival compared with those lacking Amp1q21, and in a multivariate analysis Amp1q21 was an independent poor prognostic factor.[1336] In a microarray analysis of 532 newly diagnosed patients with MM reported by Shaughnessy et al, 70 genes were linked to early disease-related death, many of them located on chromosome 1: nearly 50% of 19 underexpressed genes and 30% of 51 overexpressed genes were derived from chromosome 1p and 1q, respectively.[1337] The molecular data suggest that alterations in chromosome 1 may play a role in disease evolution by providing a growth and/or survival advantage.[1337,1338]

Monosomy 13

Monosomy 13 is one of the most frequent aberrations in MM.[266,267,304] The complete or partial deletion of chromosome 13 or translocations involving 13q by conventional cytogenetics is associated with poor outcome, a higher relapse rate (77% vs 44%), and a shorter event-free and overall survival even with high-dose treatment and autologous stem cell transplantation.[14,20,112,267,272,276] Using metaphase analysis, deletion 13 (predominantly monosomy, but sometimes interstitial deletion) is present in ~50% of patients with abnormal karyotypes, but since this method frequently fails to detect the abnormal clone, deletion 13 is seen in only 10–20% of patients overall, compared to the 40–50% usually seen by interphase FISH.[14] Kroger et al, in a multivariate analysis, showed that 13q− remained a significant risk factor for a higher relapse rate and a shorter event-free survival after dose-reduced allogeneic stem cell transplantation; two or more cycles of prior high-dose chemotherapy were associated with a significantly higher probability of death, while patients with deletion 13q had a nearly 2 times higher risk of death.[1339] In patients with MGUS monosomy 13 correlates with the risk of transformation to overt MM.[1340] In the series of Shaughnessy et al, among all MM patients with cytogenetic abnormalities and standard prognostic factors examined prior to therapy, only hypodiploidy and chromosome 13 aberrations (−13), alone or in combination, were associated with the shortest event-free survival and overall survival.[1341] The shortest postrelapse overall survival was observed with hypodiploidy and monosomy 13.[1341] A superior prognosis was associated with the absence of any chromosomal abnormalities at both diagnosis and relapse (10-year overall survival, 40%).[1341] New treatment modalities (e.g. bortezomib), however, may change the prognostic significance of monosomy 13.[1342]

Rearrangements of 14q32 (IGH gene)

The *IGH* gene (14q32 locus) is frequently involved in MM, including t(4;14), t(14;16), t(11;14), t(9;14) t(6;14), t(8;14), and t(14;2).[304,406,1343–1345] In 30% of the translocations involving *IGH*, the partner chromosomal gene is *CCND1* (11q13; *BCL1*).[430]

The other most common chromosomal partners include 6p21 (cyclin D3), 4p16 (*FGFR3* and *MMSET*), 16q23 (c-*MAF*), and 20q11 (*MAFB*).[1344, 1346–1349] The oncogene c-*MAF* is translocated in approximately 5–10% of MMs and t(8;14)$^{MYC/IGH}$ is very rare. The incidence of *IGH* translocations increases with the stage of disease: 50% in MGUS, 60–65% in intramedullary MM, and 70–80% in extramedullary MM. Some of the 14q32 abnormalities may influence the natural history of MM; t(4;14)(p16;q32) is rarely observed in MGUS, whereas t(14;16)(q32;q24) is often associated with plasma cell leukemia.[403] In the series reported by Moreau et al, patients with t(4;14) displayed a poor outcome (short event-free survival and short overall survival); those with t(11;14) displayed long survival; and patients with neither t(4;14) nor t(11;14) presented an intermediate outcome.[406] Patients lacking any 14q32 abnormality are essentially in a good prognostic group, whereas patients with t(4;14) or t(14;16) are mostly in a poor prognostic group.[403,1350]

t(4;14)

The t(4;14) occurs in 12–14% of MM patients,[304,400] and is frequently associated with del(13). In contrast to the frequent association between t(4;14) and del(13), t(4;14) and t(11;14) are never seen concurrently.[304] In a report by Garand et al, 17 out of 28 cases (61%) with t(4;14) exhibited plasma cells with a diffuse (immature) chromatin pattern; both t(4;14) translocation and immature morphology correlated with a higher incidence of high tumor mass and chromosome 13 abnormality.[1343] Patients with t(4;14) have significantly shorter progression-free survival and overall survival.[400] The t(4;14) is associated with a poor prognosis in MM patients despite intensive chemotherapy and autologous stem cell transplant. In a large series of patients, Chiecchio et al showed that the poor prognosis of t(4;14), like that of deletion 13, depends on the ability to obtain abnormal metaphase: there was no significant difference in survival between patients with a t(4;14) detectable only by interphase FISH and patients with no t(4;14) by cytogenetics.[1351]

t(9;14)

The t(9;14) and t(6;14) involve the *PAX5* and *IRF4* genes, respectively. Chromosome 8 at 8q24 (c-*MYC*) is another partner of *IGH* described occasionally in MM (although c-*MYC* appears to be dysregulated frequently in MM, it is only rarely translocated to the *IGH* locus).[430,1344] Avet-Loiseau et al reported c-*MYC* translocations in 13% of patients.[304]

t(11;14)

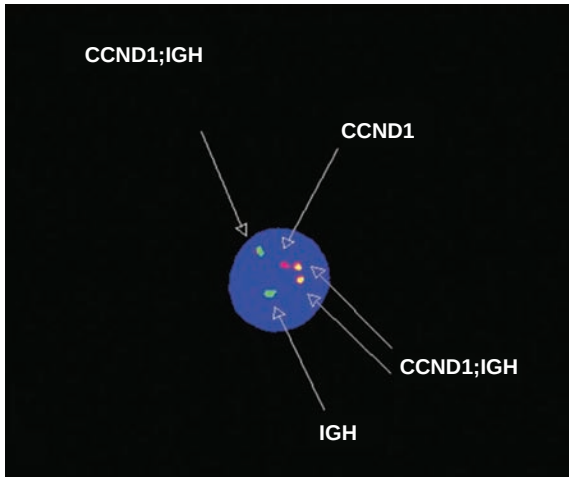
The t(11;14) occurs in ~20% of MM cases.[304] The t(11;14)(q13;q32) (Figure 3.35) results in upregulation of the *CCND1* gene (cyclin D1; *BCL1*) and is the most common translocation detected in MM.[683] The presence of this translocation correlates with lymphoplasmacytic morphology and/or small mature plasma cell morphology, frequent CD20 expression, a lower levels of serum monoclonal protein, a lower plasma cell labeling index, and less frequent appearance of a hyperdiploid DNA content.[683,1343,1352] Progression-free and overall survival are similar for patients with or without t(11;14).[400] In the series reported by Avet-Loisseau et al, the t(11;14) was associated with del(13) in 39% of patients.[304]

Deletion 17p

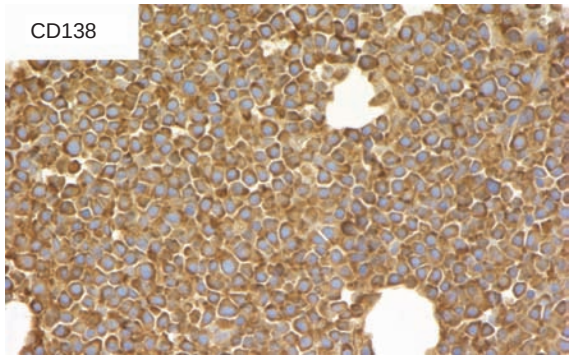
Deletion of 17p13 is seen in a subset of MM patients (11–15%).[267,304] This abnormality is associated with a poor outcome.[304] Chang et al reported a high incidence of *p53* deletions detected by FISH in CNS myeloma and suggested that it may be a marker for chromosomal instability, and may be associated with metastatic features of myeloma cells.[307] In that series, among 9 patients with CNS involvement by MM, 8 cases had hemizygous *p53* deletion and 4 had 13q deletions. Of the patients with 13q deletions, 2 had *IGH* translocations, 1 involving 4p16.3 (*FGFR3*), the other involving 16q23 (c-*MAF*).[307]

Complex abnormalities

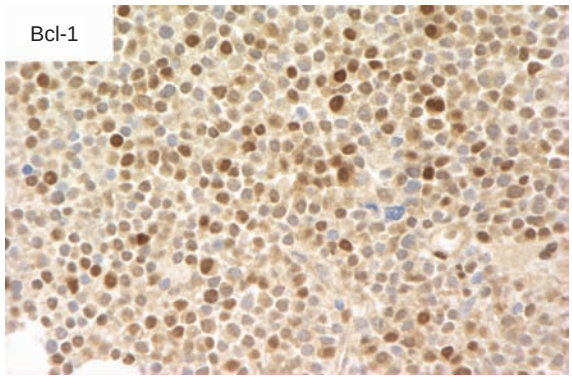
Complex chromosomal abnormalities (Figure 3.36) are associated with poorer prognosis. There is a distinct correlation between the number of aberrations



A



B



C

Figure 3.35 Multiple myeloma with t(11;14). A, FISH; B, immunohistochemical staining for CD138 (B) and cyclin D1/bcl-1 (C)

per patient and the tumor stage. In a study by Schmidt-Wolf et al, >50% patients with stage I disease had 0–3 aberrations, 47% of patients had 4–7 aberrations, and there were no stage I patients with 8 or more aberrations, whereas the proportion of MM patients with 8–12 aberrations increased from 16% in stage II to 26% in stage III.[267] There are possible differences in chromosomal aberrations between younger and older patients. Breaks in chromosomes 1p13, 6q21, and 11q13 are more common in the younger age group, whereas older patients more often show a loss of chromosome Y as the sole abnormality or +5. Trisomies of chromosomes 6, 9, and 17 are associated with prolonged survival in MM. Deletions of chromosome 11q have been reported to predict poor outcome in MM patients. The comparison of overall survival between hyperdiploid and hypodiploid MM patients showed a highly significant difference, with hypodiploidy being associated with poor prognosis and shorter overall survival.[1327,1353]

Conventional cytogenetics vs FISH

Chiecchio et al compared results obtained by FISH [chromosome 13 deletion, hyperdiploidy, *p53*, t(14;16), t(14;20), t(6;14) and t(4;14)] with those obtained by standard cytogenetics.[1351] Their findings showed that abnormal metaphase cytogenetics is associated with a poor outcome and patients with FISH deletion 13 without abnormal cytogenetics had a similar outcome to those with no deletion 13 by FISH. Similarly, patients with 'poor prognostic' FISH markers such as t(4;14) or deletion of *p53* without abnormal metaphase cytogenetics had outcomes comparable to those without these poor prognostic markers.[1351] Abnormal metaphase cytogenetics reflects the ability of myeloma cells to survive without the presence of a supporting environment (stroma-independent), which typically is associated with advanced disease (in early disease myeloma cells are stroma-dependent and taking these cells out of their supporting microenvironment in the bone marrow will result in apoptosis).[32,1354]

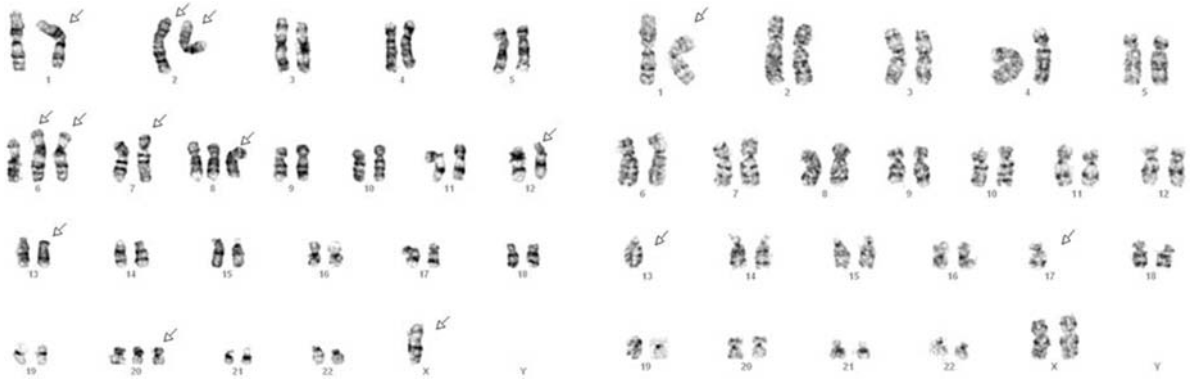


Figure 3.36 Multiple myeloma with complex chromosomal abnormalities including +6, t(1;6)(q21;21), add7(q32), +8, –17 and +20; cytogenetics (two metaphases)

Molecular markers

MM displays prominent genomic complexity as well as marked variation in clinical characteristics and patient survival. There appear to be two pathways involved in the pathogenesis of premalignant non-IgM MGUS and MM. Nearly half of tumors are nonhyperdiploid, and most have one of five recurrent *IGH* translocations: 16% 11q13 (*CCND1*), 3% 6p21 (*CCND3*), 5% 16q23 (*MAF*), 2% 20q12 (*MAFB*), and 15% 4p16 (*FGFR3* and *MMSET*).[1349,1355] The remaining hyperdiploid tumors have multiple trisomies involving chromosomes 3, 5, 7, 9, 11, 15, 19, and 21, and infrequently one of these five translocations. Translocation between the immunoglobulin heavy chain locus and *CCND1*, *CCND3*, c-*MAF*, *MAFB*, *FGFR3*, and *MMSET* represents recurrent genetic lesions in approximately 40% of MMs and, as a result of the juxtaposition of these genes to *IG* enhancer elements, hyperexpression of these genes is readily detectable in microarray studies.[904,1356,1357]

Comparative genomic hybridization showed the existence of marked differences in chromosomal imbalances between MM and plasma cell leukemia, which may help to explain the different clinical course of these disorders.[189,1358] Losses of chromosomal material are significantly more frequent in plasma cell leukemia than in MM.[1358] MM patients often show gains at chromosome 15q, 11q, 3q, 9q, and 1q, whereas all patients with plasma cell leukemia show gains in 1q.[1358] Activating mutations of Ki-*RAS* (but not N-*RAS*) represent an adverse prognostic factor in MM. Patients with *RAS* mutations had a median survival of 2.1 years; patients with wild-type *RAS* had a median survival of 4.0 years.[1359]

Based on five recurrent *IG* translocations and cyclin D expression, Hideshima et al divided MM patients into five categories: TC1 tumors (high levels of cyclin D1 or cyclin D3 as a result of an *IG* translocation); TC2 tumors (low to moderate levels of cyclin D1 despite the absence of a t(11;14) translocation); TC3 tumors (a mixture of tumors that do not fall into one of the other groups, with most expressing cyclin D2); TC4 tumors (high levels of cyclin D2, and also MMSET); and TC5 tumors (highest levels of cyclin D2, and also high levels of either c-MAF or MAFB).[1347] Patients with a t(4;14) translocation (TC4) had a shorter survival either with standard or high-dose therapy, patients with a t(14;16) (TC5) had a poor prognosis, and patients with a t(11;14) translocation (TC1) had better survival following aggressive therapy.[406,578,1341,1353,1360,1361]

Based on molecular analysis of 414 newly diagnosed patients who went on to receive high-dose therapy and tandem stem cell transplants Zhan et al

identified seven disease subtypes (MF, MS, CD-1, CD-2, HY, LB, and PR).[903] Positive t(14;16)(q32;q23) and t(14;20)(q32;q11), resulting in activation of c-*MAF* and *MAFB* proto-oncogenes, respectively, were present in 6% of cases (MF subgroup). The second group had a reciprocal t(4;14)(p16;q32) translocation, resulting in the hyperactivation of both the *FGFR3* and *MMSET* genes. The majority of cases with spiked *FGFR3* and *MMSET* expression clustered together, with *MMSET* being a dominant feature (MS subgroup; MMSET). While *FGFR3* and *MMSET* represent the top-ranked overexpressed genes in the MS group, other genes include the cadherin family member, desmoglein 2 (*DSG2*), Wnt receptors *FZD2* and *FZD8*, and the B-cell oncogene *PBX1*. Significant underexpressed genes in this group include *ICAM4*, N-cadherin (*CDH2*), cadherin 7 (*CDH7*), and the B-cell differentiation transcription factor *PAX5*.

Two cyclin D family members are activated by translocations in MM, cyclin D1 by the t(11;14)(q13;q32) in 17% (CD-1 group) and *CCND3* by t(6;14)(p21;q32) in 2% (CD-2 group). Relative to the other five groups, genes uniquely underexpressed in both the CD-1 and CD-2 subgroups include *IL6R*, *HOXB7*, *BMPR1A*, the mitotic cyclin, *CCNE*, and the cyclin-dependent kinase, *CDK6*. The biologic and clinical relevance of CD-1 and CD-2 groups is currently unclear, as there was no difference in clinical parameters or survival between the two groups. However, CD-2, but not CD-1 was associated with elevated expression of *CD20*, which has previously been shown to be associated with the t(11;14)(q13;q32) and highly correlated with CD20 protein expression in MM cells.[1362,1363]

Hyperdiploidy, most often associated with trisomies of chromosomes 3, 5, 7, 9, 11, 15, 19, and 21, represents one of two central genetic pathways in the development of MM, and this type of disease has been previously shown to have a distinct gene expression signature.[1349,1355] The presence in nearly 60% of a hyperdiploid signature (HY group) was associated with hyperdiploid karyotypes in more than 90% of the cases. Hyperdiploidy is a distinct genetic entity with a good prognosis and is largely devoid of common recurrent Ig-mediated translocations.

The HY MM subgroup was mainly characterized by overexpressed genes derived form the odd number chromosomes 3, 5, 7, 9, 11, 15, 19, and 21; however, this signature was also observed in nonhyperdiploid cases. The assignment of both hyperdiploid and nonhyperdiploid cases to the proliferation (PR) group suggests that a simple recognition of hyperdiploidy is insufficient for proper risk assessment: those with a proliferation signature and hyperdiploidy would be at higher risk than those with an HY signature alone. The LB (low bone disease) group, characterized by a low number of MRI-defined focal bone lesions, lacked any clear genetic signatures distinguishing it from other groups. The LB group had an elevated expression of endothelin 1 (*EDN1*), which has been implicated in inducing the osteoblastic phenotype of prostate cancer metastases.

The 7th group was characterized by the overexpression of numerous cell cycle- and proliferation-related genes (e.g., *CCNB2*, *CCNB1*, *MCM2*, *CDCA2*, *BUB1*, *CDC2*, and *TYMS*) and cancer-testis antigen genes (e.g. *MAGEA6*, *MAGEA3*, *GAGE1*, and *GAGE4*), and also had a significantly higher gene expression-defined proliferation index than the other group, justifying its designation as the PR group.

Dysregulated expression of one of the three cyclin D genes (*CCND1*, *CCND2*, or *CCND3*) is a feature of virtually every case of newly diagnosed MM.[1355] Hyperactivated expression of *CCND1* and *CCND2* was seen in more than 95% of cases in the study of Zhan et al, but relative levels and distribution varied across the subgroups.[903] Expression of one of the three cyclin D genes is mutually exclusive, and Zhan et al, noticed a trend for *CCND2* to be expressed in the PR, LB, and MS groups (an occasional *CCND1* spike in the PR group may reflect MM progression), and it was expressed at the highest level in the MF group.[903] The *CCND1* and *CCND3* genes exhibited mutually exclusive spiked expression in the CD-1

and CD-2 groups. With a 36-month median follow-up, the HY, CD-1, CD-2, and LB subgroups were associated with superior event-free and overall survival relative to the PR, MS, and MF groups.[903]

PERIPHERAL (MATURE) T-CELL LYMPHOPROLIFERATIVE DISORDERS

T-cell lymphomas are a diverse group of lymphoid neoplasms manifesting heterogeneous clinical, histologic, immunophenotypic, and cytogenetic features.[1364–1371] T-cell lymphomas represent approximately 10% of all non-Hodgkin's lymphomas in Western countries. The classification of T-cell lymphoma is based largely on the histomorphologic features and clinical parameters. A predominantly nodal distribution is characteristic for angioimmunoblastic T-cell lymphoma, peripheral T-cell lymphoma, unspecified (PTCL), and ALCL. Extranodal location is seen in mycosis fungoides (MF), cutaneous ALCL, extranodal NK/T-cell lymphoma (nasal type), enteropathy-type T-cell lymphoma, hepatosplenic γδ T-cell lymphoma, and subcutaneous panniculitis-like T-cell lymphoma. The diagnosis of T-cell proliferations may be difficult, especially at the early stages of the disease and in certain types of lymphomas (e.g. angioimmunoblastic T-cell lymphoma). Detection of clonal rearrangements of T-cell receptor loci γ, δ, and β (*TCRG, TCRD, and TCRB*) by PCR may be very helpful in the diagnostic process (*see* Chapter 2). Using BIOMED-2 multiplex PCR, *TCR* clonality was detected in 99% (143/145) of all definite cases of T-cell prolymphocytic leukemia, T-cell large granular lymphocytic leukemia, peripheral T-cell lymphoma (unspecified), and angioimmunoblastic T-cell lymphoma, whereas 9 of 43 ALCLs did not show clonal *TCR* rearrangements.[908]

The presence of human T-cell lymphoma virus type 1 (HTLV-1) defines adult T-cell leukemia/lymphoma (ATLL). Detection of ALK-1 by immunohistochemistry or $t(2;5)^{ALK/NPM}$ by FISH helps to diagnose ALCL. Other chromosomal abnormalities present in specific T-cell lymphoproliferations include isochromosome 7q typical for hepatosplenic T-cell lymphoma (often with trisomy 8) and inversion of chromosome 14 often seen in T-PLL.

The majority of T-cell lymphoproliferative disorders are aggressive, with only MF and cutaneous ALCL frequently following an indolent course. The response to conventional therapy is generally poor. New treatment modalities, including purine analogs, monoclonal antibodies (Campath), and stem cell transplantation, offer improved response rates and remission.[1372] Three distinct prognostic subgroups can be distinguished on survival analysis: favorable (cutaneous ALCL), 5-year overall survival 78%; intermediate (PTCL, ALCL, and angioimmunoblastic T-cell lymphoma), 5-year overall survival 35–43%; and unfavorable (NK/T-cell lymphoma, nasal type, and enteropathy-type T-cell lymphoma), 5-year overall survival 22–24%.[1364] Most of the pretreatment characteristics, including IPI risk groups, are not significantly different between B-cell and T-cell lymphomas. The rates of complete remission (71% vs 54%) and progressive disease (39% vs 63%) significantly favored patients with B-cell lymphoma. Patients with T-cell lymphoma have a poorer response to therapy and shorter survival than comparable patients with aggressive B-cell lymphoma.[1373–1377]

T-cell prolymphocytic leukemia

T-PLL is a rare mature (post-thymic) T-cell lymphoproliferative disorder which affects middle-aged to elderly adults, occurs more frequently in men, and is characterized by an aggressive clinical course and poor outcome.[1378–1381] T-PLL is composed of small to medium-sized lymphocytes with prominent nucleoli (Figure 3.37). The principal disease characteristics are organomegaly (especially splenomegaly and lymphadenopathy), anemia, thrombocytopenia, skin lesions, and prominent (often rapidly increasing) lymphocytosis in blood (most often $>100 \times 10^9$/l). A subset of patients experiences an initial (median 33 months, range: 6–103 months) indolent clinical

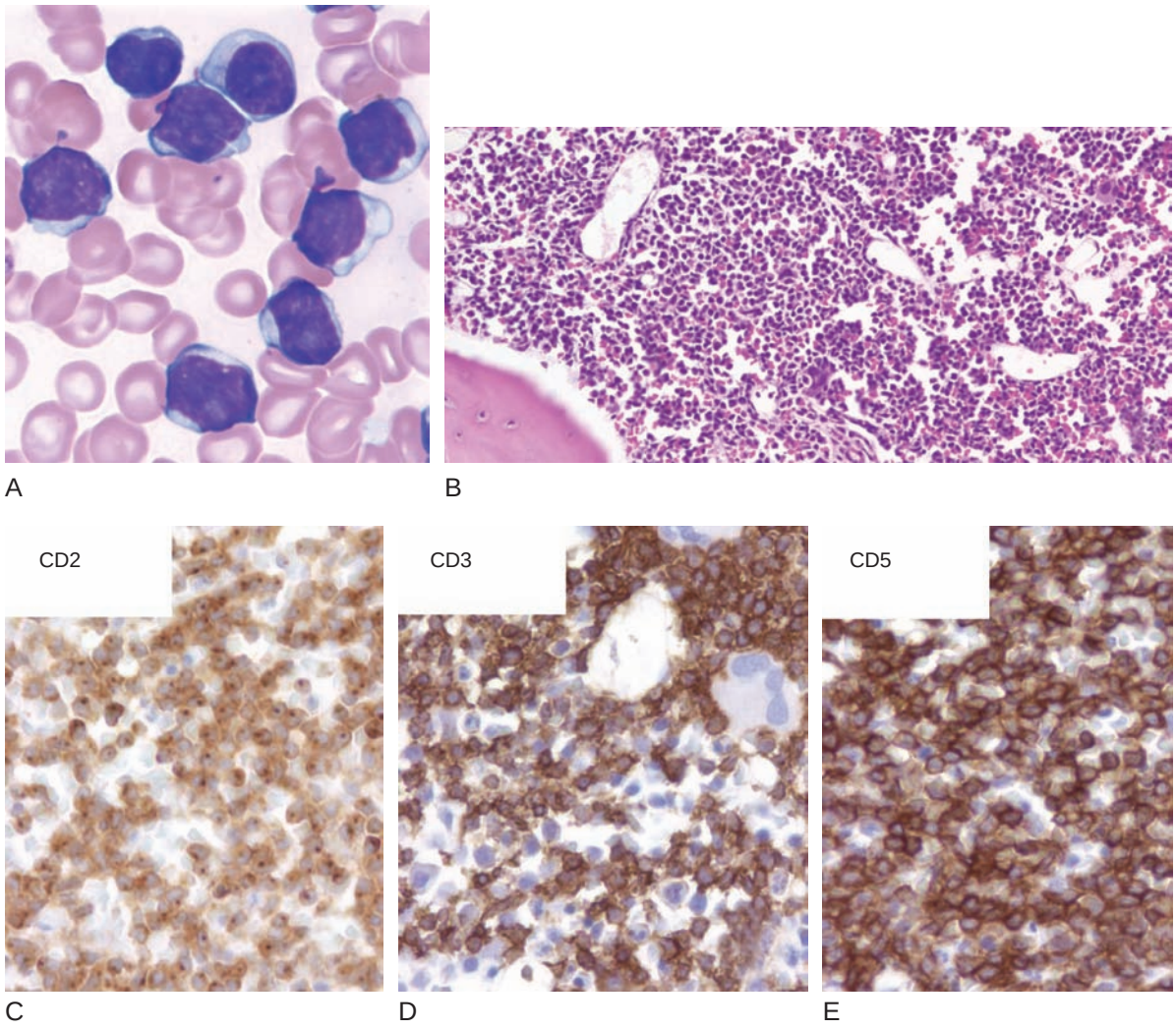


Figure 3.37 T-cell prolymphocytic leukemia. A, cytology; B, histology; C–E, immunohistochemistry. Tumor cells have irregular nuclear outlines and nucleoli (A). Bone marrow is diffusely infiltrated by leukemic cells (B), which express CD2 (C), CD3 (D), and CD5 (E)

course with stable moderate leukocytosis, with subsequent progression to the aggressive stage.[1382]

The disease is genetically characterized by the presence of complex karyotypes with recurrent alterations involving chromosomes 8, 11, and 14 (Figure 3.38) including inv(14)(q11;q32), t(14;14)(q11;q32), and i(8)(q10).[1378] The translocations involving chromosome 14 juxtapose the locus of the *TCRAB* gene with the *TCL1* and *TCL1b* genes at 14q32. Chromosome 14 abnormalities are often accompanied by a complex karyotype (Figure 3.38). Comparative genomic hybridization (CGH) of T-PLL showed that chromosomal regions most often overrepresented were 8q (75%), 5p (62%), and 14q (37%), as well as 6p and 21 (both 25%), while the chromosomal regions most often underrepresented were 8p and 11q (75%), 13q (37%), and 6q, 7q, 16q, 17p, and 17q (25%).[1383]

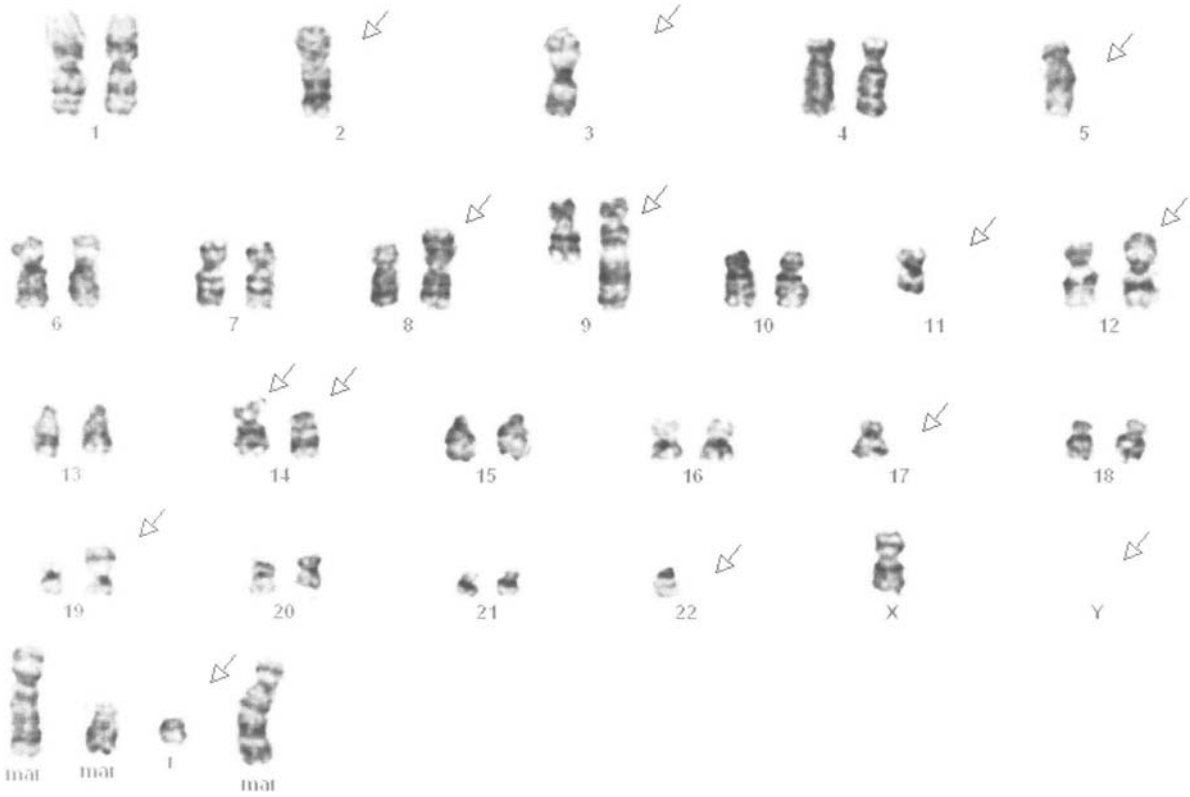


Figure 3.38 T-PLL with complex karyotype: -2, -3, -5, add(6)(q13), +del(6)(q15), t(8;8)(p11.2;q12), add(9)(q34), –11, add(11)(q23), -12, add(12(p13), add(14)(p11.2), inv(14)(q11.2q32), -17,add(19p), and -22; cytogenetics

T-large granular lymphocyte leukemia

T-large granular lymphocyte (T-LGL) leukemia is a clonal disorder of $CD3^+$ cytotoxic T-cells (large granular lymphocytes, Figure 3.39) characterized by indolent course, lymphocytosis, and cytopenia(s). They show predominantly $CD4^-/CD8^+$ phenotype (66%) or $CD4^-/CD8^-$ phenotype (33%), are positive for surface CD3, and variably positive for CD16 (45%), CD56 (45%), and CD57 (86%).[1384] Coexpression of CD56 and CD57 is seen in 43% of patients, while 41% of cases show the CD16/CD57 phenotype. Patients are asymptomatic (28%) or experience fatigue (60%), B-symptoms (12%), recurrent infections (15%) and mouth ulcers.[1385,1386] The median age is about 60 years (range: 4–88 years).[1386] T-LGL leukemia involves the peripheral blood, bone marrow, liver, and spleen. It has an indolent clinical course and may be associated with neutropenia, red cell aplasia, hypergammaglobulinemia, and rheumatoid arthritis. Neutropenia is reported in 85% of patients (severe neutropenia, $<0.5 \times 10^9$/l is seen in 50%).[1387] Splenomegaly is common, whereas lymphadenopathy is rare. In the bone marrow, T-LGLs most often display a subtle interstitial infiltrate present in approximately 70% of cases.[1388] Apart from rheumatoid arthritis, T-LGL leukemia may be associated with other systemic autoimmune diseases as well as hematologic malignancies, such as paroxysmal nocturnal hemoglobinuria, MDS, AML, and B-cell lymphoproliferative disorders. Both methotrexate and cyclosporin A are efficacious in the treatment

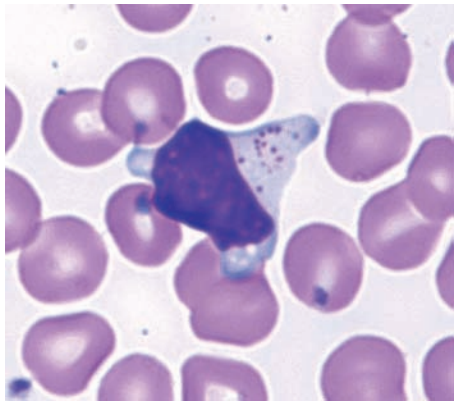


Figure 3.39 T-large granular lymphocyte leukemia, blood

of T-LGL leukemia, but generally require long-term maintenance therapy.[45,1389,1390] Cytogenetic information on T-LGL is limited. Individual cases with inv(7)(p15q22), inv(14)(q11q32), del(6q), del(1)(p32), t(11;12)(q12;q11), t(3;5)(p26;q13), and t(8;14)(q24;q32) have been described.[1391–1395]

Adult T-cell lymphoma/leukemia

Adult T-cell lymphoma/leukemia (ATLL) is a peripheral (mature/post-thymic) T-cell lymphoproliferative disorder caused by the retrovirus human T-cell leukemia virus (HTLV-1).[1396,1397] Tumor cells have prominent nuclear irregularities, often referred to as *cloverleaf* or *flower* cells (Figure 3.40). ATLL affects adults with leukemic or subleukemic presentation, cutaneous involvement, lymphadenopathy, and/or organomegaly. Hypercalcemia is present in up to 50% of patients and may result in renal failure. Patients are at increased risk for bacterial and viral infections. Several clinical variants of ATLL are recognized: chronic, smoldering, acute, and lymphomatous.[1398] Chronic and smoldering variants are clinically indolent, whereas acute and lymphomatous variants show a highly aggressive clinical behavior and a very poor prognosis (median survival 9–10 months), mostly due to chemoresistance and severe immunosuppression.[1398–1402] The 4-year survival rate is 66% for the smoldering type, 27% for the chronic type, and 5–6% for the lymphoma and acute types.[1403,1404] Indolent variants of ATLL can progress to very aggressive variants, occasionally referred to as 'crisis variants'.[1405] On the other hand, spontaneous regression can also be observed in sporadic cases.[1403,1404]

Cytogenetic analysis of ATLL shows complex and miscellaneous genomic alterations. Kamada et al studied 107 patients with ATLL and reported recurrent cytogenetic abnormalities, including gains of chromosomes 3, 7, and 21, and various losses.[169] Clonal chromosome abnormalities were found in 96% of cases, including hypotetraploidy (4 cases), trisomies for chromosomes 3 (21%), 7 (10%), and 21 (9%), monosomy for X chromosome (38%) in

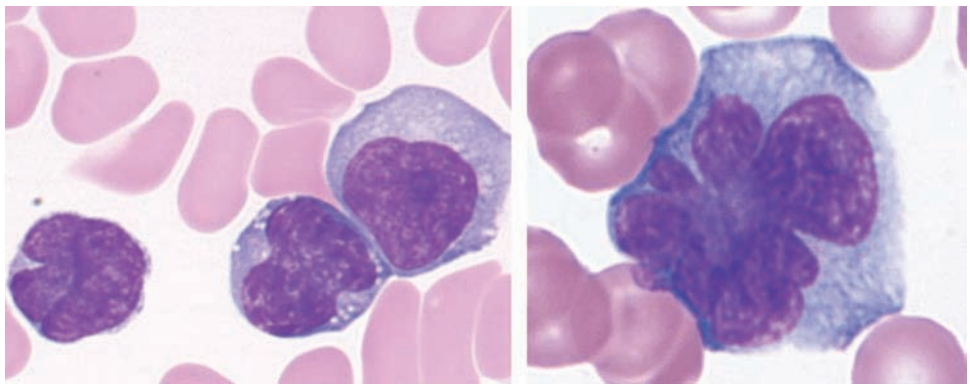


Figure 3.40 Adult T-cell leukemia/lymphoma, blood. Atypical lymphocytes with characteristic nuclear irregularities

females, and loss of a Y chromosome (17%) in male. Of 373 structural abnormalities in all 103 aneuploid cases, translocations involving 14q32 (28%) or 14q11 (14%) and deletion of 6q (23%) were most frequent, followed by deletion of 10p (9%), 3q (8%), 5q, 9q, and 13q (7% each), and 1p and 7p (6% each). The combination of rearrangement of 14q32 and monosomy X (7 cases) or deletion of 10p (6 cases), trisomy 3, and deletion in 6q21 (6 cases), occured only in the acute or lymphoma types and likely was responsible for the aggressiveness of ATLL.[169] Tsukasaki et al analyzed 46 patients with aggressive ATLL by CGH and reported frequent gains at 3p, 7q, and 14q, and losses at 6q and 13q.[1406] Aggressive ATLL showed more frequent genomic aberrations than did indolent types. Analysis of aggressive forms of ATLL by Oshiro et al, showed that the lymphoma type had significantly more frequent gains at 1q, 2p, 4q, 7p, and 7q and losses of 10p, 13q, 16q, and 18p, whereas the acute type showed a gain of 3/3p.[1407]

Peripheral T-cell lymphoma, unspecified

PTCL is a heterogeneous group of tumors with variable clinical features, histology (Figure 3.41), genetic alteration, response to treatment, and prognosis. PTCL is characterized by pleomorphic cytomorphology with an admixture of inflammatory cells including histiocytes and eosinophils. It occurs at any age, usually in older patients, and is most often a nodal disease, but any other sites including blood, bone marrow, bone, omentum, skin, CSF, soft tissues, gastrointestinal tract, spleen, liver, and lung may be involved. Histologic examination shows either a prominent paracortical infiltrate (T-zone variant) or diffuse infiltration with effacement of the normal lymph node architecture. Although $CD4^+$ lymphomas predominate (~66%), dual negative expression of CD4/CD8 is observed in a significant proportion of tumors (~20%); the remaining cases are either $CD8^+$ (~12%) or dual $CD4/CD8^+$ (~4%).[38,147]

Despite aggressive therapy the prognosis is dismal, with more than half of the patients dying of their disease.[1408] The estimated overall survival is 41–49% at 5 years.[1369,1371,1409] The IPI is a significant prognostic factor for both progression-free and overall survival.[1409–1412] Besides IPI, systemic symptoms and bone marrow infiltration have been found to correlate with prognosis.[1411]

Specific chromosomal translocations are unknown in PTCL. Based on CGH, frequent changes in PTCL include gains of 17q (17q11–q25), 8 (involving the

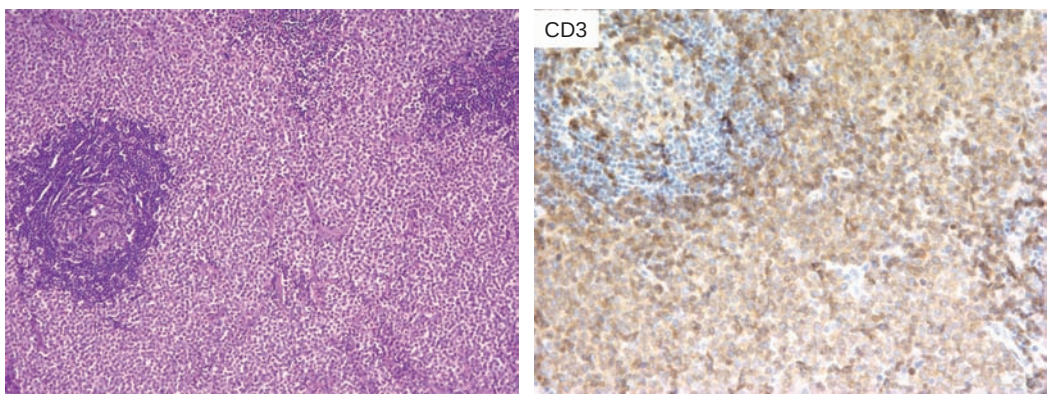


Figure 3.41 Peripheral T-cell lymphoma, unspecified with prominent T-zone pattern. A, histology; B, immunostaining with CD3

MYC locus at 8q24), 11q13, and 22q and losses of 13q, 9(9p21–q33), (6q16–q22), and 11p11.[1413] Streubel et al identified the presence of a recurrent t(5;9)(q33;q22), leading to breakpoints in the *ITK* and *SYK* genes in a subset of PTCL.[1414] Lakkala-Paranko et al described trisomy 3 in three cases of PTCL.[1415] Lepretre et al reported cytogenetic findings in 71 untreated patients with peripheral T-cell lymphoma: 57 patients (80.3%) had abnormal clones, whereas 9 karyotypes (12.7%) showed only normal metaphases; 5 karyotypes (7%) could not be analyzed.[1416] Recurrent numeric chromosomal abnormalities included +3 (21%), +5 (15.7%), +7 (15.5%), +21 (14%), −13 (14%), +8 (12.2%), +19 (12.2%), −10 (10.5%), and −Y (9% of male patients) and chromosomes involved in structural rearrangements included chromosome 6 (31.5%), mainly due to 6q deletions (19.2%), 1q (22.8%), 7q (22.8%), 9p (19.4%), 9q (19.2%), 4q (19.2%), 3q (19.2%), 2p (17.5%), 1p (17.5%), and 14q (17%) (trisomies 3 and 5 mainly correlated with angioimmunoblastic T-cell lymphoma, while isochromosome 7q, associated with trisomy 8, was present in two cases of hepatosplenic gamma/delta T-cell lymphoma).[1416] Deletions in 6q, total or partial trisomies of 7q, and monosomy 13 or changes of 13q14 are more frequent in high-grade than in low-grade T-cell lymphomas.[1417]

Angioimmunoblastic T-cell lymphoma

Angioimmunoblastic T-cell lymphoma (AILT) is a mature T-cell lymphoma characterized by a sudden onset of constitutional symptoms and lymphadenopathy. Morphologically it is characterized by the effacement of lymph node architecture by a polymorphous infiltrate that includes clusters of atypical lymphoid cells with clear cytoplasm, an admixture of small lymphocytes, histiocytes, eosinophils, and plasma cells, a marked increased in the number of arborizing vessels, and scattered EBV^+ B-immunoblasts (Figure 3.42). Patients present with generalized adenopathy, and B-symptoms, and often have hepatosplenomegaly, skin rashes and pruritus, polyclonal hypergammaglobulinemia, autoantibodies, thrombocytopenia, or hemolytic anemia. Patients with AILT have an aggressive clinical course and a poor prognosis with conventional treatment (corticosteroid and immunosuppressive agents).

The most common chromosomal changes reported in AILT include trisomy 3, trisomy 5, and an additional copy of chromosome X.[1416–1418] CGH analysis showed frequent gains of 22q, 19, and 11p11–q14 (11q13), and losses of 13q.[1413] Similarly to PTCL, recurrent gain of 11q13 can also be present in AILT.[1413] Schlegelberger et al reported a high frequency of oligoclonal proliferations in AILT: 17 of 36 patients (47%) had unrelated clones.[1419] The majority of cases are positive for *TCR* gene rearrangement, and a subset of cases (~10%) also shows clonal rearrangement of the immunoglobulin gene.[1420–1423] Using multiplex PCR assays based on the BIOMED-2 collaborative study, Tan et al detected TCRγ T-cell clones in 78 and 81% of AILT and PTCL cases, respectively, and *IGH* B-cell clones in 34 and 35% of AILT and PTCL cases, respectively.[1424] The majority of cases contained EBV-positive cells, including 50% of AILT and 57% of PTCL cases, and cases with B-cell proliferations were more often EBV-positive.[1424] Attygalle et al reported significantly higher detection of T-cell clonality in angioimmunoblastic T-cell lymphoma (90%) compared with PTCL (59%),[1425] but Bruggeman et al detected TCR clonality in 99% of all definite cases of PTCL (unspecified) and AITL.[909]

Hepatosplenic gamma/delta T-cell lymphoma

Hepatosplenic γδ T-cell lymphoma is a disease with distinct clinical, morphologic and phenotypic characteristics with poor prognosis.[1426–1430] Hepatosplenic T-cell lymphoma is an aggressive tumor of cytotoxic T-cells involving the liver, spleen, and bone marrow. It is characterized by an intrasinusoidal distribution of tumor cells. Patients present with hepatosplenomegaly and pancytopenia. The tumor cells are positive for

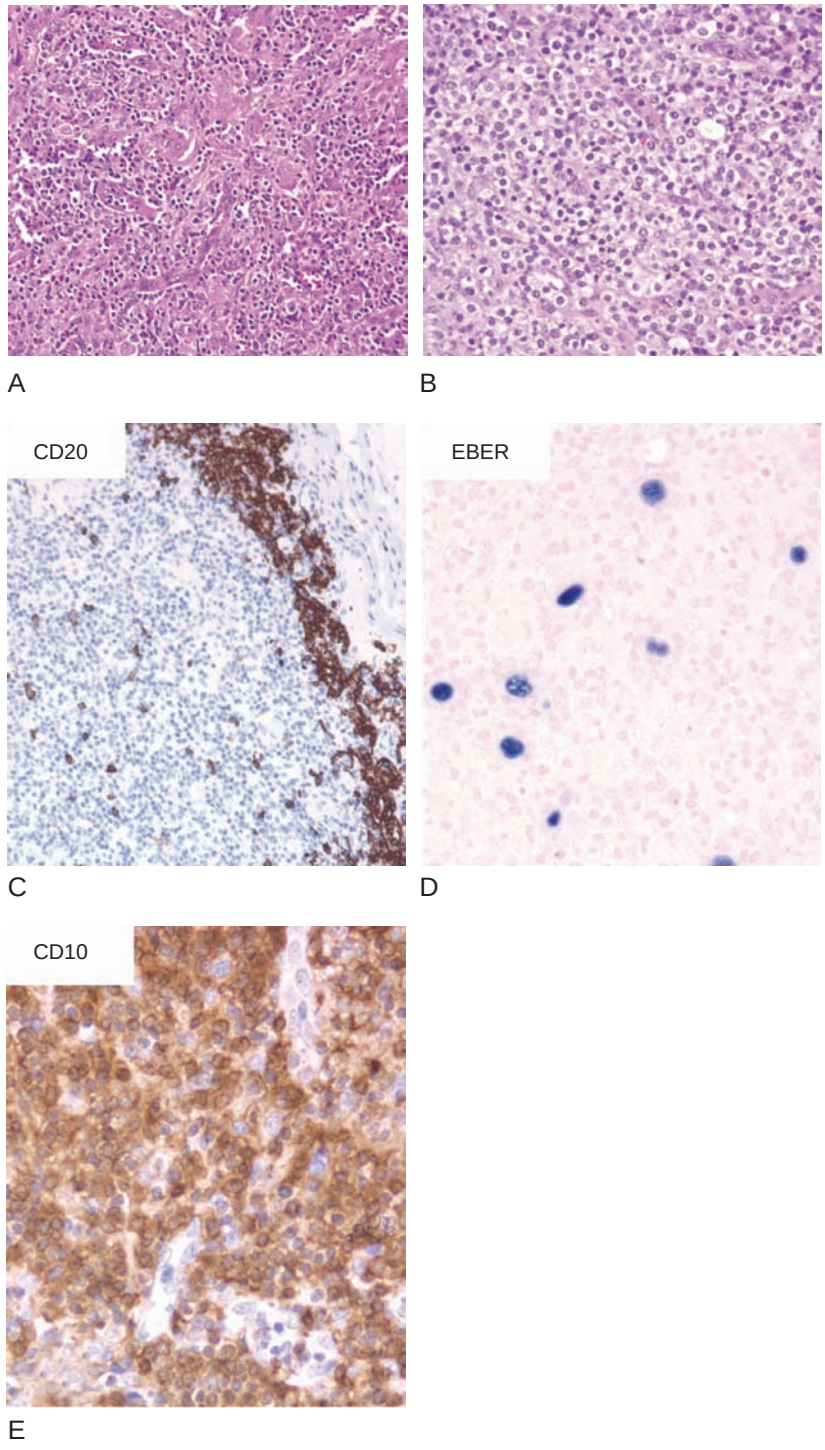


Figure 3.42 Angioimmunoblastic T-cell lymphoma. Histology (A) shows pleomorphic lymphoid infiltrate with increased vascularity. Higher magnification (B) shows atypical T-cells with abundant clear cytoplasm. B-cells (C) visualized by CD20 immunostaining are present only at the periphery of the lymph node. Rare EBV-positive B-immunoblasts are present (D; EBER staining). Lymphomatous cells show aberrant expression of CD10 (E)

CD3, CD45, and TCRγδ,[38,345,1368,1426,1427,1429,1430] but rare cases of hepatosplenic T-cell lymphoma TCRαβ+ have been reported.[1431–1434] NK-cell associated antigens are variably expressed: CD16 is positive in approximately one-quarter of cases, CD56 is positive in the majority of cases, and CD57 is negative.[38,147,1368] The median survival time is 16 months.[345] Figure 3.43 presents morphologic features of hepatosplenic lymphoma. Hepatosplenic γδ T-cell lymphoma has been associated cytogenetically with isochromosome 7q [i(7)(q10)].[346–348,1434] An increased number of 7q signals was found in three cases with cytologic features of progression, indicating a tendency of hepatosplenic lymphoma to multiply the i(7)(q10) chromosome during evolution.[348] Tamaska et al described hepatosplenic γδ T-cell lymphoma with ring chromosome 7 as an equivalent of isochromosome 7q aberration.[1435]

Enteropathy-type T-cell lymphoma

Enteropathy-type T-cell lymphoma is a large cell lymphoma with a cytotoxic T-cell phenotype occuring most commonly in the small intestine, but it may spread along the mucosa to the large intestinum and/or duodenum and stomach (Figure 3.44). Patients either have a history of celiac disease or present with the symptoms of celiac disease at the time of diagnosis.[1436] Refractory celiac sprue is strongly associated with partial trisomy of the 1q region, which may contribute to lymphomatous transformation.[1437] Involvement of sites other than jejunum or ileum, including liver, stomach, duodenum or bone marrow, is rare. Intestinal perforation is a common complication. The clinical course is aggressive. Clonal rearrangements of T-cell receptors are present and often they are similar to those found in an adjacent mucosa.

Enteropathy type T-cell lymphoma is characterized by frequent complex gains of 9q31.3 (70% of cases), or by an almost mutually exclusive 2.5 megabase loss of 16q12.1 (23% of cases).[1438] FISH analysis using a probe for 9q34 shows frequent and multiple gains of chromosomal material at 9q34 (up to nine signals per case).[1439] Gain of chromosome 1q can be found in ~15% of enteropathy-type T-cell lymphomas. In a series reported by Zettl et al, CGH revealed chromosomal imbalances in 87% of cases analyzed, with recurrent gains of genetic material involving chromosomes 9q (in 58% of cases), 7q (24%), 5q (18%), and 1q (16%).[1439] Recurrent losses of genetic material occured on chromosomes 8p and 13q (24% each), and 9p (18%).[1439] Deleeuw et al, using whole genome analysis, recognized two distinct groups of enteropathy T-cell lymphoma: type 1 linked pathogenetically to celiac disease, characterized by pleomorphic cytomorphology, CD56 negativity, and chromosomal gains of 1q and 5q, and type 2 with monomorphic small- to medium-sized tumor cell morphology, frequent CD56 expression, c-*MYC* oncogene locus gain, and rare gains of chromosomes 1q and 5q.[1438]

NK/T-cell lymphoma, nasal type

This is an aggressive lymphoma of the upper respiratory tract, especially the nasal cavity (Figure 3.45), although it may occur at different locations, including skin and subcutaneous tissue. Among T-cell lymphomas, nasal-type NK/T-cell lymphoma and enteropathy-type T-cell lymphoma belong to the most aggressive tumors, with a worse prognosis than for peripheral T-cell lymphoma, unspecified. Estimated overall survival at 5 years for extranodal NK/T-cell lymphoma, nasal type, ranges form 0%[1440] to 45%.[1441] Takahara et al showed that high LDH levels, large cells, immunoblastoid polymorphous histology, and *p53* missense mutations were significantly related to worse cause-specific survival in nasal NK/T-cell lymphoma.[1442] Multivariate analysis showed that *p53* missense mutation was the most independent among these three factors.

Chromosomal abnormalities are commonly found in these tumors, and aberrations involving chromosome 6q are most frequent. Other non-random abnormalities include +X, i(1q), i(7q), +8, del(13q), del(17p), i(17q), and 11q23 rearrangement.[195,1443]

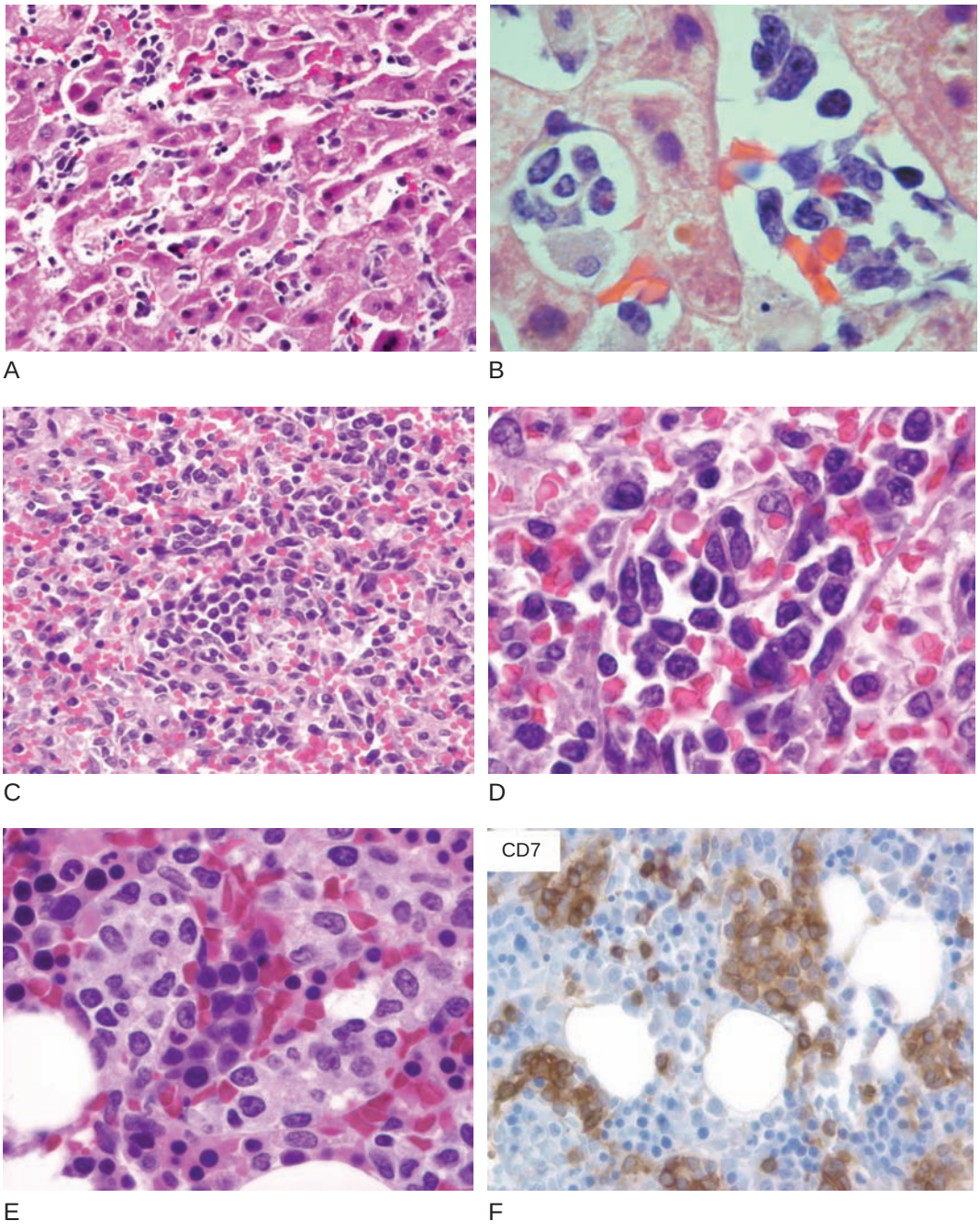


Figure 3.43 Hepatosplenic T-cell lymphoma involving liver (A, B), spleen (C, D) and bone marrow (E, F). Note prominent intrasinusoidal pattern of involvement (A–E, H&E; F, immunostaining with CD7)

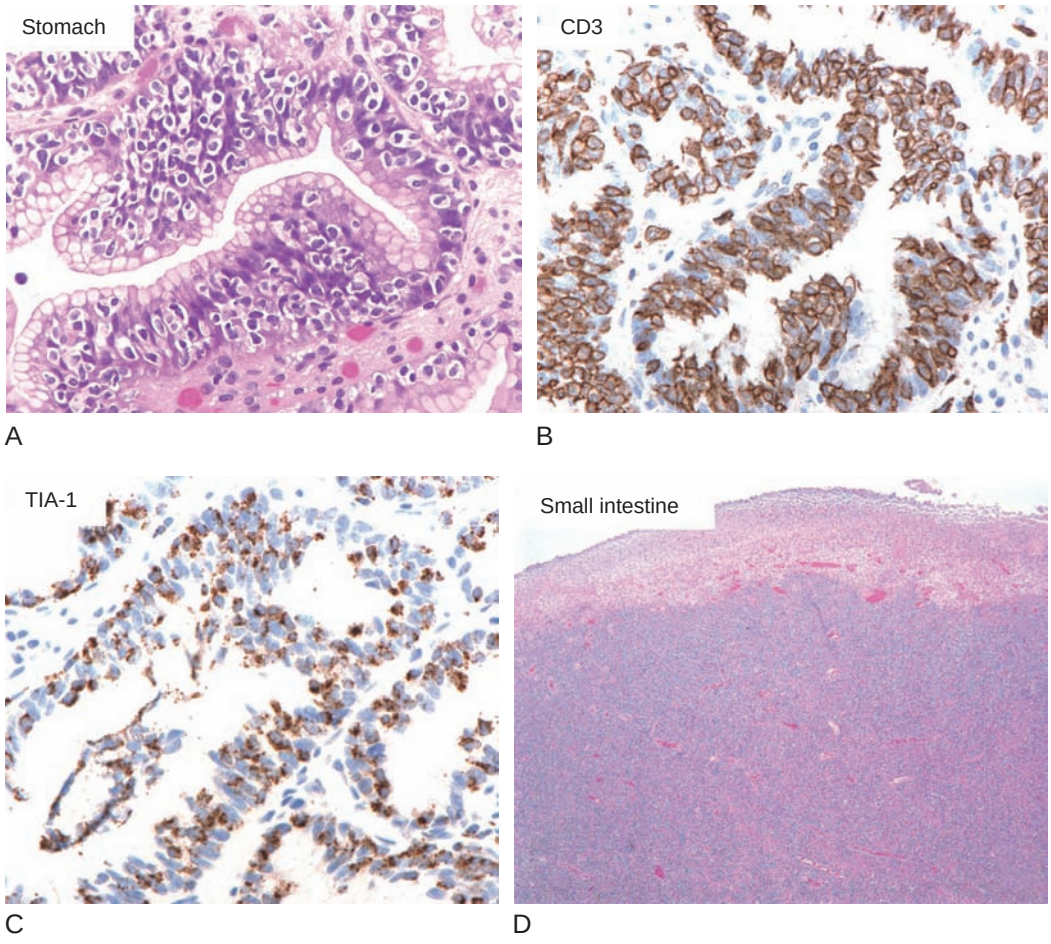


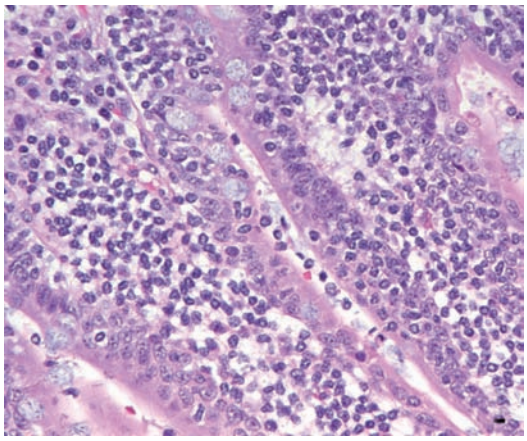
Figure 3.44 Enteropathy-type T-cell lymphoma with involvement of stomach (A–C), small intestine (main tumor mass; D–F) and large intestine (G–I). Neoplastic cells display prominent tropism to epithelium, invading glands (A, E, H; H&E sections). The lymphomatous cells are positive for CD3 (B), TIA-1 (C), and CD2 (F). Cytokeratin staining (I) shows prominent intraepithelial lymphocytosis with destruction of normal glandular structures

Nakashima et al showed recurrent regions characteristic of the extranodal NK/T lymphoma, nasal-type, compared with those of the aggressive NK-cell leukemia: gain of 2q, and loss of 6q16.1–q27, 11q22.3–q23.3, 5p14.1–p14.3, 5q34–q35.3, 1p36.23–p36.33, 2p16.1–p16.3, 4q12, and 4q31.3–q32.1.[1444]

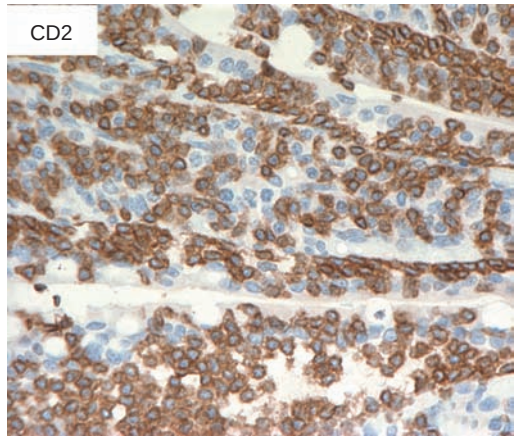
Mycosis fungoides

Mycosis fungoides (MF; Figure 3.46) is a neoplastic proliferation of small to medium-sized $CD4^+$ T-lymphocytes with cerebriform nuclei characterized by multifocal disease and a protracted clinical course, except for cases with large cell transformation.[1268, 1445–1449] At diagnosis, most MF patients present with early stage disease and a high probability of long-term survival. MF is the most common type of cutaneous T-cell lymphoma and accounts for almost 50% of all primary lymphomas involving skin. MF typically affects older adults (median age at diagnosis 50–60 years), but may occur in children and adolescents.

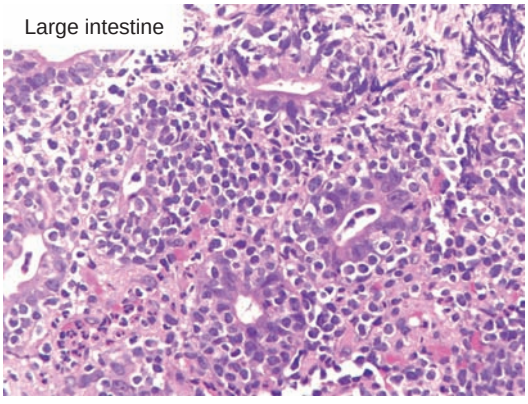
Chromosomal abnormalities, mostly complex, are seen in about 50% of patients with MF/Sézary's



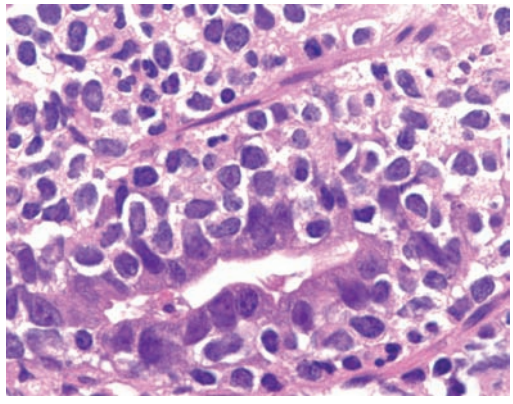
E



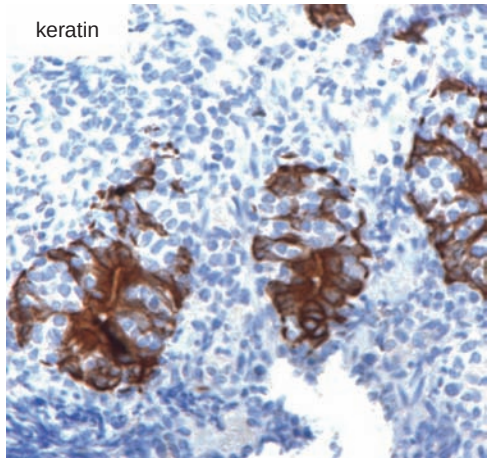
F



G



H



I

Figure 3.44, cont'd

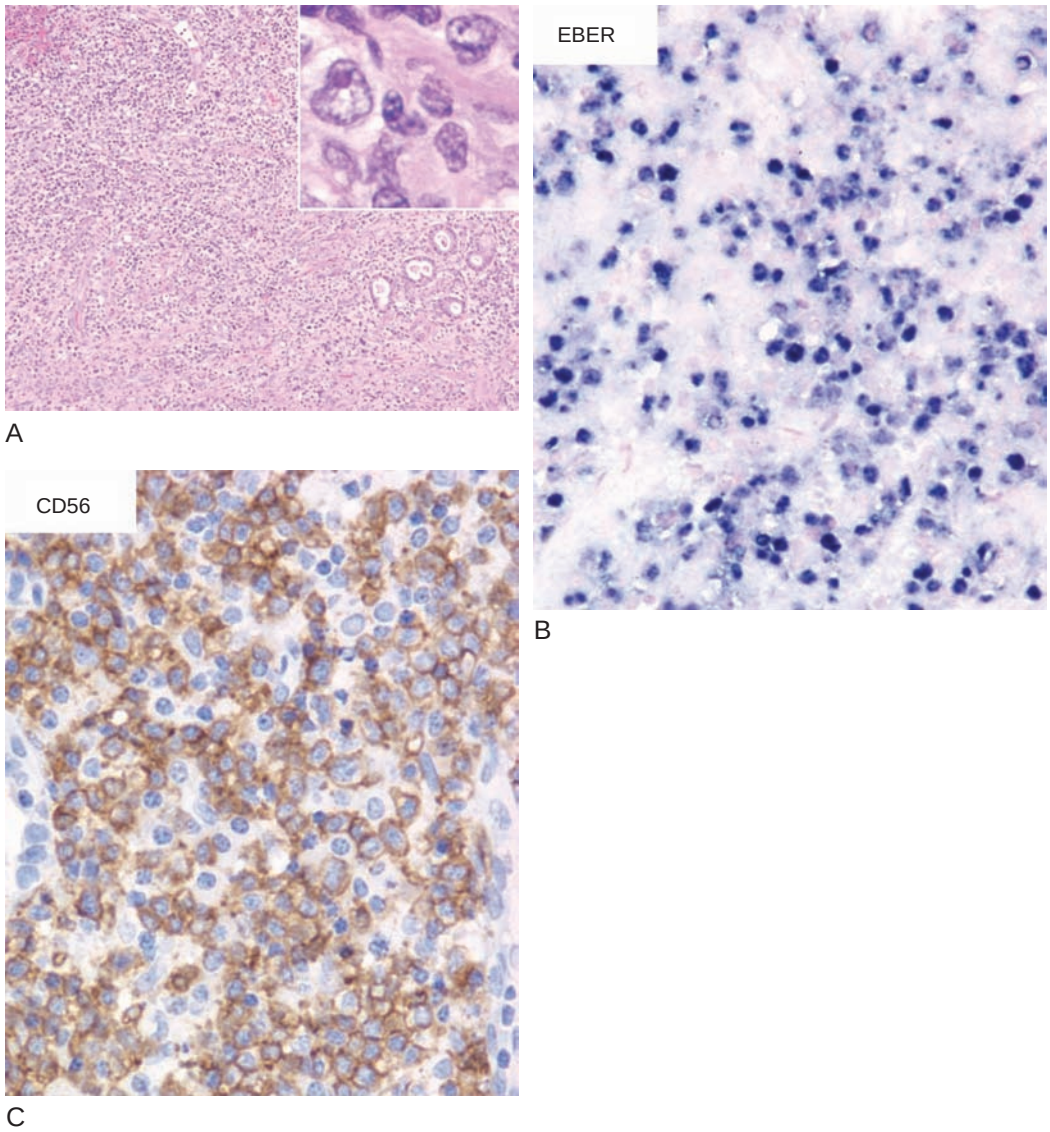


Figure 3.45 Extranodal NK/T-cell lymphoma, nasal type. The biopsy shows diffuse lymphoid infiltrate within nasal mucosa (A; *inset*: pleomorphic lymphoid cells). Lymphomatous cells are positive for EBER (B) and CD56 (C) by immunohistochemistry

syndrome (SS), but recurrent rearrangements are rare. Batista et al revealed an abnormal karyotype in 9 of 19 (47%) blood samples from patients with MF/SS.[1450] The most frequent abnormalities, in 7 of 9 cases, involved chromosome 10, followed by chromosome 6, in 6 of 9 cases; chromosomes 3, 7, 9, 17, and 19, in 5 of 9 cases; chromosomes 1 and 12, in 4 of 9 cases; and chromosomes 8, 11, and 13, in 3 of 9 cases.[1450] In the same series, recurrent rearrangements included deletion of chromosomes 6 and 13, and recurrent breakpoints at 1p32–36, 6q22–25, 17p11.2–13, 10q23–26, and 19p13.3. In a study reported by Mao et al, the most frequent losses detected by CGH involved chromosomes 1p (38%),

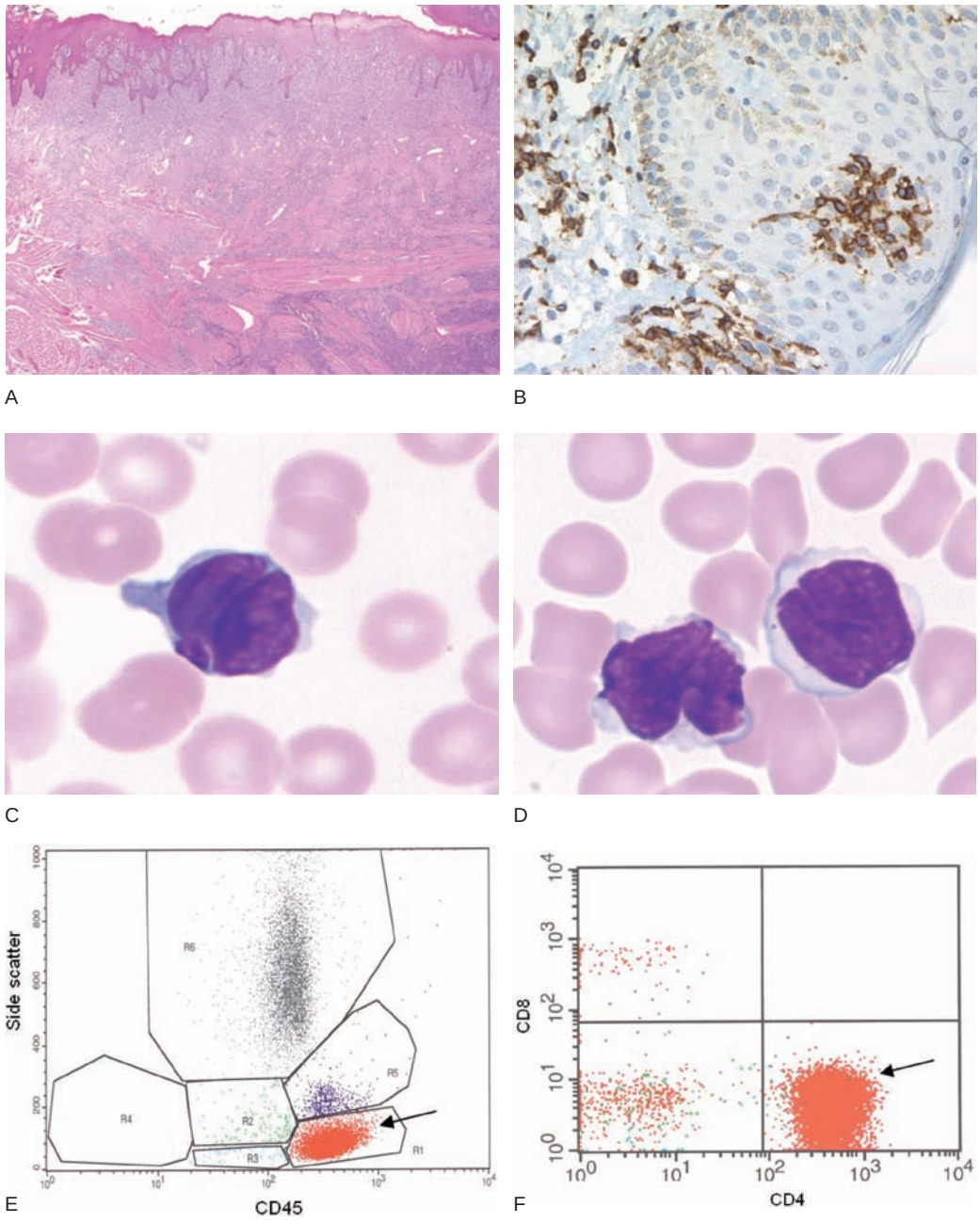


Figure 3.46 Mycosis fungoides and Sézary's syndrome. A, B, mycosis fungoides, tumor stage (A, low power showing dense dermal lymphoid infiltrate; B, immunohistochemical staining for CD3 showing epidermotropism with cluster of T-cells within epidermis). C, D, Peripheral blood films with atypical lymphocytes with cerebriform nuclei. Flow cytometry (E, F) shows T-cells with CD4 restriction (arrow)

17p (21%), 10q/10 (15%), and 19 (15%), with minimal regions of deletion at 1p31p36 and 10q26.[297] The commonly detected chromosomal gains involved 4/4q (18%), 18 (15%), and 17q/17 (12%).[297] In a cytogenetic analysis reported by Thangavelu et al, clonal abnormalities in blood samples from MF/SS patients were observed in 53% of cases, including one with monosomy for the sex chromosome as the sole abnormality.[1451] The abnormalities involving chromosomes 1 and 8 were observed in six cases, chromosomes 10 and 17 in five cases, chromosome 2 in four cases, and chromosomes 4, 5, 6, 9, 13, 15, 19, and 20 in three cases; in two cases a der(8)t(8;17)(p11;q11) was observed.[1451]

PCR analysis of *TCR* gene rearrangements can demonstrate the clonality in up to 99% of cases.[908,1452–1454] Detection of a predominant T-cell clone at the initial diagnosis is an independent negative predictive marker of treatment response in MF.[1455] The application of multiplex PCR with heteroduplex analysis is associated with a high specificity and sensitivity for cutaneous T-cell lymphoma diagnosis,[947] and is very helpful in differential diagnosis between lymphoma and benign inflammatory disease. The percentage of clonality in benign conditions is low: Ponti et al reported a clonal T-cell population in only 2.3% of inflammatory changes.[947] A slightly higher clonality rate can be achieved using capillary gel electrophoresis (GeneScan) for detection of PCR products when compared to polyacrylamide- and agarose-gel electrophoresis.[1456] In a series by Costa et al, five cases identified as clonal by gel electrophoresis, but negative by GeneScan, showed no clonality on sequencing of the PCR products.[1456]

The frequency with which a T-cell clone is detected in lymph nodes from patients with MF increases with extent of skin involvement, overall clinical stage, and histomorphology. Involvement of lymph nodes is relevant for therapeutic options, response to treatment, and overall prognosis.[917,1446,1457] Histologic or molecular evidence of lymph node involvement is associated with a poor prognosis.[917,1458] The involvement of the lymph node by a neoplastic T-cell infiltrate can be assessed by histologic examination, especially when combined with immunophenotyping. However, early lymph node involvement may be difficult for morphologic assessment, especially differentiating it from dermatopathic changes. Assaf et al, using *TCRB*-PCR and *TCRG*-PCR methodology, confirmed T-cell clonality in 22 of 22 lymph nodes with histologic involvement, as well as in 7 of 14 histologically negative lymph nodes with dermatopathic changes, and the clonal T-cell populations in the lymph nodes were in all cases identical to those detected in the corresponding skin lesions.[917] Using the Southern blot technique, Lynch et al found T-cell clonality in 47% of dermatopathic lymphadenopathy cases in MF patients.[1459]

TCR gene analysis by PCR is also a sensitive methodology for assessment of blood involvement. Blood is involved in a large proportion of patients with MF (46–7%).[1460–1462] The significance of identification of the circulating tumor cells to predict clinical course and prognosis remains controversial. Fraser-Andrews et al showed that the presence of a blood clone was an independent poor prognostic variable in patients with MF after correcting for age and skin/lymph node stages.[1462] Also Beylot-Barry et al showed that an identical cutaneous and blood T-cell clone was an independent prognostic factor for disease progression of MF/SS patients.[1463] However, Muche et al could not confirm such a correlation in a series of 67 patients analyzed by *TCRG*-PCR and temperature gradient gel electrophoresis.[1464,1465] Similarly, Laetsch et al reported that the clinical course of patients with demonstrable blood involvement did not differ from PCR-negative cases.[1466] Independent of clinical stage, patients who had the same gene rearrangement detected in multiple concurrent biopsy specimens at the time of diagnosis were more likely to have progressive disease than those who had different gene rearrangements.[1467] In a report by Beylot-Barry et al, a blood T-cell clone was detected in 42% of early MF stages and in 74% of late MF stages, but was identical to the cutaneous

one in 15% and in 63%, of cases, respectively.[1463] Among the patients who had identical circulating and cutaneous T-cell clones, 56% had erythroderma.[939] A significant subset of patients with MF shows distinct T-cell clones in blood when compared to cutaneous tumors.[939,1463] The finding of a dominant clone in the blood but not in the skin was frequent, regardless of the classification; it occured in 30% of patients with cutaneous T-cell lymphoma, 41% with nonlymphomatous malignant infiltrates, and 34% with benign infiltrates.[939] The expansion of a clonal T-cell population (usually $CD8^+$) in a healthy individual was significantly more frequent in patients over 60 years of age.[939,943–946,1461]

Anaplastic large cell lymphoma

ALCL is an aggressive T-cell lymphoma characterized by large pleomorphic cells with irregular nuclei ('hallmark' cells; Figure 3.47), preferential paracortical and intrasinusoidal lymph node involvement, and expression of CD30.[1468] A subset of the tumors carries the t(2;5)(p23;q35).[1468] The t(2;5) disrupts the *NPM* gene at 5q35 and the *ALK* gene at 2p23, generating a novel *NPM/ALK* fusion gene (Figure 3.48). *NPM/ALK* fusion leads to a chimeric mRNA molecule and a unique 80 kDa NPM/ALK fusion protein refered to as p80.[560] The t(2;5)(p23;q35) and variant translocations involving 2p23 result in overexpression of ALK protein, which can be detected by routine immunohistochemistry.[367,368,564,565,1469] ALCL with *NPM/ALK* fusion shows strong nuclear, nucleolar, and cytoplasmic ALK staining. Several cytogenetic and molecular studies have demonstrated that chromosomal aberrations other than the t(2;5)(p23;q35) may give rise to *ALK* fusion in ALCL. These alternative partners to *NPM* gene include *TPM3* (nonmuscle tropomyosin) associated with t(1;2)(q21;p23), *TFG* (*TRK*-fused gene) associated with t(2;3)(p23;q21), *CLTC* (clathrin heavy chain gene) associated with t(2;17)(p23;q23), and *MSN* (moesin).[564–566] Contrary to the nuclear and cytoplasmic distribution of the NPM/ALK protein, variant ALK fusion proteins show a variable subcellular localization, for example in ALCL with t(1;2)*[TPM3/ALK]* ALK expression is restricted to cytoplasm with strong membrane staining.[565,1470] As shown by Liang et al, ALK variants are not uncommon: results among 21 patients showed seven cases (33%) with t(2;5), six (29%) with variant gene rearrangements, seven (33%) with uncharacterized rearrangements, and one with ALK protein expression, but no ALK rearrangement.[1471] Among six variant gene rearrangements, one had t(2;19)(p23;p13)*[TPM4/ALK]* and two had inv(2) with the breakpoint proximate to *ATIC/ALK* and an unknown partner gene separately, while the genetic features of the remaining three cases were as follows: ins(8;2) with an unknown partner gene; conversion from ALK^- at diagnosis to ALK^+ at recurrence with unspecified gene rearrangement, and complex karyotype without involvement of 2p23, suggesting a cryptic translocation.[1471] ALK identification by immunostaining on routine tissue sections (immunohistochemistry) or ALK FISH with break-apart probes is most commonly applied to identify ALK^+ ALCL.

ALK^+ ALCL occurs most commonly in the first three decades of life, is more common in men, presents as aggressive stage III to IV disease with systemic symptoms, frequently has extranodal involvement, and has a good response to chemotherapy.[1469,1472–1475] The ALK^+ ALCL shows a wide range of the morphologic spectrum, including common, lymphohistiocytic, small cell, giant cell, and Hodgkin-like types.[1475] ALCL without ALK– expression occurs in older patients, with a similar distribution in male and female patients, and is associated with a lower incidence of stage III to IV disease and extranodal involvement. Supervised analysis with microarray gene expression profiling showed that ALK^+ ALCL and ALK^- ALCL have different gene expression profiles, further confirming that they are different entities.[562] Among the most significantly differentially expressed genes between ALK^+ and ALK^- samples, Lamant et al found *BCL6*, *PTPN12*, *CEBPB*, and *SERPINA1* genes to be overexpressed in ALK^+ ALCL.[562]

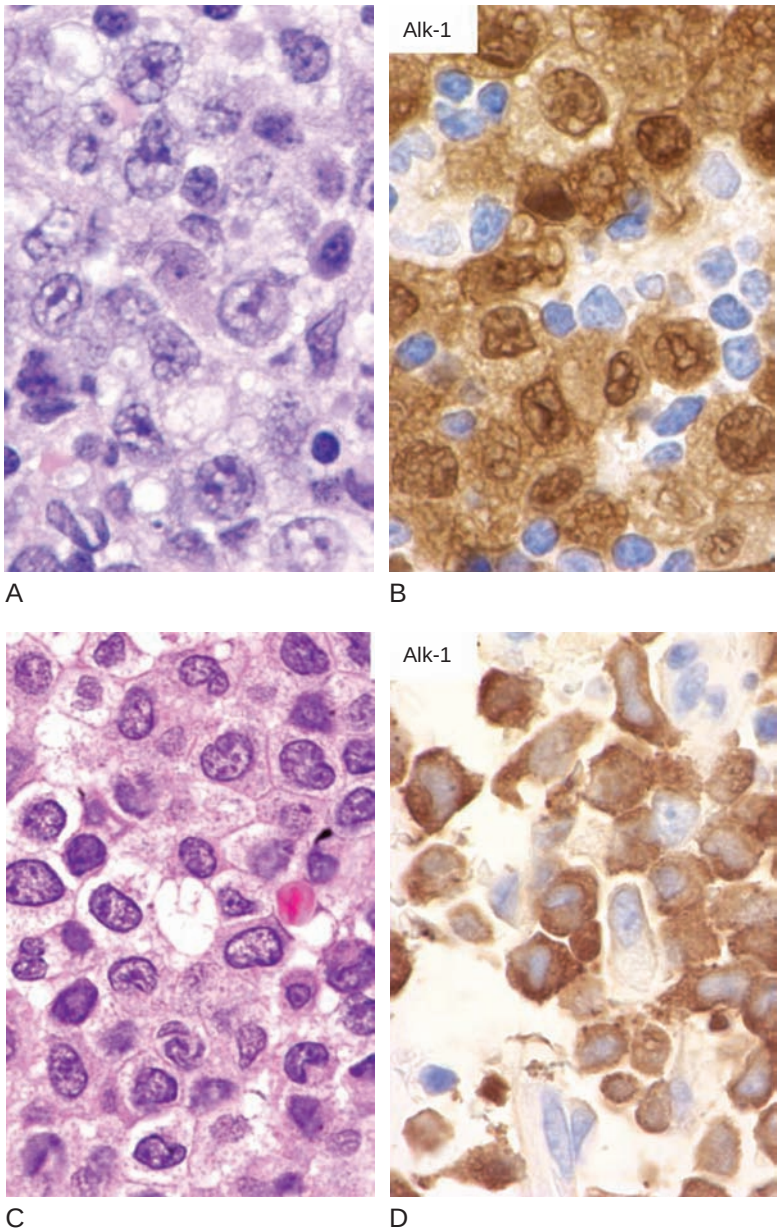


Figure 3.47 Anaplastic large cell lymphoma (two cases). ALCL with large cells showing nuclear and cytoplasmic expression of ALK1 (A, histology; B, immunostaining). Another case composed of smaller cells with cytoplasmic expression of ALK-1 (C, histology; D, immunostaining)

Pediatric ALCL cases frequently have complex karyotypes and usually involve *ALK/NPM* translocations.[1476] ALK-negative ALCL may carry trisomy 2.[1477] Overexpression of c-*MYC* has been shown to be a consistent finding in ALK$^+$ ALCL, and the c-*MYC* gene is considered a downstream target of deregulated *ALK* signaling. Monaco et al described a pediatric ALK$^+$ ALCL with a leukemic phase at relapse with t(2;5), c-*MYC* overexpression, and t(3;8)(q26.2;q24) which followed an aggressive clinical course despite multiple regimens of chemotherapy and bone marrow transplantation.[769]

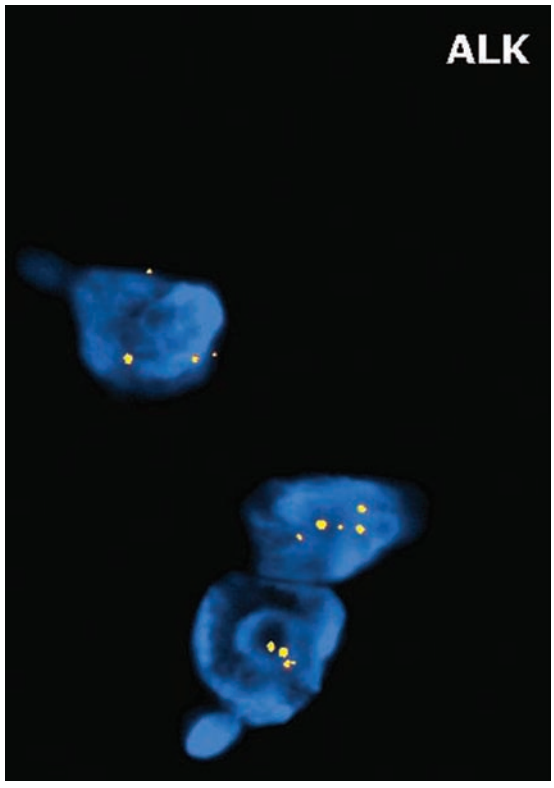


Figure 3.48 Anaplastic large cell lymphoma with ALK1; FISH

Primary cutaneous ALCLs are negative for t(2;5) (ALK expression). Cutaneous ALCLs analyzed by CGH showed gains of 1/1p and 5 (50%) and 6, 7, 8/8p, and 19 (38%).[1478] In the same study, microarray-based CGH analysis of six DNA samples from five cases with chromosomal imbalances revealed genomic imbalances in all of the cases studied. Those abnormalities included oncogene copy number gains of *FGFR1* (8p11) in three cases, and *NRAS* (1p13.2), *MYCN* (2p24.1), *RAF1* (3p25), *CTSB* (8p22), *FES* (15q26.1), and *CBFA2* (21q22.3) in two cases.[1478]

The majority of DLBCL cases with granular cytoplasmic ALK expression are positive for t(2;17)(p23;q23) involving the clathrin gene (*CLTC*) at chromosome band 17q23.[370,371]

HODGKIN LYMPHOMA

Classic Hodgkin lymphoma

Hodgkin lymphoma (Figure 3.49) is a malignant lymphoma of B-cells characterized by Reed–Sternberg and Hodgkin cells accompanied by reactive lymphocytes, granulocytes, eosinophils, histiocytes, and plasma cells, and often variable amounts of fibrosis. Tumor cells are positive for CD30, and often CD15 and Pax-5. A subset of cases expresses CD20, EBER/EBV, and bcl-2. CD45, T-cell antigens, and ALK-1 are not expressed. Neoplastic cells in classic Hodgkin lymphoma originate from germinal center B-lymphoid cells with defective immunoglobulin transcription which escape from apoptosis. Based on the number of neoplastic cells, amount of fibrosis, and composition of background cells, Hodgkin lymphoma is divided into nodular sclerosis (NS), mixed cellularity, lymphocyte-rich and lymphocyte-depleted subtypes. With the modern therapeutic approach, Hodgkin lymphoma is now considered to have a favorable prognosis.

Molecular markers

Tissue microarray analysis revealed deregulation of the genes involved in the G_1/S and G_2/M transitions and inactivation of the tumor suppressor pathways including $p14^{ARF}$-p53–$p21^{WAF1}$, $p16^{INK4a}$-Rb, and $p27^{KIP1}$.[1479] The abnormalities of $p14^{ARF}$ are associated with Hdm2 overexpression and subsequently *p53* inactivation, whereas the $p16^{INK4a}$-Rb pathway is inactivated by loss of $p16^{INK4a}$ and/or cyclin D overexpression. Additionally, neoplastic cells in Hodgkin lymphoma show high expression of cyclins and cdk involved in G_1/S and G_2/M transitions and have defective regulation of apoptosis due to overexpression of bcl-2 (23%), Bcl-X_L (19%), and survivin (89%), loss of BAX expression (3%), and increased nuclear expression of p65/RelA.[1479] Bcl-2, Bcl-X_L, and NF-κB activation are involved in the resistance to apoptosis by Hodgkin and Reed–Sternberg cells. The presence of NF-κB and its activation are related to changes in the expression of a set of proteins that

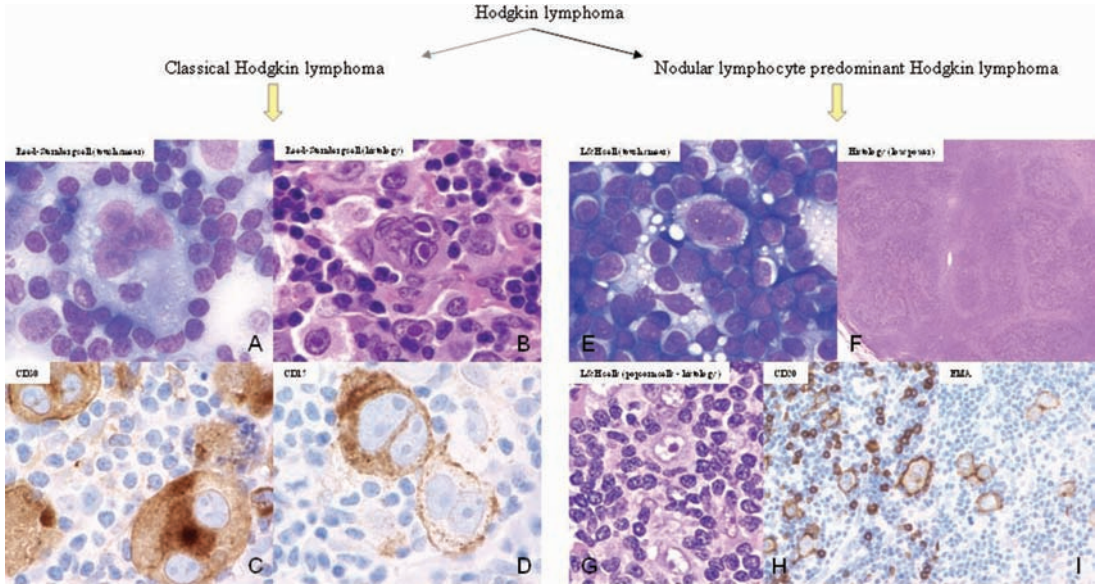


Figure 3.49 Classification of Hodgkin lymphoma. A–D classic Hodgkin lymphoma: A, touch imprint with Reed–Sternberg cells; B, histologic section with typical multinucleated R-S cell; C, expression of CD30; D, expression of CD15. E–I Nodular lymphocyte predominant Hodgkin's lymphoma: E, touch imprint showing an atypical cell with large nucleus, F, low magnification of the lymph node showing nodular pattern; G, high magnification showing typical 'popcorn' cells, H, expression of CD20; I, expression of EMA

play a role in the control of cell cycle progression, apoptosis, and gene transcription, such as p21, p16, p27, pRb, cyclin E and D3, cdk1, cdk2, and survivin.[1479] Shorter survival of patients with Hodgkin's lymphoma is correlated with overexpression of bcl-2, p53, Bcl-X_L, and BAX, and high proliferative (Ki-67/MIB1) and apoptotic indexes.[1479]

Nodular lymphocyte predominant Hodgkin's lymphoma

Nodular predominant Hodgkin lymphoma (NLPHL) is a distinct type of Hodgkin lymphoma representing approximately 5% of all Hodgkin lymphoma cases.[1480] It is characterized by nodular architecture on low magnification and the presence of large neoplastic cells with irregular multilobated nuclei, often referred to as popcorn cells or L&H cells (Figure 3.49).

The *BCL6* rearrangement is identified in 48% of NLPHL cases and is not detected in classic Hodgkin lymphoma cases.[1481] In a case reported by Wlodarska et al, each of the analyzed NLPHL cases showed a different type of *BCL6* rearrangement that included the t(3;22)(q27;q11) targeting the *IG* alpha chain locus, the complex t(3;7;3;1) involving the 7p12/*Ikaros* gene region, t(3;9)(q27;p13) affecting an unknown gene in the vicinity of *PAX5*, and t(3;4)(q27;q32) showing the alternative 3q27 breakpoint outside *BCL6*, and possibly an internal deletion of *BCL6*.[1481,1482] Translocations juxtaposing the *BCL6* oncogene next to the *IGH* locus are the most common recurrent translocations in NLPHL.[1483] These findings further support the hypothesis of a germinal center B-cell-derived origin of NLPHL.

ACUTE MYELOID LEUKEMIA

AML represents a heterogeneous group of disorders with variable clinical presentation, cellular morphology, immunophenotype, therapeutic response, and

overall prognosis. Generally, AML can be defined as a clonal malignancy of transformed multipotent hematopoietic progenitor cells leading to accumulation of immature cells in the bone marrow which replace normal elements, causing cytopenias and their complications (e.g. fatigue due to anemia, infections due to granulocytopenia and bleeding due to thrombocytopenia). The diagnosis and prognosis are most accurately provided by pretreatment assessment of the morphology, immunophenotype, and underlying chromosomal/molecular aberrations. Patients with APL are treated with an ATRA, whereas patients with other types of AML are typically given conventional chemotherapy (e.g. 3 days of anthracycline +7 days of ara-C). Over the last decades, improvement in the diagnosis (especially identification of prognostically relevant cytogenetic groups), new treatment strategies, and advances in supportive care have increased the survival rate in patients with AML.[1484,1485] Despite this, however, nearly two-thirds of patients diagnosed with AML will die of the disease and/or complications of treatment. The prognosis of patients aged 60 years or older with AML remains extremely poor, with 5 to 15% or fewer patients alive beyond 5 years.[1486,1487]

Cytogenetic features

Table 3.7 presents the most common chromosomal abnormalities in AML. Cytogenetic abnormalities in AML at presentation have been identified as one of the most important prognostic factors and have been shown to be independent of age, WBC count, and type of leukemia.[1,15,18,24,80,1488] Approximately 30% of AML patients carry recurrent chromosomal abnormalities associated with different clinical features and prognosis.[1,18] Cytogenetic risk status is a significant factor in predicting response of AML to therapy,[1489] for example, the outcome of patients with APL has substantially improved with the use of ATRA in combination with chemotherapy.[1490–1493] AML with multilineage dysplasia commonly displays an abnormal karyotype; the changes are similar to chromosomal abnormalities seen in MDS: −5/del(5q), −7/del(7q),

Table 3.7 Most common chromosomal abnormalities in AML

Cytogenetic abnormality	*Genes*	*prognosis*
t(1;3)(p36;q21)	*MEL1*; *ribophorin*	poor
t(1;7)(q10;q10)		poor
t(1;11)(p32;q23)	*AF1p*, *MLL*	poor
t(1;11)(p21;q23)	*AF1q*, *MLL*	poor
t(1;22)(p13;q13)	*OTT; MAL*	poor
inv(3)(q21;q26), t(3;3)(q21;q26)	*EVI1*; *ribophorin*	poor
t(3;5)(q25, 1; q35)	*MLF1*; *NPM*	intermediate to poor
t(3;21)(q26;q22)	*EVI1* or *MDS1; RUNX1*	poor
+4		poor
del(5q)		very poor
t(5;17)(q35;q12)	*NPM*; *RARα*	poor
t(6;9)(p23;q34)	*DEK*; *CAN*	poor
t(6;11)(q27;q230	*AF6*; *MLL*	poor
t(7;11)(p15;p15)	*HOXA9*; *NuP98*	intermediate
−7/del(7q)		very poor
+8		intermediate to poor
t(8;16)(q11;q13)	*MOZ*; *CBP*	poor
t(8;21)(q22;q22)	*ETO*; *RUNX1 (AML1)*	good
t(9;11)(p21–22;q23)	*AF9*; *MLL*	intermediate
t(9;22)(q34;q11)	*ABL*; *BCR*	poor
t(10;11)(p12;q23)	*AF10*; *MLL*	poor
+11	*MLL*	poor
t(11;16)(q23;p13)	*MLL*; *CBP*	poor
t(11;17)(q23;q25)	*MLL*; *AF17*	poor
t(11;17)(q23;q21)	*PLZF*; *RARα*	intermediate
t(11;17)(q13;q21)	*NUMA; RARα*	good
t(11;19)(q23;p13)	*MLL*; *ENL*	poor
i(12)(p10)		poor
t(12;22)(p13;q11)	*TEL*; *MN1*	poor
+13		poor
t(15;17)(q22;q11)	*PML*; *RARα*	good
inv(16)(p13)(q22)	*MYH11*; *CBFB*	good
t(16;16)(p13;q22), del(16)(q22)		
t(16;22)(p11;q22)	*FUS*; *ERG*	poor
iso(17)(q10)		poor
t(17;17)(q11;q21)	*STAT5b; RARα*	
del(20q)		poor
+21		intermediate
+22		intermediate
−Y		intermediate
Complex (> 3)		very poor

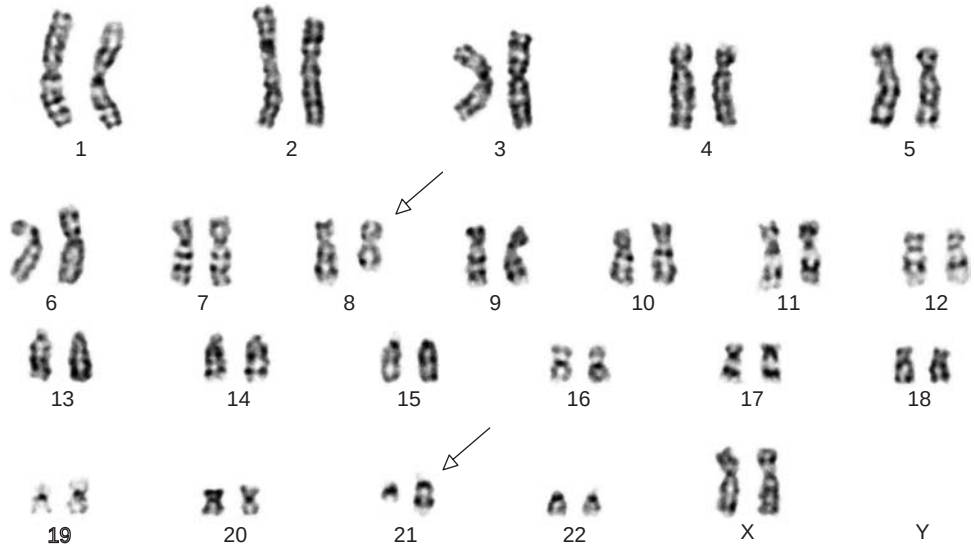


Figure 3.50 AML with t(8;21); cytogenetics

del(11q), del(12p), del(20q), −18, +8, +9, +11, +19, and +21. The t(3;21), which disrupts CBF, is typically present in therapy-related AML.

Three chromosomal abnormalities, t(8;21)*RUNX1/ETO*, t(15;17)*PML/RARα*, and inv(16)/ t(16;16) are regarded as favorable prognostic factors (Figures 3.50–3.53). Patients with inv(16)/t(16;16) or t(15;17) have a favorable prognosis regardless of additional abnormalities.[18,323,1489] Neither complex karyotype nor secondary aberrations affected the outcome of patients with t(8;21), inv(16)/t(16;16), or t(9;11) in a series reported by Byrd et al,[323] but there are some conflicting data on the prognostic impact of secondary aberrations in t(8;21)$^+$ AML. For example, loss of the Y chromosome or del(9q) was reported to be associated with a worse prognosis and shorter overall survival.[449,1494] Additionally, Fenaux et al showed that the t(8;21) might be associated with a high incidence of early relapses.[889] AMLs with t(8;21) and inv(16) comprise the group of so-called CBF leukemias (*see below*). The CBF is a protein complex involved in normal hematopoiesis, which is partially encoded by *RUNX1* on 21q22 and *CBFβ* at 16q22.

Trisomy 8 is the most frequently reported numeric cytogenetic abnormality in patients with AML.

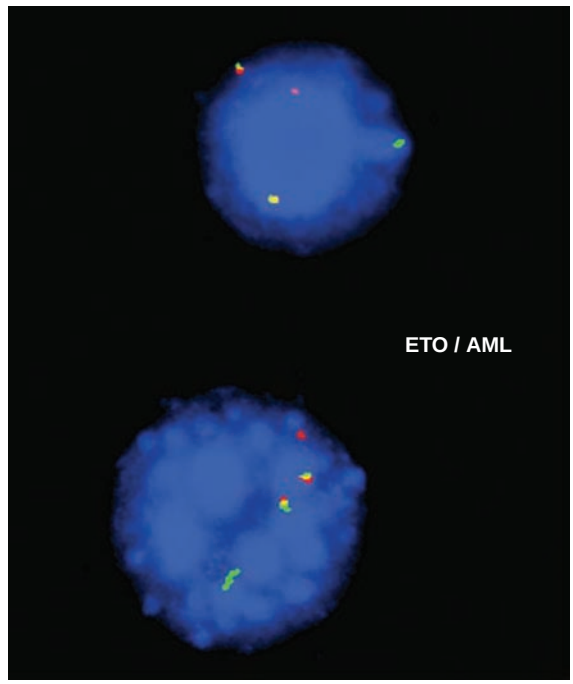


Figure 3.51 AML with t(8;21) [ETO]; FISH

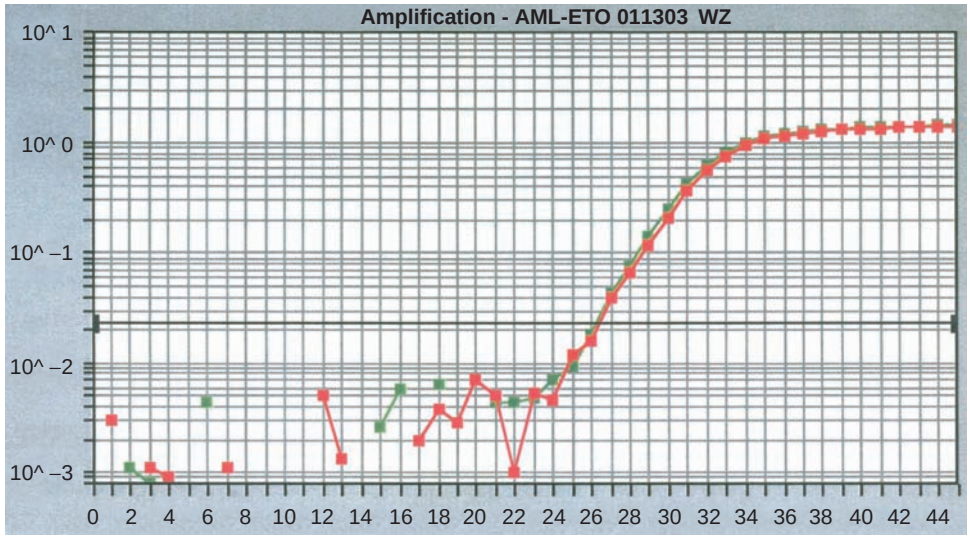


Figure 3.52 AML with t(8;21). Molecular analysis (PCR) shows ETO mRNA

Patients with sole +8 and +8 with additional abnormality other than t(8;21), inv(16)/t(16;16), and t(9;11) had significantly inferior overall survival, but not complete remission rate, while patients with +8 and a complex karyotype with three or more abnormalities had a significantly inferior complete remission rate and overall survival.[323] The impact of trisomy 8 on AML patients is best predicted by the presence and nature of abnormalities that accompany it.[108,323]

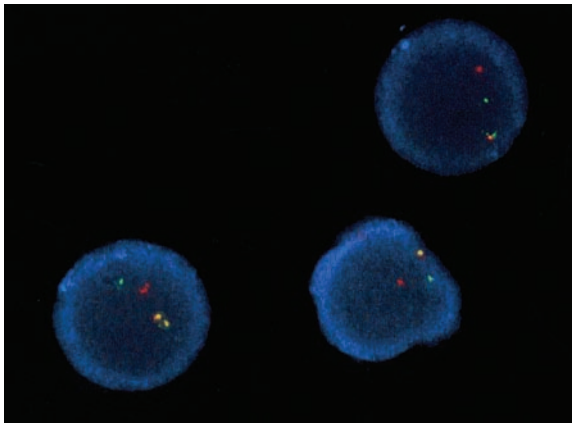


Figure 3.53 AML with inv(16), FISH

Patients with normal karyotype, –Y, t(9;11), del(11q), +13, del(20q), and +21 belong to the intermediate prognostic category.[18,323,1489] Patients with AML with 5(q)– and additional aberrations (Figure 3.54) have a worse prognosis than those with 5(q)– as a single abnormality. Complex karyotype (≥3 abnormalities; Figure 3.55) predicts a significantly worse outcome when compared to cytogenetically normal patients.[323] Older patients (>60 years old) with ≥5 abnormalities have an extremely poor prognosis. In a study by Farag et al, no patients 60 years of age or older with ≥5 abnormalities was alive at 5 years, despite intensive chemotherapy.[1487]

Other adverse cytogenetic changes include: inv(3) or t(3;3), t(6;9), t(6;11), and –7.[323] The outcome of patients with balanced 11q23 translocations depends on which partner chromosome is involved.[24,323,1495] While the prognosis for patients with t(9;11) is not different from those with a normal karyotype (intermediate prognostic category), the overall survival of patients with t(6;11) or t(11;19)(q23;p13.1) is significantly shorter compared to the normal group.[323] Therefore, the prognosis of patients with 11q23 aberrations is regarded as either unfavorable.[1489,1496] or intermediate.[18] These inconsistencies in outcome

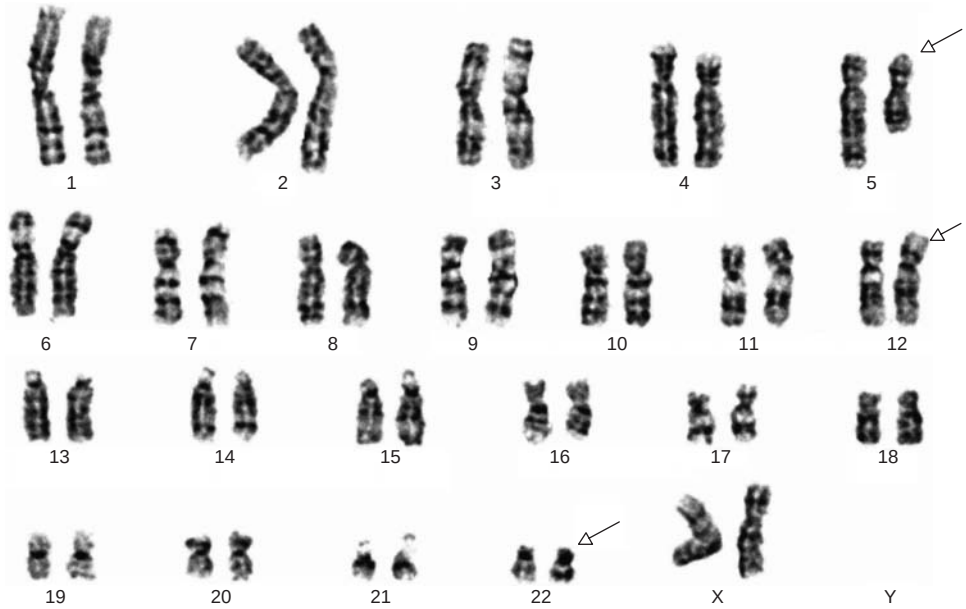


Figure 3.54 AML with 5q– and t(12;22); cytogenetics

may result from the multiplicity of 11q23 aberrations resulting in different fusion partners, variable molecular rearrangements, or the presence of additional chromosomal alterations imparting the prognostic impact. Young patients with *de novo* presentation of t(9;11) belong to the intermediate category. Patients with t(6;9) have a dismal prognosis.[323] The prognosis of patients with del(9q) is intermediate/unfavorable, but varies depending on the genotypic makeup.[1489]

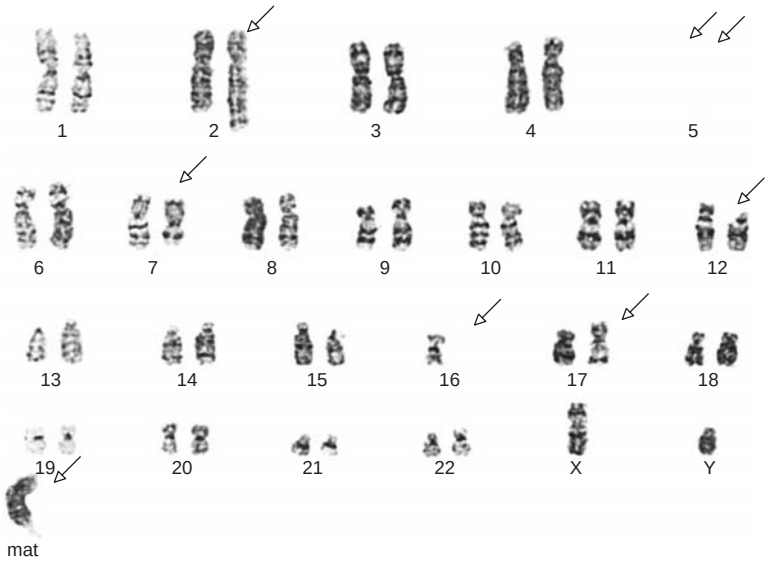


Figure 3.55 AML with complex karyotype; cytogenetics

AML with t(9;22)$^{BCR/ABL}$ may occur *de novo* or represent the blast phase of CML. *BCR/ABL*$^{+}$ AML is a rare disease, characterized by a poor prognosis, with resistance to induction chemotherapy and frequent relapses in responsive patients. In sporadic patients the Ph chromosome may be observed as an additional, late-appearing cytogenetic change during the evolution of AML. Ph^{+} AML may respond to tyrosine kinase inhibitors (e.g. imatinib), which may induce long-lasting remission in some patients.[1497] Soupir et al, however, reported a median survival of less than 1 year for *BCR/ABL*$^{+}$ AML patients.[1498]

Molecular markers

As mentioned above, only ~30% of AML patients carry recurrent chromosomal abnormalities associated with different clinical features and prognosis, and an additional 10–15% have nonrandom chromosomal abnormalities.[1,18] Based on cytogenetic studies, 40–50% of *de novo* AML cases have a normal karyotype and these leukemias often show genetic abnormalities by molecular testing. They include internal tandem duplication of the *FLT3* gene (*FLT3*-ITD), mutations of the *NPM1* gene, partial tandem duplication of the *MLL* gene (*MLL*-PTD), high expression of the *BAALC* gene, *FIP1L1/PDGFRA* fusion, mutations in the *CEBPA* gene, and abnormalities involving the *ERG*, *MN1,* and *RAS* genes.[25,778,1499,1500] Structural and molecular changes contribute to inappropriate proliferation in the absence of normal growth signals, indefinite self-renewal, escape from programmed cell death (apoptosis), inhibition of differentiation, aberrant cell cycle checkpoint control, genomic instability, and multiorgan dissemination of leukemic cells.

Transcription factors are commonly disrupted in AML, either by their fusion as a result of chromosomal translocation or by point mutation. Factors affected by chromosomal rearrangement include the CBF complex, the retinoic acid receptor (RAR), the MLL protein, and Hox proteins. The CBF complex is disrupted by at least three different translocations: t(8;21), which generates the *RUNX1/ETO* fusion; inversion of chromosome 16, yielding the *CBFβ/MYH11* fusion; and t(3;21), which generates the *RUNX1/EVI1* fusion. The t(8;21) can be identified by conventional cytogenetics, but for inv(16) and t(3;21) RT-PCR and/or FISH are more reliable.

APL (*see below*), an example of differentiation blockage in AML, is associated with rearrangement of the retinoic acid receptor alpha (*RARα*). The t(15;17) seen in most of APL patients leads to *PML/RARα* fusion. A small subset of APL (less than 1%) carries *PZLF/RARα* fusion associated with t(11;17), which is associated with a lack of response to retinoic acid. Rare fusions of *RARα* to *STAT5b*, *NPM* (a gene mutated in a substantial number of AML cases), as well as the *NuMA* (nuclear matrix) gene have been reported as well (*see below*).

*FLT*3 mutation status has a major impact on remission duration and overall survival in AML patients with normal cytogenetics. The *FLT*3 mutations are present in 27 to 32% of the patients and are associated with leukocytosis and a high percentage of blasts in bone marrow.[57,1501] *FLT*3 mutations occur predominantly by internal tandem duplications (ITDs) and, less commonly, through Asp835 mutations. *FLT*3 mutations do not correlate with the complete remission rate, but they predict relapse and are associated with a shorter remission duration, shorter event-free survival, and shorter overall survival when compared to AML patients without *FLT*3 mutations.[57,1501–1503] ITD mutations have been associated with an increased risk of treatment failure after conventional chemotherapy.[55,1501,1502]

The c-*KIT* tyrosine kinase is expressed in 60–80% of AML patients, and this kinase is activated by mutation in mast cell leukemia and some cases of AML (~1%). The *RAS* mutations occur in 20 to 44% of AML patients, may be associated with lower blood and bone marrow blast percentages, and have not consistently predicted the prognosis.[61,1504] *NRAS* is mutated and constitutively activated in 10–20% of AMLs, *KRAS* in 5–15% of patients, and *HRAS* is rarely mutated.[1505] AML samples that

contain *RAS* mutations do not have kinase fusions or activating mutations, suggesting that *RAS* and tyrosine kinase molecules act separately.

Partial tandem duplications of the *MLL* gene (*MLL*-PTDs) occurs in 5–10% of AMLs and have been associated with trisomy 11 in AML and, similarly to *FLT3*-ITD, preferentially occur in karyotypically normal AMLs.[66,68,1506] AML may be positive for both *MLL*-PTD and *FLT3*-ITD.[1506] In a series reported by Schnittger et al, of the 16 *MLL*-duplication positive cases, seven were classified as FAB M2 (AML with maturation), two as M1 (AML without maturation), five as M4 (acute myelomonocytic leukemia), one as M0, and one as M5 (acute monoblastic leukemia).[68] The median survival and relapse-free interval of the *MLL*-PTD$^+$ group were significantly worse than those of an age-matched karyotypically normal control group. AMLs with *MLL* gene amplification/tandem repeats (Figure 3.56) have a poor response to treatment and a dismal prognosis.[66,68]

The *NPM1* gene encodes for a multifunctional nucleocytoplasmic shuttling protein that is localized mainly in the nucleolus. *NPM1* mutations occur in 50–60% of adult AMLs with normal karyotype (~35% primary AMLs).[782,1507] NPM1 protein exchanges between the nucleus and cytoplasm and binds to several proteins including p53 and proteins interacting with and regulating *p53* (e.g. Rb, p19^{ARF}, HDM2). In patients with AML, mutations in exon 12 of the *NPM1* gene on chromosome 5q35 lead to a frameshift and an elongated protein, which is retained in the cytoplasm, hence the term NPM-cytoplasmic-positive leukemia (NPMc$^+$ AML).[782,1507] Falini et al showed that *NPM1* mutations are associated with several features, including normal karyotype, female > male, multilineage involvement, low or absent CD34 expression, an increased prevalence of *FLT3*-ITD mutations, and a higher rate of complete remissions after standard induction chemotherapy.[782] Using fragment analysis for *NPM1* exon 12 mutations in 1485 patients with AML, Thiede et al detected *NPM1* mutations (an insertion of four bases, either between nucleotides 960–961 or between 964–965) in 408 patients (27.5%).[789] *NPM1* mutations were most prevalent in patients with normal karyotype (45.7% of patients with normal karyotype had *NPM1* mutations compared to only 8.5% among patients with abnormal karyotype).[789] Like *FLT3*-ITD mutations, *NPM1* mutations were associated with a higher bone marrow blast count, and high WBC and platelet counts, and female sex.[789] *NPM1* mutations are often associated with *FLT3*-ITD mutations (*NPM1*$^+$/*FLT3*-ITD 43.8% *NPM1*–/*FLT3*-ITD 19.9%).[789] Patients having only an *NPM1* mutation (no *FLT3*-ITD) have a significantly better overall and disease-free survival and a lower cumulative incidence of relapse (improved response to treatment) and comprise a prognostically favorable subgroup in the heterogeneous group of AML patients with normal karyotype.

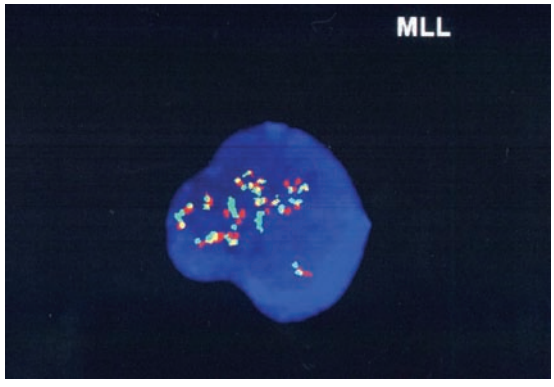


Figure 3.56 *MLL* amplification in AML; FISH

The *p53/TP53* is involved in cell cycle regulation and apoptotic signaling. Mutations in the tumor suppressor gene *TP53* (*p53*) are associated with a poor response to chemotherapy and poor outcome.[1508] The *RUNX1/MTG8* fusion protein represses the expression of p14^{ARF} and promotes destabilization of *p53*. The *MOZ/TIF2* fusion binds to CBP and indirectly attenuates the transcriptional activity of *p53*. The co-expression of the apoptosis-related genes *BCL2* and

WT1 predicts for poor survival in AML patients.[1509] Expression of *MDR* genes has been identified as an adverse prognostic marker in AML.[724,728,1510,1511] Expression of MDR-1/P-glycoprotein is more commonly found in older patients, often in conjunction with aadverse cytogenetics, and is associated with a lower complete remission rate.[724,728]

A subset of AML is associated with eosinophilia. The majority of these cases belong to the category of acute myelomonocytic leukemia with eosinophilia (AML-M4Eo) and are associated with inv(16)/t(16;16) (variant of CBF^{+} AML). Occasional AMLs with eosinophilia, especially those preceded by CMPDs associated with eosinophilia (e.g. chronic eosinophilic leukemia and mastocytosis with eosinophilia) may be associated with *FIP1L1/PDGFRA* fusion or show recurrent breakpoint clusters at chromosome bands 5q31–33, 8p11, and 9p24, which are linked to tyrosine kinase genes *PDGFRB*, *FGFR1*, and *JAK2*, respectively. As shown by Metzgeroth et al, patients with eosinophilia-associated hematologic malignancies (including AML) with *FIP1L1/PDGFRA* fusion respond very well to treatment with tyrosine kinase inhibitors (e.g. imatinib).[1500]

Acute myeloid leukemia (M0/M1/M2)

AML, minimally differentiated (AML-M0) is an acute leukemia with no cytomorphologic evidence of myeloid differentiation (<3% blasts react for myeloperoxidase). FC immunophenotyping reveals expression of one or more pan-myeloid antigens (CD13, CD33), CD34, CD117, and HLA-DR. TdT may be positive, but B- and pan-T markers are negative. AML without maturation (AML-M1) is defined by ≥90% blasts of nonerythroid lineage (less than 10% of the marrow cells manifest evidence of maturation to promyelocytes or more mature granulocytes). At least 3% of blasts are positive for MPO. AML with maturation (AML-M2) is defined by ≥20% blasts and evidence of myeloid maturation (promyelocytes and subsequent stages) in ≥10% of the bone marrow cells.

Both AML-M0 and AML-M1 do not carry specific chromosomal abnormalities, and may show complex karyotype, +4, +8, +13, and −7. AML with maturation (AML-M2) is the predominant type among AMLs with $t(8;21)^{RUNX1/ETO}$. Other chromosomal changes, apart from those seen in less differentiated subtypes, include t(6;9), t(8;16), and del(12p). AMLs with t(8;16) are often associated with erythrophagocytosis (more often seen in acute monoblastic leukemia) and those with del(12p) with marrow basophilia.[1,24,80]

Acute promyelocytic leukemia (AML-M3)

APL (Figure 3.57), which accounts for approximately 10% of AML cases, is a subtype of AML characterized by abnormal promyelocytes and response to treatment with all *trans*-retinoic acid, which induces maturation. Distinctive features of APL include leukopenia co-existing with a marrow replacement by atypical promyelocytes, disseminated intravascular coagulopathy (DIC), and lack of HLA-DR antigen expression by immature cells. APL is characterized by a translocation between the long arms of chromosomes 15 and 17 (Figure 3.58), which fuses the promyelocytic leukemia gene (*PML*) on chromosome 15 to the *RARα* gene on chromosome 17, resulting in the chimeric gene encoding *PML/RARα* fusion protein.[1512,1513] The introduction of ATRA and arsenic trioxide (As_2O_3), which target the underlying molecular changes, dramatically changed the clinical course of APL from invariably fatal to one of the most curable types of acute leukemia.

Cytogenetics/molecular markers

The balanced translocation between chromosomes 15 and 17 characterizes >95% cases of APL.[77] The t(15;17) disrupts the *PML* gene on chromosome 15 and the *RARα* gene on chromosome 17. The t(15;17) leads to the formation of two reciprocal fusion genes, *PML/RARα* on chromosome 15 and *RARα/PML* on

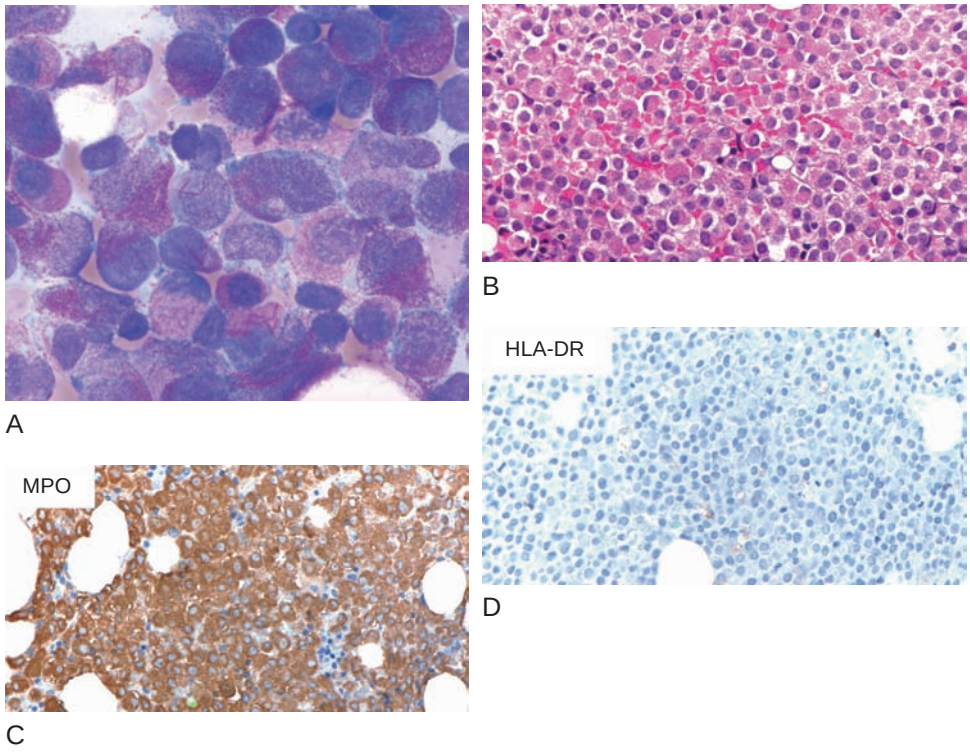


Figure 3.57 Acute promyelocytic leukemia (APL). A, Bone marrow aspirate smear shows atypical hypergranular promyelocytes with numerous Auer rods; B, histology section shows replacement of the bone marrow by immature mononuclear cells; C, D, immunohistochemical stainings show expression of MPO (C) and lack of HLA-DR (D)

chromosome 17. The resultant *PML/RARα* fusion protein, which retains the retinoic acid receptor-binding domain, plays a role in leukemogenesis, but also mediates the response to retinoids.[468] The fusion of *PML* and *RARα* genes may result in some cases from insertion of complex rearrangements.[16,1514] Variant translocations in which *RARα* is fused to a gene other than *PML* have also been identified.[468] In these variant translocations, *RARα* is fused to the *PLZF* (promyelocytic leukemia zinc finger) [t(11;17)(q23;q21)], *NPM* (nucleophosmin) [t(5;17)(q35;q21)], and *NuMA* (nuclear mitotic apparatus) [t(11;17)(q13;q21)].[469,810,1515] The nature of the fusion partner has a significant impact on the biology of the disease. The t(15;17). translocation is associated with a favorable prognosis.[15,18,1516] APL associated with t(11;17)(q23;q21) and fusion of the *PLZF* and *RARα* genes is a discrete clinicopathologic syndrome which is resistant to the differentiation effects of ATRA and has a distinctly worse prognosis than APL with t(15;17).[469] The *PLZF*/*RARα* cases are often characterized by a predominance of immature precursors with regular nuclei, an increased number of pelgeroid features, and expression of CD56.[1517] With conventional cytogenetics, these translocations are detected in about 70–90% of patients, with most of the negative results due to technical problems or cryptic variants. Cryptic t(15;17) are generated by submicroscopic insertions of *PML* or *RARα*, or complex rearrangements.[16,806,807] These masked *PML/RARα* fusions can be identified by molecular analyses such as PCR and FISH. PCR testing can be used to monitor the response to treatment (Figure 3.59).

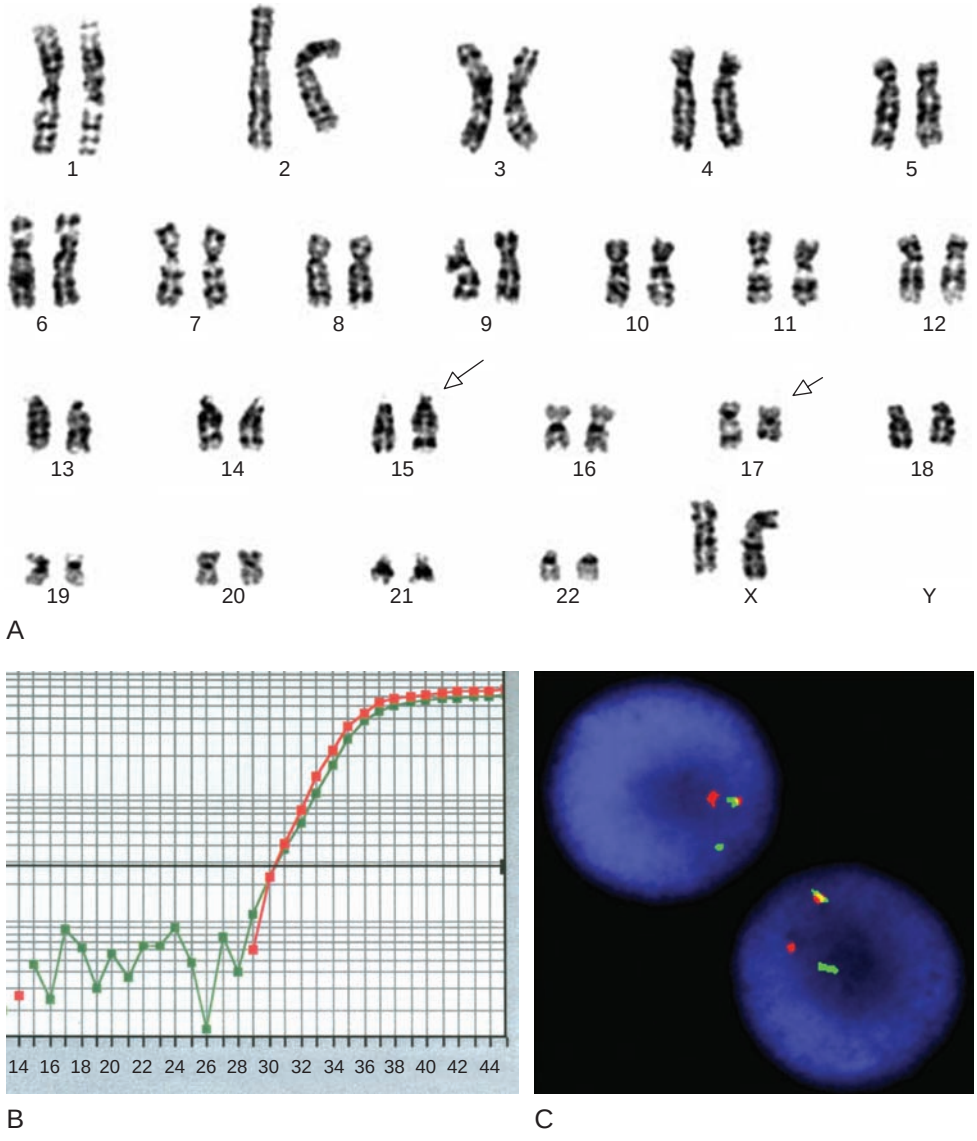


Figure 3.58 Detection of translocation t(15;17) [*PML-RAR(* fusion] in acute promyelocytic leukemia; A, cytogenetics; B, PCR; C, FISH

The secondary chromosomal changes in addition to t(15;17) occur in 26–40% of APLs and include most often trisomy 8 (46% of all cases with secondary abnormalities; 15–30% of all cases), followed by abnormalities of chromosomes 6, 7, 9, 12, 16, 17, and 21.[516] The presence of additional chromosomal changes in APL has no impact on prognosis in patients treated with ATRA and chemotherapy.[516] Mutation of the *PML* gene leading to loss of *PML* function is associated with ATRA resistance and poor prognosis.[813]

In a study of 82 patients with APL, the presence of *FLT3*-ITD had a significant negative impact on overall survival.[670] A retrospective study from the

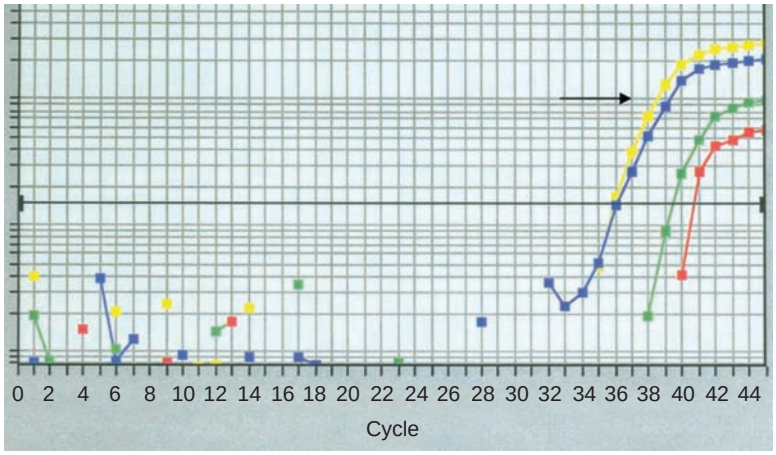


Figure 3.59 Acute promyelocytic leukemia. MRD detected by PCR analysis. Note positive reaction at the very late stages of amplification (arrow)

European APL group indicated that the *FLT3*-ITD correlated with poor post-relapse survival and the presence of *FLT3*-ITD was associated with high WBC count, high Sanz index, M3-variant subtype, and V/S *PML/RARα* isoforms.[668] Another study, however, showed that cases with *FLT3*-ITD had a higher rate of induction death, but no significant difference in relapse risk or overall survival.[669]

Acute myelomonocytic leukemia (AML-M4)

This acute leukemia is characterized by the proliferation of both neutrophil and monocyte precursors with ≥20% of myeloblasts in the bone marrow. Both monocytic and granulocytic lineages comprise at least 20% of marrow cells. The number of monocytic cells and their precursors in peripheral blood is usually greater than 5×10^9/l. The bone marrow is hypercellular and contains a mixed population of monocytes and their precursors and granulocytes and their precursors (Figure 3.60). The monoblasts are slightly larger than myeloblasts, have abundant cytoplasm, irregular nuclei, and prominent nucleoli. The myeloblasts and maturing granulocytes show morphologic features similar to AML with maturation. At least 3% of blasts are MPO^+. Monocytic precursors are positive for NSE. AMLs with monocytic features often display 11q23 abnormalities. A variant of acute myelomonocytic leukemia characterized by an increased number of abnormal eosinophils (AML-M4Eo) is positive for t(16;16)(p13;q22) or inv(16)(p13q22).

Acute monoblastic leukemia (AML-M5)

Acute monocytic (monoblastic) leukemia is defined as AML in which 80% or more of the leukemic cells are of monocytic lineage (monoblasts, promonocytes, and monocytes). The bone marrow core biopsy is hypercellular and usually completely replaced by monoblasts, which are large and have prominent nucleoli. Leukemic monocytes have abundant cytoplasm which may show irregular borders with pseudopods, cytoplasmic vacuoles, and strong NSE positivity (Figure 3.61). MPO is characteristically negative. Based on the proportions of monoblasts and promonocytes, AML-M5 can be subdivided into acute monoblastic leukemia (AML-M5a; monoblasts predominate) and acute monocytic leukemia (AML-M5b; promonocytes predominate).

Acute monoblastic leukemia often shows complex chromosomal changes (Figure 3.62). There is a strong association between AML-M5 and deletions or translocations involving the *MLL* gene at chromosome

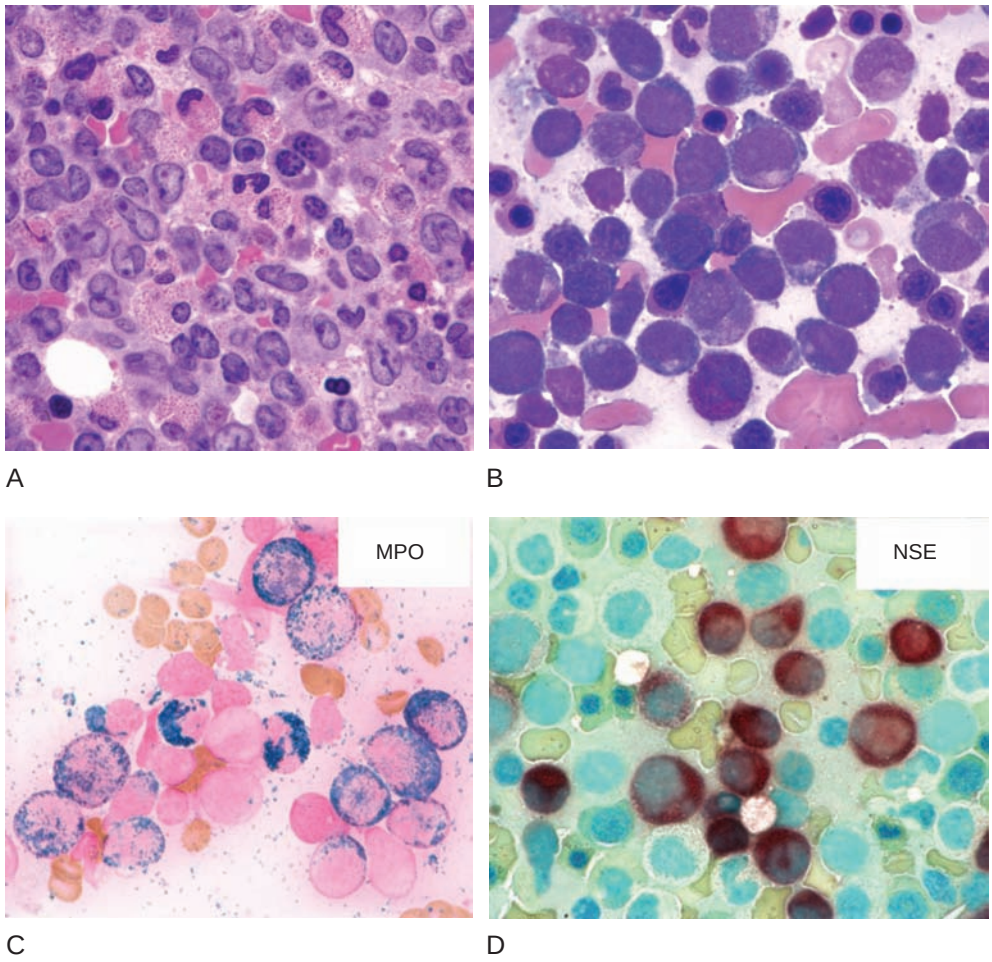


Figure 3.60 Acute myelomonocytic leukemia (AML-M4). Bone marrow is hypercellular (A, histology) with increased number of immature mononuclear cells. Aspirate smear (B) shows mixed population of blasts, monocytes, scattered erythroid precursors, and occasional maturing myeloid cells. Myeloblasts are positive for myeloperoxidase (C), and monocytes are positive for NSE (D)

11q23, which are identified in 12–31% of patients.[1518] Occasional cases demonstrate t(8;16), rare translocations that may also be seen in AML-M4.[1,1519] AMLs with t(8;16) and their variant, inv(8)(p11q13), have a poor prognosis, and apart from erythrophagocytosis, may be associated with disseminated intravascular coagulation and extramedullary leukemic infiltrates.[1519,1520] Haferlach et al analyzed 58 patients with *de novo* AML-M5a and 66 patients with *de novo* AML-M5b[1518] and found an aberrant karyotype in ~76% of AML-M5a and ~29% of M5b.[1518] Trisomy 8 as the sole cytogenetic aberration was found in 22.4% of M5a, in 3% of M5b, and in 2.5% of all other AML subcategories.[1518] *FLT3* mutations were rare in AML-M5a (6.9%), but occurred more often in the M5b subtype (~29%) and non-M5 AML (23.5%).[1518]

Acute erythroid leukemia (AML-M6)

Acute erythroid leukemia (erythroleukemia; AML-M6) is characterized by a predominance of abnormal

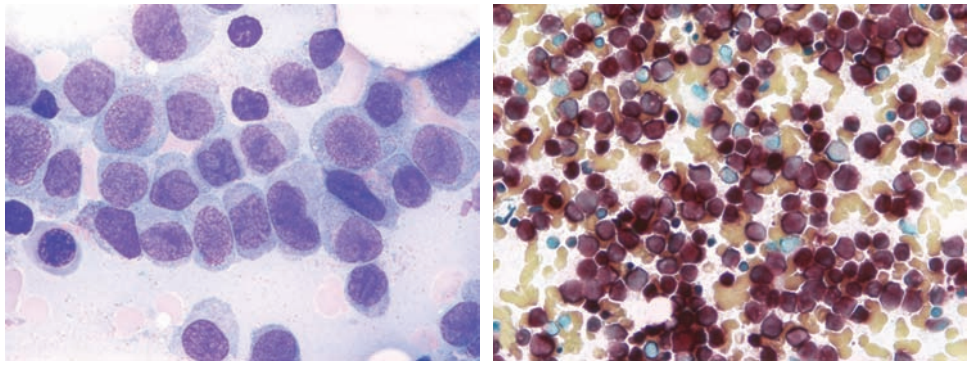


Figure 3.61 Acute monocytic leukemia (AML-M5). Aspirate smear (A) shows large immature monocytic cells with irregular nuclei and prominent nucleoli. Leukemic cells stain strongly with NSE (B)

erythroid cells (Figure 3.63). The WHO recognizes two subtypes of AML-M6: erythroleukemia (erythroid/myeloid leukemia) and pure erythroid leukemia. Erythroleukemia is defined by ≥50% erythroid precursors (among all nucleated cells) and ≥20% myeloblasts of nonerythroid cells. Pure erythroid leukemia is defined by >80% erythroid precursors among all nucleated marrow cells. MDS differs from erythroleukemia by <20% myeloblasts of non-erythroid cells (proerythroblasts are not included in the blast percentage). Erythroblasts are positive for glycophorin A (GPHA) and hemoglobin A. They lack MPO, CD34, CD45, and pan-myeloid antigens. CD117, CD43, and epithelial membrane antigen (EMA) are often positive.

Acute erythroid leukemia has no specific chromosomal changes but may show numerous unbalanced and balanced abnormalities, the former being

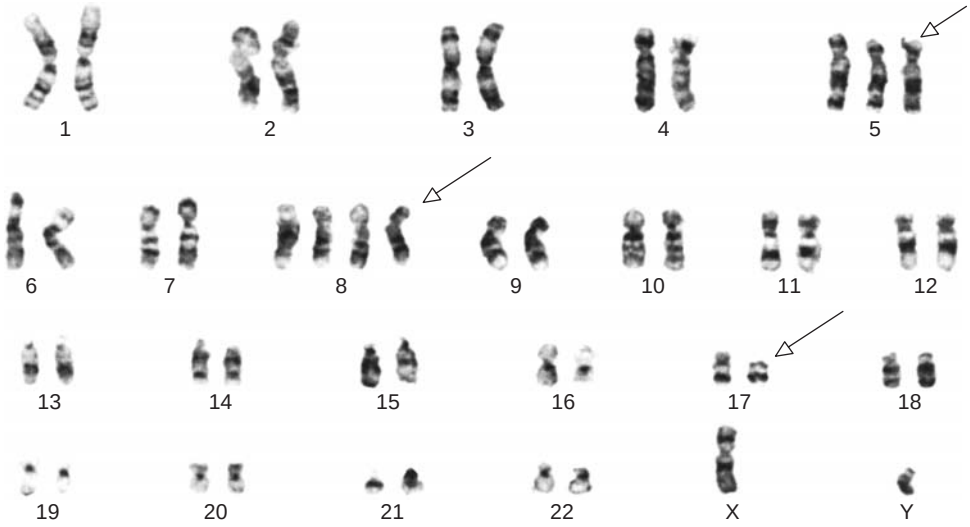
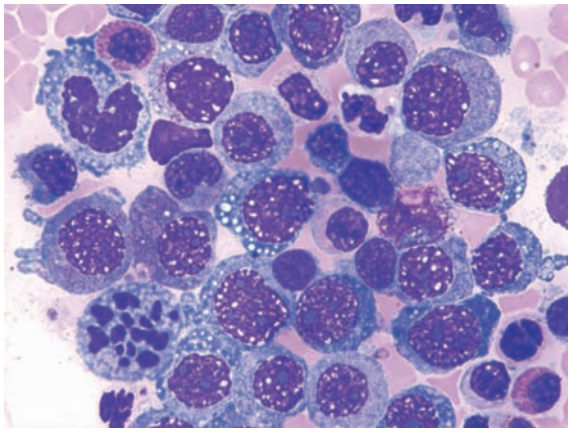
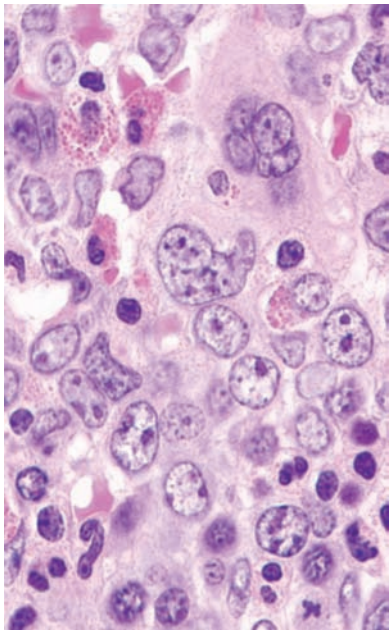


Figure 3.62 Acute monoblastic leukemia (AML-M5) with complex karyotype: +5, +8, +8, del(17)(p11.1) [trisomy 5 and trisomy or tetrasomy 8 are often associated with monocytic leukemia and deletion of the short arm of chromosome 17 in AML usually confers a poor clinical outcome]; cytogenetics



A



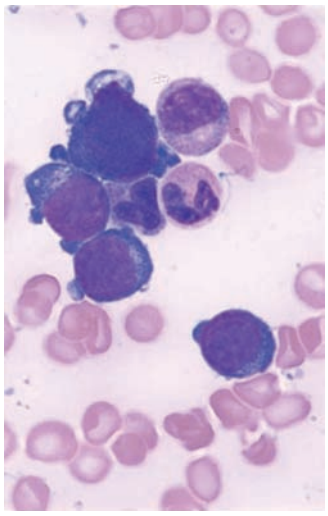
B

Figure 3.63 Acute erythroid leukemia (AML-M6). Cytology (A) shows immature erythroblasts with cytoplasmic vacuoles. Histology (B) shows replacement of the bone marrow by sheets of atypical erythroid precursors

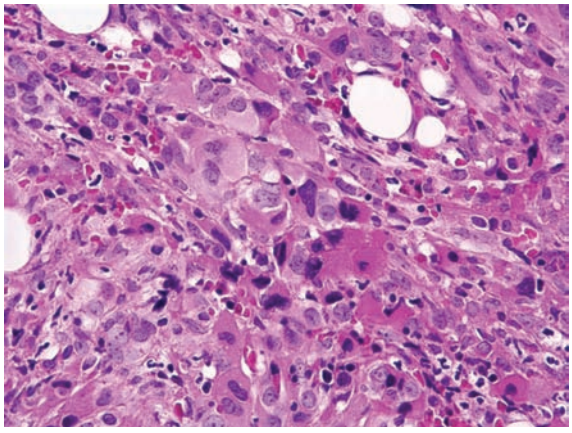
more common.[1521–1524] In a series of 75 cases reported by Lessard et al, hypodiploidy was the most frequent numeric abnormality (48% of cases with an abnormal karyotype), associated with complex karyotypes (37%).[1521] Chromosomes 5 and 7 are most frequently involved (complete monosomy 5 in 26% and complete monosomy 7 in 38%), followed by chromosomes 8, 16, and 21.[1521] Trisomy 8 is the most frequent complete trisomy in erythroleukemia. Sporadic changes reported in erythroleukemia include: −1, −2, −3, −4, −5, −10, −13, −14, −15, −16, −17, −19, −21, −22, +5, +6, +8, +10, +18, +19, +21, del(4)(q21), del(5)(q31q35), del(12)(q24), i(13)(q10), i(17)(q10), i(21)(q10), i(22)(q10), inv(1)(p21q22), inv(3)(q21q26), inv(6)(p22q22), inv(7)p11q21), t(2;7)(q31;q35), t(3;3)(q21;q26), t(3;4)(q13;q25), t(3;8), t(3;11)(p14;p11-12), t(4;8)(q27;q24), t(6;8)(p22;q12), t(8;9)(p22;p23), t(8;13)(q22;q14), and t(9;22)(q34;q11).[1521,1523,1525-1528] Cases of (*de novo*) pure erythroid leukemia may display t(9;22)$^{BCR/ABL}$. The karyotypic changes are somewhat similar to those observed in high-grade MDS (refractory anemia with excess blasts), with the following changes observed only in erythroleukemia (AML-M6): der(9)t(1;19)(q11;p13), i(3)(q10), t(3;5)(q25;q35), inv(6)(p23q12), and t(7;11) (q11;p15).[1521]

Acute megakaryoblastic leukemia (AML-M7)

Acute megakaryoblastic leukemia (AML-M7) is a rare variant of AML with ≥20% blasts, of which 50% or more are of megakaryocyte lineage. Cytomorphologic features vary, depending on the degree of maturation of megakaryoblasts. In the more differentiated cases, megakaryoblasts (Figure 3.64) are medium-sized to large with round or slightly irregular nuclei and basophilic, agranular cytoplasm with distinct blebs (pseudopods). In poorly differentiated cases, megakaryoblasts resemble myeloblasts or lymphoblasts and their lineage can be revealed by immunophenotyping. The spectrum of morphologic features of acute megakaryoblastic leukemia in bone marrow core biopsy varies from a predominance of poorly differentiated blasts to sheets of atypical megakaryocytes with a predominance of micromegakaryocytes. FC analysis shows blasts with moderate CD34, dim CD117, negative to dim



A



B

Figure 3.64 Acute megakaryocytic leukemia (AML-M7). A, Cytology; B, histology. Atypical megakaryocytes and immature forms (megakaryoblasts) are present

HLA-DR, negative CD13, bright CD33, dim CD64, and positive CD41 and 61.

Acute megakaryoblastic leukemia is characterized by a higher complexity and incidence of chromosomal abnormalities than in the other subtypes of AML.[1529–1531] In a series of 281 patients with AML reported by Athale et al, acute megakaryocytic leukemia was diagnosed in 14.6% (six patients with Down's syndrome, six had secondary leukemia, and 29 represented *de novo* leukemia).[1533] The most frequent chromosomal changes reported in AML-M7 include −7/del(7q), −5/del(5q), +8, +19 (or +19q), and +21, followed by i(12)(p10), t(9;22)(q34;q11), t(10;22)(q26;q11), t(13;20), del(20)(q11), der(7)/t(7;17), t(1;22)(p13;q13), t(6;11)(q24.1;p15.5), t(17;22)(q21;q13), and 11q23 abnormalities.[460,1529, 1530,1534–1538] Dastugue et al, identified nine cytogenetic groups in patients with AML-M7: normal karyotypes (group 1); patients with Down's syndrome (group 2); numeric abnormalities only (group 3); t(1;22)(p13;q13) or *OTT/MAL* transcript (group 4); t(9;22)(q34;q11) (group 5); 3q21q26 (group 6); −5/del(5q) or −7/del(7q) or both (group 7); i(12)(p10) (group 8), and other structural changes (group 9).[1529] Groups 1, 2, 3, and 4 were exclusively seen in children (except one adult in group 3), whereas groups 5, 6, 7, and 8 were mainly made up of adults.[1529]

Extramedullary myeloid tumor (myeloid sarcoma; granulocytic sarcoma; chloroma)

Extramedullary myeloid tumor (EMT; granulocytic sarcoma, myeloid sarcoma) is a tumor mass composed of immature myeloid cells (myeloblasts or monoblasts), which occur outside the bone marrow. EMT may precede or occur concurrently with AML, MDS, or a CMPD. EMT may be the first evidence of AML or precede it by months or years.[1,1539–1541] It involves skin, lymph nodes, bone, paranasal sinuses, testis, GI tract, and the CNS. The most common type of EMT is the granulocytic sarcoma which has a phenotype similar to AML, with or without maturation. EMT with the phenotype similar to AML-M5 may be termed monoblastic sarcoma. Occasional EMTs may display features of biphenotypic acute leukemia.

In a series of EMT patients reported by Pileri et al, 27% (25 patients) presented as *de novo* EMT, 35% (32 patients) had simultaneous AML (M1, 3; M2, 5; M4, 8; M5 10), CMPD (1), or MDS (RAEB-I, 3; RAEB-II, 1; 5q−, 1) and the remaining 38% (35 patients) had a previous history of AML (12),

P. vera (2), ET (1), CIMF (3), CML (7), mastocytosis (1), or MDS (9).[1540] Cytogenetic analysis revealed a normal karyotype in 13/28 patients (46%), whereas 15/28 patients (54%) showed chromosomal abnormalities which included add(6)(q24), add(8)(p23), del(9), del(2), inv(16), −16, −18, t(1;12), t(9;11), t(9;22), t(11;11), t(15;17), +8, +10, and +21.[1543] FISH analysis showed clonal abnormalities in 25/49 cases (54%). Based on the FISH and cytogenetic studies, monosomy 7 (10.8%), trisomy 8 (10.4%) and MLL splitting (8.5%) were the commonest abnormalities, whereas t(8;21) was rare (2.2%).[1543]

PRECURSOR LYMPHOBLASTIC LYMPHOMA/LEUKEMIA (ACUTE LYMPHOBLASTIC LEUKEMIA)

Childhood ALL is a heterogeneous disease consisting of various genetic subtypes, which are used to stratify the patients to particular therapies. Recent treatment strategies for children with ALL advanced the cure rate to near 80%, and molecular genetic analyses of leukemic cells as well as monitoring of early treatment response, as defined by the measurement of MRD, become important prognostic indicators to guide the treatment and predict the overall outcome.[744,1545–1549] Chromosome ploidy status and karyotypic abnormalities are routinely used in clinical decision making. ALLs with t(9;22)$^{BCR/ABL}$, t(12;21)$^{ETV6/RUNX1}$, hyperdiploidy (>50 chromosomes), 11q23^{MLL} rearrangements or t(1;19)$^{E2A/PBX1}$, and T-lineage differ markedly in treatment response.[1548,1549] Hyperdiploidy, which occurs in 25% of children, is associated with a good response to therapy. The presence of t(12;21)(p13;q22)$^{ETV6/RUNX1}$ is associated with a good prognosis in most cases, whereas t(9;22)$^{BCR/ABL}$ and 11q23-associated *MLL* rearrangements relate to a poor prognosis. In adult ALL, the frequency of the t(9;22)$^{BCR/ABL}$ correlates with age and is reported in 50% of patients older than 55 years, while the prognostic relevance of other abnormalities is less obvious (*see below*).

Precursor B-lymphoblastic leukemia/lymphoma (acute lymphoblastic leukemia)

Precursor B-lymphoblastic leukemia/lymphoma (acute lymphoblastic leukemia; B-ALL) is a lymphoproliferative disorder of immature B-cells with blastic morphology (Figure 3.65) which often express

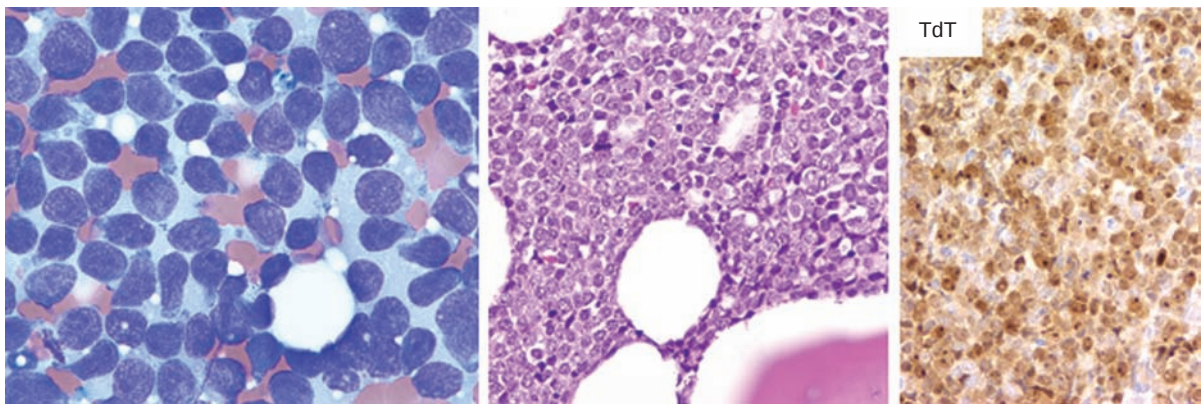


Figure 3.65 Precursor B-lymphoblastic leukemia; bone marrow. Leukemic cells are medium-sized with high nuclear–cytoplasmic ratio and immature chromatin pattern (A). Histologic section shows replacement of the normal bone marrow elements by lymphoblasts (B), which express TdT (C)

CD10, CD34, and TdT. A number of clinical, phenotypic, and genetic features are prognostically significant in B-ALL. The total WBC count at the time of diagnosis is the single most powerful clinical predictor of remission induction and duration, and long term survival for all age groups.[1547–1549] Patients with high WBC counts often have extramedullary disease at diagnosis and are at high risk for relapse in the CNS and testes. Age at diagnosis, certain chromosomal changes, immunophenotype, and persistence of leukemia after induction therapy comprise other important prognostic parameters.[1550–1552] B-ALLs are divided into early pre-B-ALLs (pro-B-ALL; $TdT^+/CD19^+/CD10^-$), common ALL ($CD10^+/CALLA^+$), pre-B-ALL ($CD10^{+/-}$; cytoplasmic IgM^+), and mature B-ALL (surface IgM^+). Mature B-ALL occurs in older children and is characterized by L3 morphology and a higher incidence of CNS involvement. In a series by Cimino et al, adult patients with early-pre-B-ALL had the *ALL1/AF4* fusion transcript, originating from the t(4;11) translocation in 36.4%, and the $t(9;22)^{BCR/ABL}$ in 9%.[397] B-ALLs with t(9;22) often display aberrant expression of pan-myeloid antigens (CD33 or less often CD13). Pediatric patients with ALL blasts possessing myeloid antigens may represent a high-risk group for length of remission and survival.[1556] Adult patients with early-pre-B-ALL and t(4;11) or t(9;22) have a poor prognosis and the absence of both of these translocations correlates with a significantly better clinical outcome after intensive polychemotherapy treatment.[397] CD10 (CALLA) expression does not appear to have independent prognostic significance. [1557–1559] Early pre-B ALL is associated with a favorable prognosis.[1560,1561] The outcome of patients with pre-B-ALL and common ALL is similar.

Cytogenetic and molecular features

Precursor B-lymphoblastic leukemia with hyperdiploidy (>50 chromosomes) has a favorable prognosis, [28,73,1557,1562,1563] especially when there is a concurrent trisomy of chromosomes 4, 10, and/or 17.[1561–1563] Near-tetraploid tumors (92–94 chromosomes) are associated with a high frequency of treatment failure and therefore differ in prognosis from other hyperdiploid ALLs.[1564] Hypodiploid tumors with less than 45 chromosomes, especially those with 24 to 28 chromosomes, had a significantly worse prognosis despite intensive treatment.[94,1565–1568]

Chromosomal translocations are strong predictors of adverse treatment outcome in ALL. The $t(9;22)(q32;q11)^{BCR/ABL}$ is present in approximately 20% of adults and 5% of children with ALL.[31,1569–1571] Features associated with the $t(9;22)^+$ ALL include high WBC count, older age, male predominance, and FAB L2 blast morphology.[1569] There is a strong adverse prognostic significance of t(9;22) in ALL, even after adjustment for other prognostic features.[73,456,1572] Irrespective of the breakpoint site, the presence of any *BCR/ABL* transcript predicts a lower chance of initial treatment response and a lower probability of disease-free survival at 3 years. This poor outcome is not influenced by post-induction high-dose treatment stratifications.[456] In $t(9;22)^+$ ALL patients with otherwise favorable prognostic features (age between 1 and 9, low WBC count) the outcome is better because the disease can be controled by transplantation and intensive chemotherapy.[1571]

The t(1;19)(q23;q13) translocation, associated with fusion of the *E2A* and *PBX1* genes on chromosomes 1 and 19, respectively, is present in 20 to 30% of pre-B-ALL,[1576] and is associated with a poor prognosis.[95,361]

Rearrangements of the mixed-lineage leukemia gene *MLL* (also known as *ALL1*, *Htrx*, and *HRX*) on 11q23 are associated with aggressive acute leukemias and poor prognosis in both children and adults. The t(4;11)(q21;q23), similarly to the Ph chromosome, is associated with high-risk features (high WBC count, age <1 year) and a poor prognosis,[1574,1575] although recent treatment protocols improved the outcome. The *MLL/AF4* rearrangements are the molecular hallmark of ALL with t(4;11). The $t(4;11)^{MLL}/AF4$ is identified in approximately 50% of B-ALLs in

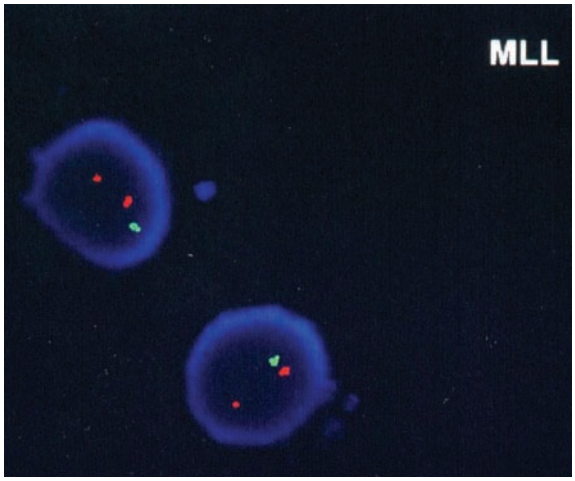


Figure 3.66 B-ALL with deletion of the *MLL* gene; FISH

infants below 6 months of age,[1576] and is associated with poor prognosis.[64,395,396] Figure 3.66 presents B-ALL with deletion of the *MLL* gene.

The cryptic translocation t(12;21) is common in childhood ALL. The presence of a reciprocal translocation of chromosomes 12p13 and 21q22 results in a fusion of the *ETV6* gene (also known as *TEL*) on chromosome 12 and the *RUNX1* gene (also known as *AML1*) on chromosome 21. *ETV6/RUNX1* transcripts, detectable by PCR and/or FISH, are associated with early-pre-B immunophenotype, younger children, and nonhyperdiploidy, and have a favorable prognosis, although relapse may occur in a subset of patients.[1577–1579] It is suggested that a favorable prognosis of t(12;21)$^{ETV6/RUNX1}$ may be associated with intensity of therapy.[1580,1581] The *ETV6/RUNX1*$^{+}$ patients are relatively more sensitive to L-asparaginase.[657] The presence of an intrachromosomal amplification of chromosome 21 (iAMP21), which is associated with multiple extra copies of *RUNX1*, defines a subgroup of pediatric B-ALL with pre-B-ALL immunophenotype, lower WBC count, older age (9 years vs 5 years), and a significantly inferior event-free and overall survival at 5 years (29% vs 78%, and 71% vs 87%, respectively.[1582]

Intensive chemotherapy with regimens appropriate for Burkitt lymphoma reversed the poor prognosis previously associated with ALL positive for the translocations involving the c-*MYC* locus at 8q24 [t(8;14), t(2;8), or t(8;22)].[1583–1585]

The gene expression profile can differentiate lineage and molecular subtypes of ALL,[63,1586,1587] but can also identify genes associated with drug resistance and treatment outcome.[1588] Genes involved in multidrug resistance, cell cycle control, DNA repair, drug metabolism, and apoptosis have been associated with prognosis in ALL.[657,735,843,1588–1592]

Akasaka et al described a new subgroup of B-ALL with *IGH* translocations involving the *CEBP* gene family, which included patients with t(8;14)(q11;q32) involving *CEBPD* on chromosome 8, t(14;19)(q32;q13) involving *CEBPA*, inv(14)(q11q32) or t(14;14)(q11;q32) involving *CEBPE,* and t(14;20)(q32;q13) involving *CEBPB.*[645]

In childhood ALL, MRD is an independent prognostic factor allowing for risk stratification.[986,1593] The most widely applied MRD assays in ALL are FC identification of leukemia immunophenotypes and PCR amplification of antigen–receptor genes, both yielding concordant results in the vast majority of cases.[150,1045] Early elimination of leukemic cells is a favorable prognostic indicator. A ximately half of the children with ALL achieve significant clearance of leukemic cells after 2 to 3 weeks of remission-induction chemotherapy, and these patients have an excellent treatment outcome.[1594] The level and kinetics of MRD by qRT-PCR correlates better with the risk of relapse than qualitative results (MRD^{+} vs MRD^{-}); patients with low levels of MRD and those with decreasing levels after treatment have a lower risk of relapse.[1052–1056] Bone marrow and peripheral blood MRD in patients with ALL is associated with a very high risk for disease recurrence.[1595] In a series by Coustan-Smith et al, the 4-year cumulative incidence of relapse in patients with B-ALL was 80% for those who had peripheral blood MRD at the end of remission induction therapy, but only

13% for those with MRD confined to the marrow.[1595]

Adult acute lymphoblastic leukemia

ALL is uncommon in adults. The major prognostic factors include age, WBC count, time of response to treatment, chromosomal aberrations, MRD, and drug resistance. A better prognosis is associated with age <30 years, WBC count <30 000, t(10;14), and complete remission within 4 weeks. Factors associated with poor a prognosis include elevated total WBC count (>50 × 10^9/l), t(9;22), t(4;11), t(1;19), hypodiploidy, −7, +8, expression of myeloid antigens (CD13, CD33), and persistent MRD.[1593,1596–1599] The 5-year survival rate is 24% and it decreases markedly with age (37% for patients 15–45 years old and 3% for patients ≥75 years old).[1600] The difference in prognosis for elderly patients is most likely associated with reduced tolerance to intensive therapy, co-morbidity and a higher incidence of unfavorable genetic changes (e.g. Ph chromosome).[1601–1603] Similarly to pediatric patients, adults with leukemia harboring aberrations of 19p13 usually do not respond to intensive therapy and have a short survival.[1607]

Based on the cytogenetic-molecular aberrations and disease-free survival (DFS), Mancini et al divided adult ALL into three prognostic categories: (a) no genetic abnormalities or isolated del(9p)(p15-p16) predicted relatively favorable outcome (median DFS > 3 years); (b) the t(9;22)$^{BCR/ABL}$, t(4;11)$^{MLL/AF4}$, or t(1;19)$^{E2A/PBX1}$ predicted a highly adverse prognosis (median DFS 7 months), and (c) 6q deletions, other miscellaneous structural aberrations, and hyperdiploidy predicted an intermediate prognosis (median DFS 19 months).[1605]

The incidence of Ph chromosome$^+$ ALL increases with age: 4% for 15–19 years, 14% for 20–29 years, 24% for 30–39 years, 33% for 40–49 years, and 26% for >50 years.[224] The minor *BCR* breakpoint is more prevalent in ALL (70%).[224] Among adult ALL patients with t(9;22)$^{BCR/ABL}$ additional cytogenetic abnormalities occur in 67% and include gain of a Ph chromosome, −7, +8, +X, and del(9p).[224] In a study of 1522 adult patients reported by Moorman et al, patients with a Ph chromosome, t(4;11)-(q21;q23), t(8;14) (q24.1;q32), complex karyotype (five or more chromosomal abnormalities), or low hypodiploidy/near triploidy all had inferior rates of event-free and overall survival when compared with other patients.[224] In contrast, patients with high hyperdiploidy or a del(9p) had a significantly improved outcome. Multivariate analysis demonstrated that the prognostic relevance of t(8;14), complex karyotype, and low hypodiploidy/ near triploidy was independent of sex, age, WBC count, and T-cell status among Ph-negative patients.[224]

In adult patients, analysis of DFS rates for MRD$^+$ and MRD$^-$ patients demonstrated that MRD positivity is associated with increased relapse rates at all times, being most significant at 3–5 months post-induction and beyond.[1059] For autologous stem cell transplantation, MRD tests before the procedure are predictive of outcome, which differs from allogeneic stem cell transplantation in which outcome is related to results of the tests after the procedure rather than before.[1059] The status of MRD can be used in risk stratification of patients with ALL in conjunction with conventional prognostic factors such as WBC, age, immunophenotype, cytogenetics, and molecular genetics.[1606,1607]

Precursor T-lymphoblastic leukemia/lymphoma (acute lymphoblastic leukemia)

T-ALL accounts for approximately 20–25% of patients with ALL (15% of childhood and 25% of adult ALL).[1546] T-ALL has clinical, immunologic, cytogenetic and molecular features that are distinct from those with B-ALL.[1586,1608–1612] Precursor T-ALL/LBL is a neoplasm of T-lymphoblasts which involves bone marrow and blood, mediastinum and less commonly lymph nodes, skin, gonads, and CNS.[1,1548,1549,1584,1613–1615] Mediastinal

involvement and adenopathy are more common in younger patients than in patients older than 60 years. Morphologically, T-lymphoblasts are similar to B-lymphoblasts. T-ALL/LBL, regardless of the site of involvement, shows replacement of normal structures by a diffuse monotonous infiltrate, often with a 'starry-sky' pattern (Figure 3.67). T-lymphoblasts are positive for TdT, CD1a, pan-T antigens, and CD45. Among pan-T antigens, CD7 and cytoplasmic CD3 are most often expressed and surface CD3 is most often absent.[38,147,148,1368] Most of the cases are dual CD4/CD8$^-$ or dual CD4/CD8$^+$, and only rare cases are either CD4$^+$ or CD8$^+$. T-ALL/LBL most often lacks TCR expression; about one-third of cases are TCR$\alpha\beta^+$, and only rare cases TCR$\gamma\delta^+$. A subset of T-ALL/LBL is positive for CD10, CD56, and pan-myeloid antigens (CD13, CD33).

Chromosomal abnormalities and molecular genetics

T-ALL has been associated with a normal karyotype in 30 to 45% of cases.[395,1476,1614,1616] Recurrent chromosomal translocations are reported in 25–50%.[1618] Most frequent abnormalities include: del(6q), t(10;14), and t(9;22). Pediatric advanced T-lymphoblastic lymphomas (T-ALL/LBL) have a high frequency of chromosome abnormalities and often

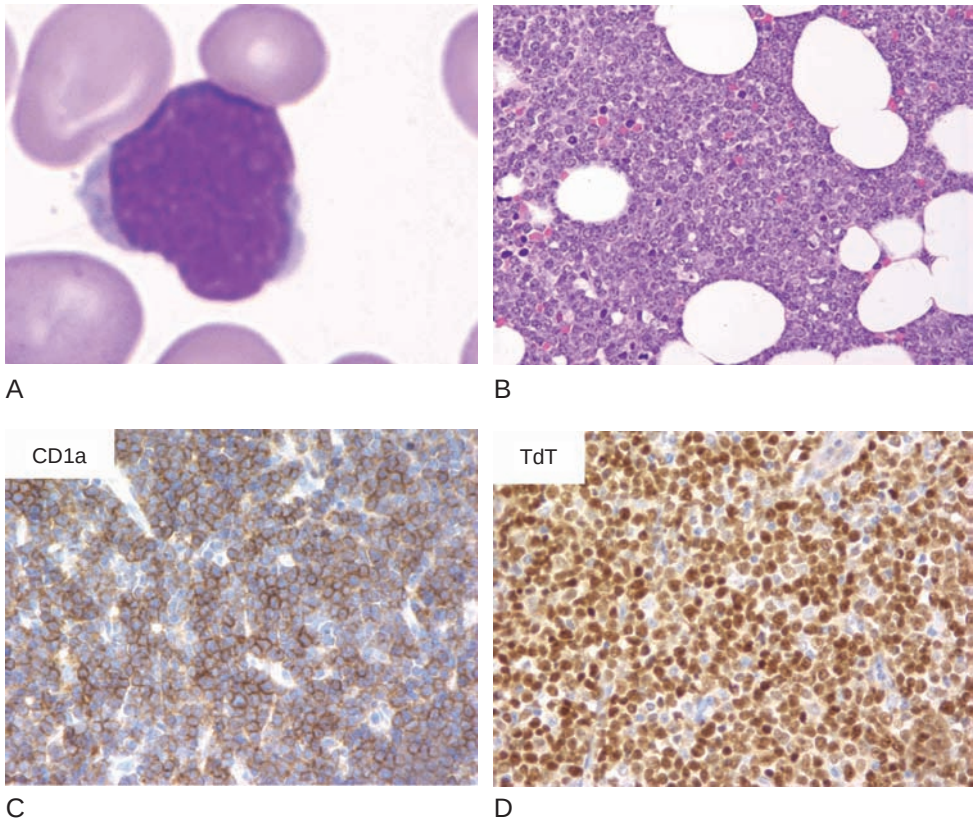
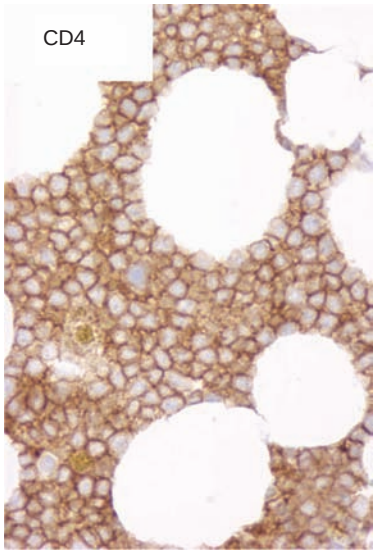
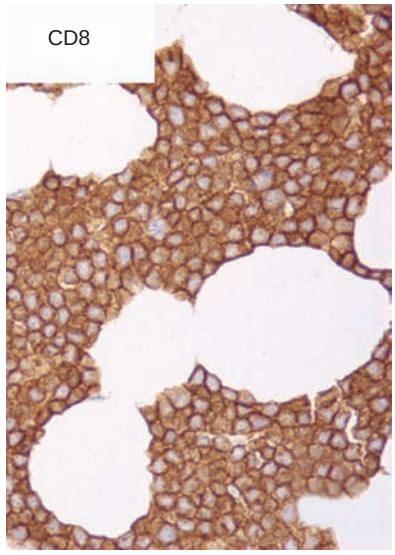


Figure 3.67 Precursor T-lymphoblastic leukemia (bone marrow). Lymphoblasts (A) have delicate chromatin, scanty pale basophilic cytoplasm and nucleoli. Histologic section shows total bone marrow replacement by neoplastic cells (B). Immunohistochemistry shows expression of CD1a (C), TdT (D), CD4 and CD8 (E, F) and CD3 (G)

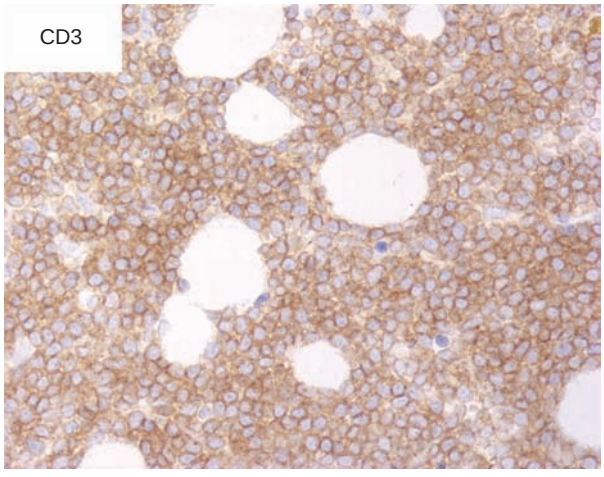


E



F

Figure 3.67, cont'd



G

include translocations involving 14q11.2 (the site of *TCRA/D*).[1476] Tetraploidy is seen in 5% of cases, and does not have a prognostic significance. Lones et al reported numeric chromosomal abnormalities in 5/11 (45%) of children with T-lymphoblastic lymphoma; these included −9, −13, +4, +8, +17, +20, +22, and +mar.[1476] The t(9;22)(q34;q11) encoding the BCR-ABL1 fusion kinase, characteristic of CML, is found in 25% of precursor B-ALL and only rarely (1%) in T-ALL.[1546,1619]

Translocations involving the *TCR* loci (14q11$^{TCRA/D}$ and 7q34^{TCRB}) are found in about 35% of T-ALL/LBL.[325,1476] The translocations involving *TCRA/D* include t(14;20)(q11.2;p11.2), t(11;14)(p13;q11.2), t(1;14)(p32;q11.2), and t(8;14)(q24;q11.2).[1476]

A high percentage of cryptic abnormalities are revealed by FISH, mainly cryptic deletions at 9p21 and 1p32. Cryptic interstitial deletion at 1p32 leading to the *SIL/TAL1* fusion gene is found in 9–30%

of childhood T-ALL. The t(10;11)(p13;q14) encoding *CALM/AF10* is found in 10% (often cryptic); translocations involving *MLL* with various partners represent about 8% of cases and translocations of *ABL1* are rare, except for *NUP214/ABL1* fusion identified in 6%.[393,558,898,1620]

Among the homeobox genes, *HOXA* genes can be upregulated in T-ALL as a consequence of often cryptic inv(7) or t(7;7), which brings the *TCRB* enhancer within the *HOXA* locus.[324,326] *TCRB/HOXA* rearrangements are identified in up to 5% of T-ALLs, which are characterized by mature phenotype with negative CD2, positive CD4, and low or lack of TCR$\alpha\beta$ or TCR$\gamma\delta$ surface expression.[324,326] Chromosome 7 aberrations were found in 4/14 cases (two with inversion 7 and two with deletion 7p15) reported by Cauwelier et al, and only a few additional chromosomal abnormalities were found in five patients: add(6), del(6)(q14), add(5)(q31), del(9)(p21), del(9)(p12.p24), +11, and +21.[324] *TLX3* (class II homeobox gene) expression in T-ALL is due to the cryptic translocation t(5;14)(q35;q32) juxtaposing *TLX3* to the distal region of *BCL11B*. The t(5;14) is found in approximately 20% of childhood T-ALLs and in 13% of adult cases. *TLX3* expressing T-ALLs do not have the favorable outcome reported for *TLX1*$^+$ cases.

Better survival in T-ALL is associated with normal karyotype and with the t(10;14)(q24;q11.2) translocation (involving the *TLX1* gene, also known as *HOX11*), whereas the presence of any derivative chromosome is associated with worse survival.[1609] Five different T-cell oncogenes (*HOX11*, *TAL1*, *LYL1*, *LMO1*, and *LMO2*) are often aberrantly expressed in the absence of chromosomal abnormalities. Using oligonucleotide microarrays, Ferrando et al identified several gene expression signatures that were indicative of leukemic arrest at specific stages of normal thymocyte development: *LYL1*$^+$ signature (pro-T), *HOX11*$^+$ (early cortical thymocyte), and *TAL1*$^+$ (late cortical thymocyte).[744] Hierarchical clustering analysis of gene expression signatures grouped samples according to their shared oncogenic pathways and identified *HOX11L2* activation as a novel event in T-cell leukemogenesis. These findings have clinical importance, since *HOX11* activation is significantly associated with a favorable prognosis, while expression of *TAL1*, *LYL1,* or *HOX11L2* confers a much worse response to treatment.[744] T-ALL with expression of *HOX11* as a result of *MLL/ENL* fusion has a favorable prognosis.[744] *NUP214/ ABL1* expression defines a new subgroup of individuals with T-ALL who could benefit from treatment with imatinib.[558]

In T-ALL, *NOTCH1* was identified as a fusion partner of *TCRB* in the rare t(7;9)(q34;q34.3) leading to the formation of N-terminally truncated constitutively active NOTCH1 peptides. Recently, *NOTCH1* activating mutations have been found in 56% of T-ALLs from all molecular subtypes, suggesting that they occur in immature progenitors.

The *MLL* gene at chromosome band 11q23 is known to rearrange with more than 50 partners in several translocations encoding chimeric proteins. Translocations involving *MLL* are seen in both myeloid and lymphoid leukemias, and are frequent in infants and in secondary leukemias in patients treated with topoisomerase inhibitors. *MLL* fusions are found in 4–8% of T-ALLs.[63,393,1621] The 11q23/*MLL* rearrangements are associated with a poor prognosis. Translocation t(11;19)(q23;p13.3) encoding *MLL/ENL* is often found in young adolescents and carries a better prognosis than other *MLL* rearrangements.[1545]

Plasmacytoid dendritic cell leukemia/lymphoma (blastic NK-cell lymphoma/leukemia; CD4$^+$/CD56$^+$ hematodermic neoplasm)

Plasmacytoid dendritic cell leukemia/lymphoma (blastic NK-cell lymphoma/leukemia; DC2 acute leukemia) is a highly aggressive neoplasm which involves the skin and often disseminates into other organs with leukemic blood and bone marrow involvement. Its ontogenic origin has not been

clearly identified, but it has been suggested that it arises from transformed CD56+ 'plasmacytoid' monocyte-like dendritic cells (pDC; DC-2).[1622–1625] Recently, the WHO/European Organization for Research and Treatment of Cancer (EORTC) classified this entity as CD4+/CD56+ hematodermic neoplasm or early plasmacytoid dendritic cell leukemia/lymphoma.[1626,1627] Integrated genomic analysis using expression profiling and array-based CGH demonstrated that plasmacytoid dendritic cell leukemia/lymphoma shows distinct gene expression profiles and distinct patterns of chromosomal aberrations when compared to acute myelomonocytic leukemia involving skin; plasmacytoid dendritic cell leukemia/lymphoma was characterized by recurrent deletion of regions on chromosomes 4 (4q34), 9 (9p13–p11 and 9q12–q34), and 13 (13q12–q31), that contain several tumor suppressor genes with diminished expression (*Rb1*, *LATS2*).[1628] Tumor cells express CD56, CD43, HLA-DR, and CD4 (Figure 3.68), as well as the following markers: CD45 (dim to moderate expression), CD116, CD123 (IL-3α receptor), CD45RA, BDCA-2 (blood dendritic cell antigen-2; CD303), BDCA-4 (CD304), and ILT-3 (immunoglobulin-like transcript-3).[168,1623,1624,1629–1632] A case with a lack of CD4 expression has been reported.[1633] The 5-year survival is 0%.[1440]

Leroux et al reported clonal, mostly complex chromosome aberrations in 66% of patients with plasmacytoid dendritic cell leukemia/lymphoma.[1629] The recurrent abnormalities involved long arm of chromosomes 5 (targeting two regions, 5q21 or 5q34; 72%), 12p (64%), 13q (64%), −6q/del (6q23–qter) (50%), −15q, (43%), and −9 (28%).[1629] Recurrent aberrations of chromosomes 4, 9, and 13 have also been reported.[1628]

CHRONIC MYELOPROLIFERATIVE DISORDERS

CMPDs, also termed chronic myeloproliferative neoplasms (MPN) in forthcoming WHO nomenclature, represent a heterogeneous range of clonal hematopoietic stem cell diseases which include CML; three major non-CML categories; P. vera; essential

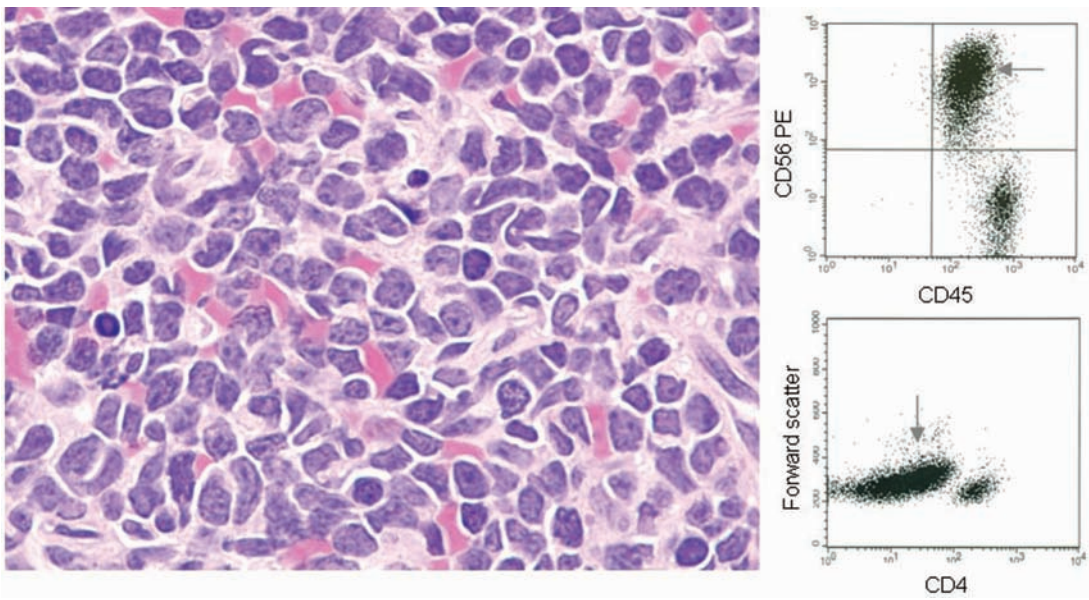


Figure 3.68 Blastic NK-cell lymphoma, skin. Tumor cells are positive for CD4 and CD56

thrombocythemia (ET), and chronic idiopathic myelofibrosis (CIMF); and several less common entities, including chronic neutrophilic leukemia and chronic eosinophilic leukemia/hypereosinophilic syndrome (CEL/HES) (Figure 3.69 and Table 3.8). Generally, CMPDs are characterized by hyperplasia of the at least one of the bone marrow main lineages (granulocytic, erythroid and/or megakaryocytic), leading to increased production of granulocytes (most typical for CML), platelets (a major feature of ET), and red cells (a major feature of PV). Chronic idiopathic myelofibrosis (CIMF) is characterized by bone marrow fibrosis, cytopenia(s), and splenomegaly. The histomorphologic and clinical features of CMPD often overlap, for example thrombocytosis, apart from ET, is often present in P. vera and CML. Marrow fibrosis may be seen in CML, mastocytosis, and P. vera (but not ET). In addition to thrombotic and hemorrhagic complications, CMPD may progress to acute leukemia (blast phase; blast crisis).

CML is associated with t(9;22)(q34;q11.2), resulting in *BCR/ABL* fusion. Chronic eosinophilic leukemia with systemic mastocytosis (CEL-SM), which is often associated with abnormalities of *PDGFRA,* is an example of another CMPD with specific molecular changes. Additionally, there is a correlation between del(13)(q12q14) and CIMF, t(5;12)(q33;p13) and CEL, and del(20q11)/+8/+9 and P. vera, but these anomalies can be seen in various hematologic malignancies.[319] The most common chromosomal anomalies among CMPD in order of

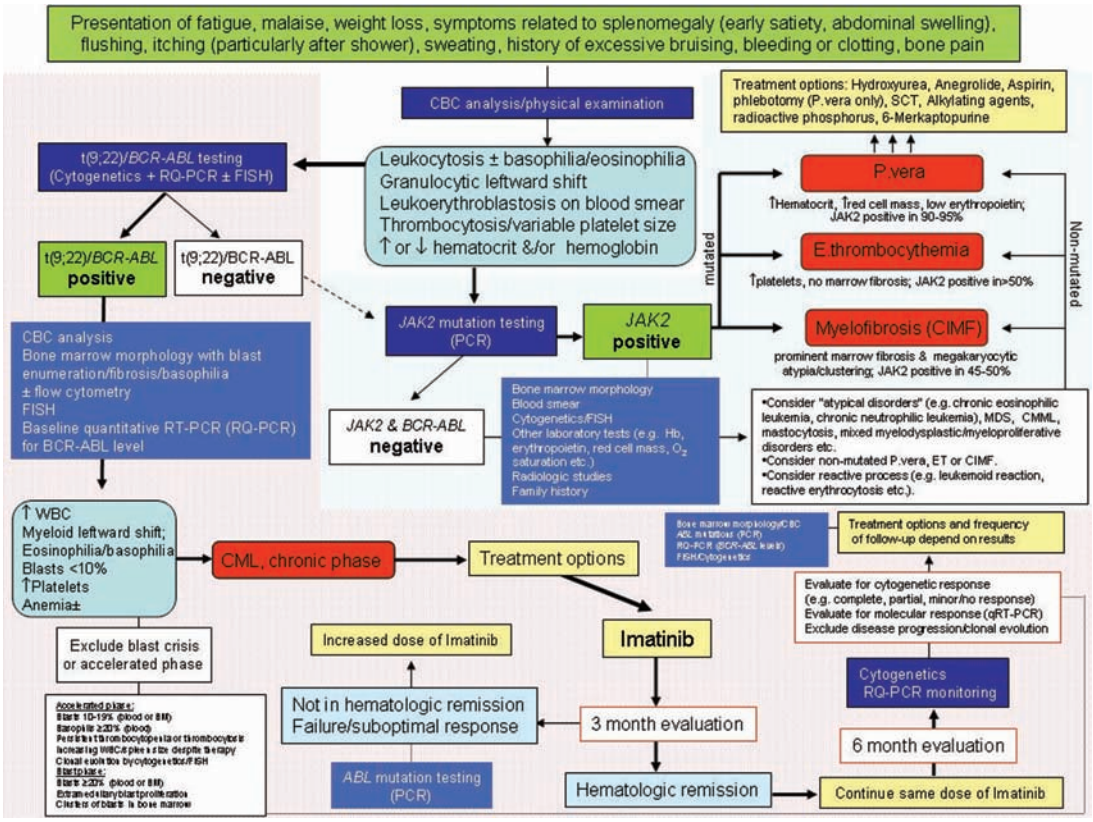


Figure 3.69 Algorithm for the diagnosis of chronic myeloproliferative disorders

Table 3.8 Differential diagnosis of the most common CMPDs

Disorder Feature	*CML*	*P. vera (polycythemic stage)*	*P. vera (classic; advanced stages)*	*CIMF (prefibrotic)*	*CIMF (fibrotic)*	*ET*
t(9;22) [*BCR/ABL*] (Ph chromosome)	Yes	No	No	No	No	No
JAK2^{V617F} mutation	No	Yes (>95%)		Yes (~50%)		Yes (~50%)
c-*MPL*^{515} mutation	No	Yes (occasionally)		No		Yes (occasionally)
PRV-1 overexpression	No	Yes (80%)		Yes (40%)		Yes (30%)
WBC	↑↑↑	Normal	Normal/↑	Normal/↑		Normal/↑
Hemoglobin	↓/Normal	↑ (0.45–0.5 in men; 0.43–0.48 in women)	↑↑↑ (>0.51 in men; >0.48 in women)	Normal	↓/Normal	Normal/↑
Platelets	Normal/↑	Normal	↑	↑↑	↑	↑↑↑
Marrow cellularity	↑↑↑	↑	↑↑	↑↑	Variable	Normal/↑
Myeloid hyperplasia	++	+		↑↑	↑	↑
Erythroid hyperplasia	No	+/++		Normal/↑	Normal/↑	Normal/↑
Megakaryocytic hyperplasia	+/++	++		+++	++/++	+++
Megakaryocytic clustering	+	++		+++		++ (loosely clustered)
Megakaryocytic atypia	+ (‘dwarf’ hypolobated micromegakaryocytes)	++ (pleomorphic, small to giant megakaryocytes with hyperlobulated nuclei)		+++ (very pleomorphic, often bizarre megakaryocytes with hyperlobulated nuclei)		+ (enlarged megakaryocytes with hyperlobulated nuclei)
Iron storage	Variable	Absent		Variable		Variable
Osteosclerosis	No	No		No	Yes	No
Ectasia of sinuses	–/+	+		+	+++	+
Fibrosis	Variable	Variable		–/+	+++	No or borderline increase in reticulin

frequency are t(9;22)(q34;q11.2), –Y, +8, +9, –7, del(20) (q11q13), del(13)(q12q14), del(5)(q13q33), and del(12)(p12).[319] Identification of the molecular equivalent to the Philadelphia chromosome (*BCR/ABL* fusion transcript) made it possible to develop a specific tyrosine kinase inhibitor, imatinib (Gleevec), which is used successfully as the first-line treatment in CML. Leukemic cells in a subset of *FIP1L1/PDGFRA*$^{+}$ CEL/HES are even more sensitive to imatinib, responding to 100 mg/day (CML is most often treated with 400 mg/day while 800 mg/day as a standard therapy is being investigated currently in clinical trials). The cause of Ph chromosome-negative CMPDs is much less certain or unknown, but other kinases are likely candidates, since they have been associated with hematopoietic tumors, e.g. *FLT3* in AML, platelet-derived growth factor receptor beta (*PDGFRB*) in CMML, *PDGFRA* in CEL/HES, and c-*KIT* in systemic mastocytosis (e.g. recurrent missense mutation D816V).[798,799,1634] This is important from the clinical point of view since tyrosine kinase inhibitors (e.g. imatinib), apart from ABL, can target disorders with *ARG*, *PDGFRA*, *PDGFRB*, and c-*KIT* abnormalities.

The mutations of *JAK2*, a cytoplasmic tyrosine kinase with a key role in signal transduction from multiple hematopoietic growth factor receptors, have been implicated in the pathogenesis of CMPDs other than CML.[710] The *JAK2*^{V617F} mutation is observed in the majority of patients with P. vera (≥95%), in ~50% (49–57%) of patients with ET, and in ~50% (44–55%) of patients with CIMF.[35,36,706–708,710,1635] The frequency of the *JAK2*^{V617F} mutation in different CMPDs is presented in Table 3.9. The homozygous *JAK2*^{V617F} mutation is observed in 3–4% of ET, 24–27% of P. vera, and 6–18% of CIMF.[35,36] Two recent studies showed a strong correlation between *JAK2*^{V617F} mutation and PRV-1 overexpression in P. vera, ET, and CIMF, suggesting an allele dose-dependent effect of *JAK2*^{V617F} mutation on granulocyte PRV-1 overexpression.[1635,1636] High levels of *JAK2*^{V617F} expression or progression from heterozygous to homozygous status correlate with PRV-1 overexpression on granulocytes and progressive post-P. vera myelofibrosis.[1637,1638] Kralovics et al reported that patients with *JAK2*^{V617F} mutations had a significantly longer duration of disease, more treatment with cytoreductive agents, and higher rates of complications (myelofibrosis, thrombosis, and bleeding) than did those with wild-type *JAK2*.[708]

Recently, two mutations in the thrombopoietin receptor (c-*MPL*) have been found in patients with CMPDs: W515L and W515K.[747,748,750] These mutations have been evident in patients with ET and CIMF but not in P. vera.[749] *MPL*$^{W515L/K}$ and *JAK2*^{V617F} may occur concurrently, suggesting that these alleles may have functional complementation in myeloproliferative disease.[747,750] Multiple molecular abnormalities are involved in the pathogenesis of

Table 3.9 Frequency of the overall *JAK2*^{V617F} mutation in Ph^{-} CMPD

Study group	*No. of patients*	*PV (%)*	*ET (%)*	*CIMF (%)*
James et al[706]	45	89	—	—
Baxter and scott[710]	140	97	57	50
Kralovics et al[708]	244	64	23	57
Zhao et al[709]	24	83	—	—
Levine et al[707]	325	74	32	35
Jones et al[1635]	166	81	41	43
Goerttler et al[1835]	78	100	33	57
Total incidence		**79**	**35**	**45**
Diagnostic specificity		**64–100**	**23–57**	**35–57**

the CMPDs and aberrant *MPL* expression may be a common denominator of aberrant signaling in both the *JAK2*^{V617F}-positive and *JAK2*^{V617F}-negative CMPDs.[751]

Chronic myeloid leukemia

CML is a clonal stem cell disorder characterized by a proliferation of myeloid cells at all stages of differentiation and the t(9;22)(q34;q11) leading to the formation of the *BCR/ABL* fusion gene.[53,1639,1640] The critical causative event in CML is the formation of the Ph chromosome, which results in the fusion of the *BCR* gene with the *ABL* gene. The c-abl protein, a tightly regulated tyrosine kinase, is predominantly present in the nucleus and plays a key role in cell cycle control. The fused oncoprotein bcr-abl, an activated tyrosine kinase, is mainly present in the cytoplasm.[1641–1646] Introduction of the tyrosine kinase inhibitor, imatinib mesylate (Gleevec) revolutionized the treatment of CML,[1060,1647] (*see below*).

The diagnostic process for CML includes correlation of clinical features, including laboratory findings (e.g. WBC count), evaluation of bone marrow aspirate smear (and often bone marrow trephine biopsy), and most importantly, cytogenetic and molecular studies. Peripheral blood shows leukocytosis with a predominance of neutrophils, absolute basophilia, and eosinophilia. Granulocytic cells show a leftward shift with a 'nonsymmetric' distribution (myelocytes > metamyelocytes). Blasts do not exceed 2%. Platelets may be slightly increased or normal. The bone marrow aspirate is hypercellular with an increased M:E ratio due to both myeloid hyperplasia and erythroid hypoplasia (the myeloid to erythroid ratio is usually around 10:1 to 30:1) (Figure 3.70). Granulocytic cells show full maturation to

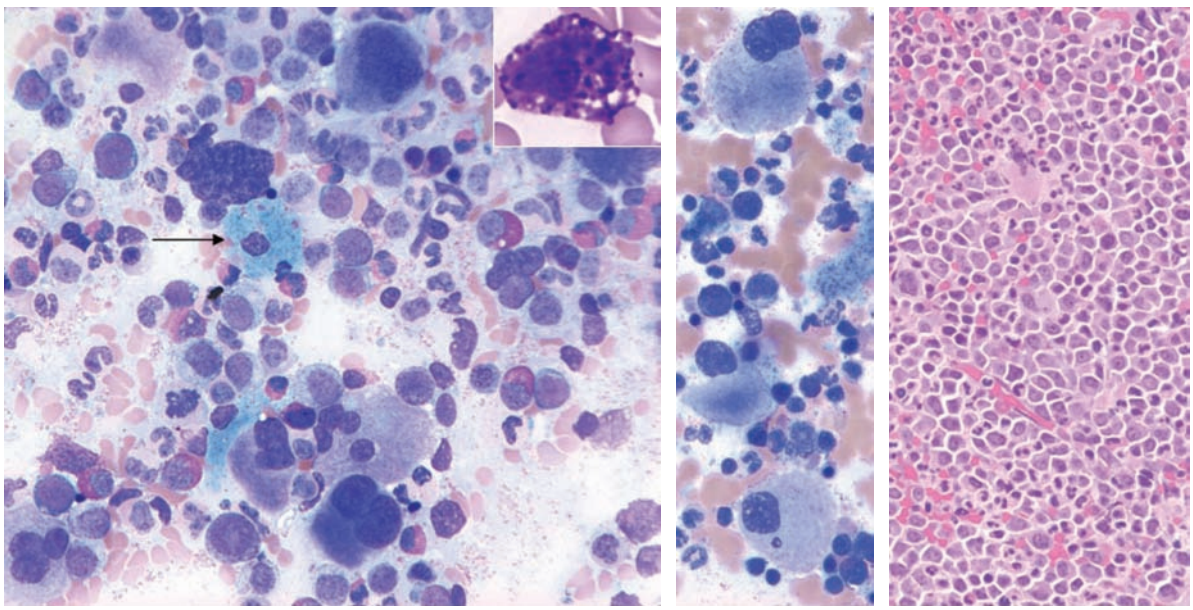


Figure 3.70 Chronic myeloid leukemia (CML). Bone marrow (A, B, aspirate smear; C, histology of core biopsy) is hypercellular with trilineage maturation, myeloid hyperplasia with leftward shift, scattered sea-blue histiocytes (arrow), eosinophilia, atypical megakaryocytes, and occasional basophiles (inset). Typically for CML hypolobated micromegakaryocytes can be identified (B)

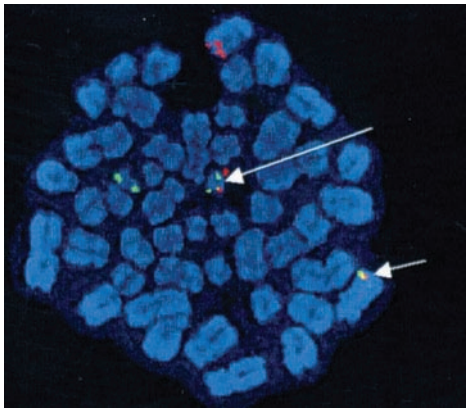
segmented forms with a leftward shift and a predominance of myelocytes ('myelocyte bulge'). Erythroid and granulocytic series do not exhibit overt dyspoiesis. Megakaryocytes may be increased in number and display atypia, most characteristically in the form of small megakaryocytes with hypolobated nuclei. Blasts do not exceed 5% of the marrow cells. Eosinophils and basophils are increased in number. Bone marrow biopsy reveals hypercellular marrow with trilineage maturation, increased M:E ratio, and megakaryocytosis with atypia. Based on histomorphologic features, CML may display three variants comprising the granulocytic category, the megakaryocyte-rich variant, and the myelofibrotic variant.[1641,1645,1648–1652] The megakaryocyte-rich subtype is associated with an increased risk of transformation into myelofibrosis.

The diagnosis of CML is usually based on detection of the Ph chromosome, first described as a shortened chromosome 22 and then as a t(9;22) translocation (Figure 3.71). This abnormality is present in the leukemic cells of more than 95% of patients with CML.[1653] Remaining patients have complex or variant translocations involving additional chromosomes. The translocation between the *ABL* (Ableson leukemia virus) gene on chromosome 9 and the *BCR* (breakpoint cluster region) gene on chromosome 22 leads to the *BCR/ABL* fusion (Figure 2.26; Chapter 2). The encoded fusion protein, bcr-abl, is a constitutively active cytoplasmic tyrosine kinase. The breakpoints may occur at three different regions of the *BCR* gene: major (M-bcr), minor (m-bcr), and micro (μ-bcr). Depending on the site of the breakpoint in the *BCR* gene, the fusion protein can vary in size from 190 kD ($p190^{BCR-ABL}$; m-bcr), to 210 kD ($p210^{BCR-ABL}$; M-bcr), to 230 kD ($p210^{BCR-ABL}$; μ-bcr). The $p190^{BCR-ABL}$ is found most often in patients with ALL. Conventional cytogenetics has been the gold standard for the diagnosis of CML and has been effectively used for monitoring the response to therapy. However, cytogenetic analysis requires fresh samples (bone marrow aspirate), depends on the presence of actively dividing cells, may miss submicroscopic *BCR/ABL* translocations, and its sensitivity is too low for the detection of minimal residual disease (MRD). FISH and PCR methods offer a more sensitive way to diagnose and monitor CML, and can be performed on the blood sample.[1654] FISH analysis can detect large DNA deletions involving chromosome 9 [der(9)], which can be seen in ~10–20% of CML patients.[1655,1656] The presence of der(9) does not correlate with response to therapy or survival in imatinib-treated patients.[1656,1657]

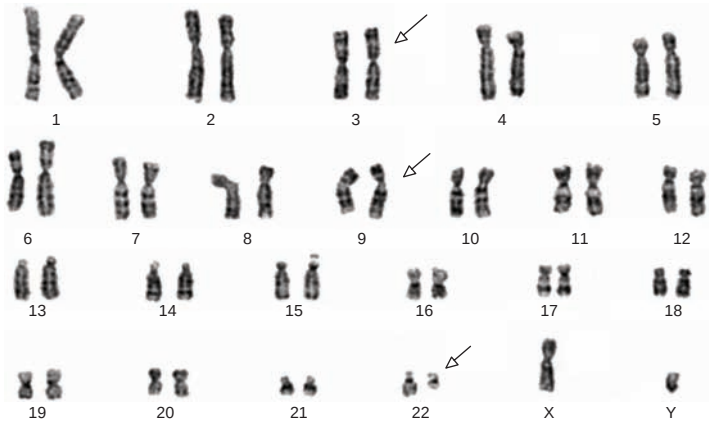
Disease progression

CML usually has a biphasic or triphasic course, with disease progression from the chronic phase (CP) to the accelerated phase (AP; Figure 3.72) and/or the blast phase (BP; Figure 3.72). The majority of patients with CML are diagnosed in CP (~80%).[43,1658] In most untreated patients, a progressive increase in the leukocyte count is observed. Eventually the chronic phase of the disease progresses into an accelerated phase and/or blast phase (blast crisis) of the myeloid or, less often, the lymphoid phenotype. In untreated patients transformation to BP occurs after a median of 3 years. With new treatments (especially imatinib), the course of CML may differ from that observed under prior treatment protocols. The progression is frequently preceded or accompanied by recurring secondary chromosomal abnormalities and oncogene alteration, most often including mutation or deletions of *p53*/*TP53*, trisomy 8, upregulation of *Rb*, *MYC*, and *RAS* genes, doubling of the Ph chromosome (Figure 3.73), i(17q), trisomy 19, and loss of the Y chromosome.[1659–1665] The rate of transformation is initially low (approximate rate of 5% in the first year) and increases gradually to 20 to 25% in each year thereafter.[1669,1670] Patients who had a complete cytogenetic response or in whom levels of *BCR/ABL* transcripts had fallen by at least 3 log had a significantly lower risk of disease progression than did patients without a complete cytogenetic response.[1066]

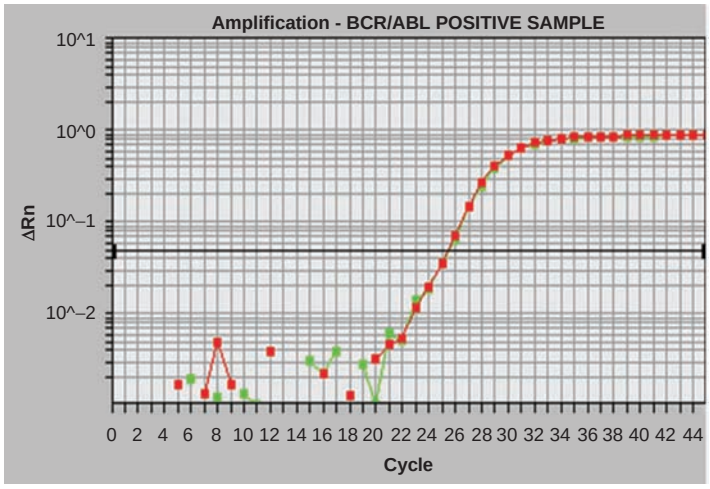
Signs and symptoms of the accelerated phase include: increasing blood basophilia (≥20%),



A



B



C

Figure 3.71 CML with t(9;22) [*BCR/ABL*]; A, FISH; B, cytogenetics; C, PCR

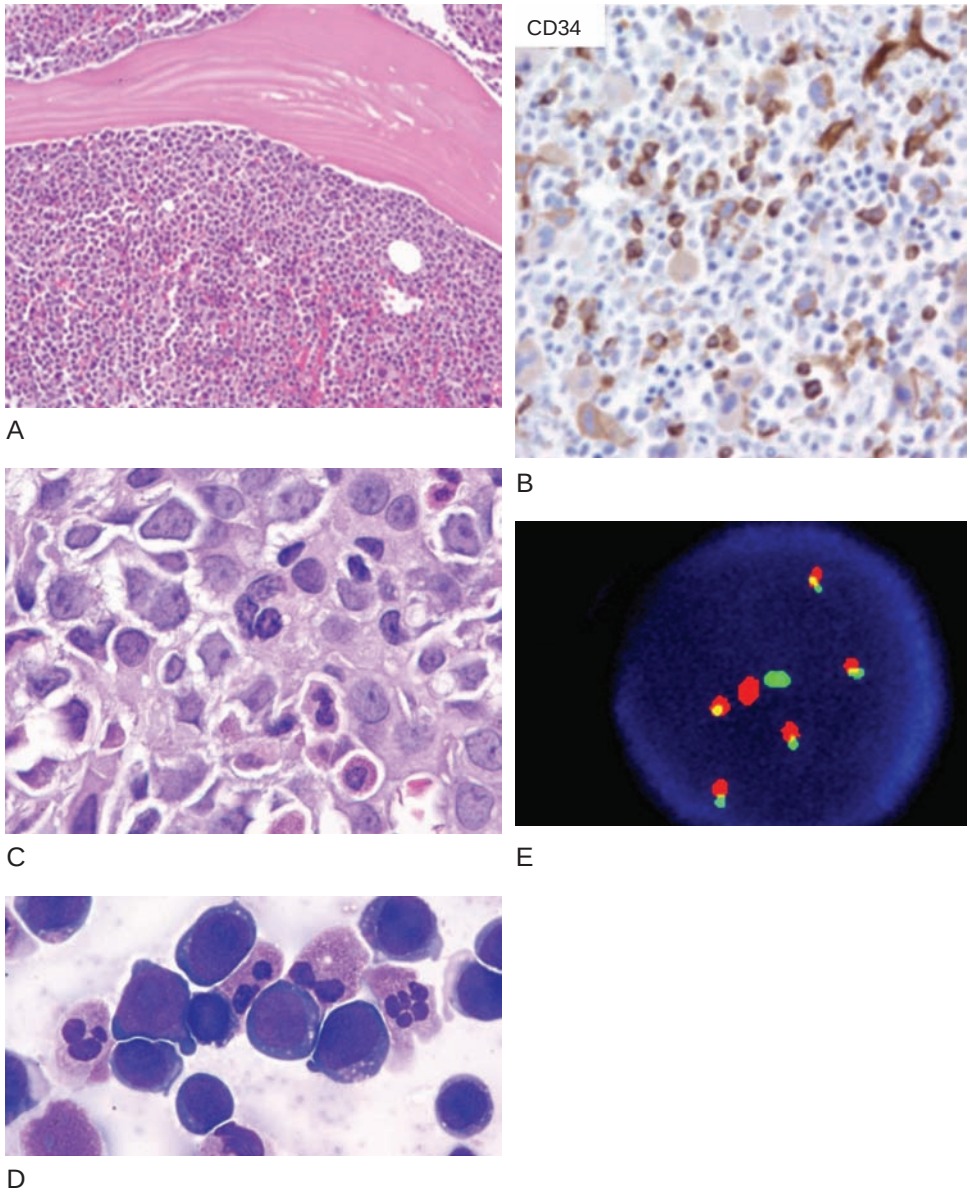


Figure 3.72 CML, accelerated phase (A, B). Histology shows hypercellular marrow with myeloid leftward shift (A). The immunohistochemical staining with CD34 confirmed an increased number of blasts (B). Chronic myelogenous leukemia, myeloid blast crisis (C–E). Bone marrow shows increased number of immature cells (C–D). FISH studies revealed increased number of *BCR/ABL* copies (E)

10%–19% blasts in bone marrow or blood (WHO criteria), cytogenetic evolution (the appearance of additional genetic abnormalities that were not present at the time of diagnosis; clonal evolution), difficult to control WBC, marrow fibrosis, thrombocytosis (>1000 × 10^9/l) unresponsive to therapy, increasing spleen size unresponsive to therapy, and thrombocytopenia <100 × 10^9/l unrelated to therapy.[1] Recent reports of patients treated with imatinib (e.g. MD Anderson Cancer Center,

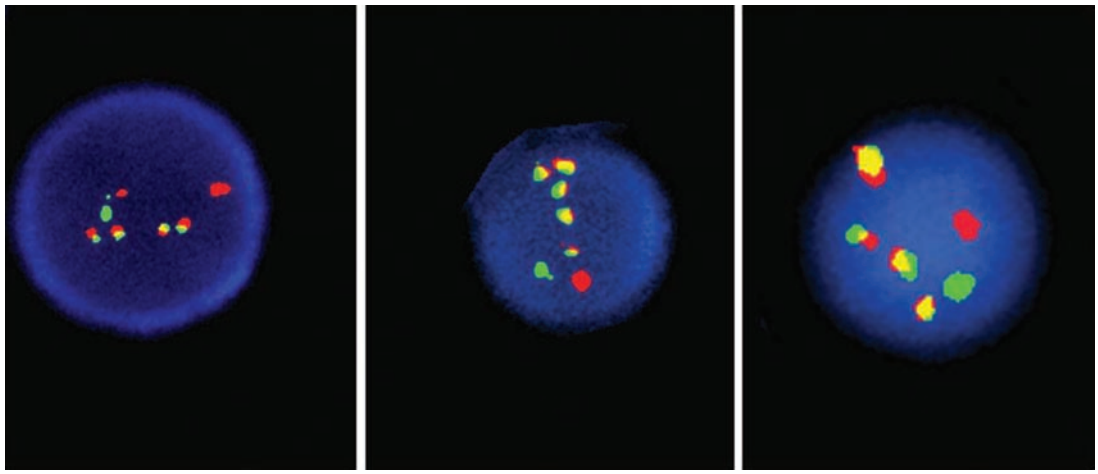


Figure 3.73 Multiple *BCR/ABL* fusion signals, suggesting CML progression (FISH analysis)

International Bone Marrow Transplant Registry, European Leukemia Net) define AP by the presence of the following criteria: 15–29% blasts in blood or bone marrow; >30% blasts + promyelocytes in blood or bone marrow (blasts <30%), ≥20% basophils in blood or bone marrow, cytogenetic clonal evolution, and persistent thrombocytopenia ($<100 \times 10^9$/l).[41,42,50,1061–1063] Cytogenetic clonal evolution, a constant criterion among most AP classifications, occurs in 20-40% of patients.[41] The genomic *BCR/ABL* amplification leading to a *BCR/ABL* overexpression may be seen in patients undergoing acceleration during imatinib treatment.[1671] A negative relationship of additional chromosome abnormalities with imatinib response has been shown, including a lower cytogenetic response rate,[1672] a higher hematologic relapse rate (50% vs 9%),[1673] and a shorter overall survival (75% vs 90% at 2 years).[11]

Signs and laboratory features of BP (blast crisis) include: >20% blasts in marrow or blood, clumps of blasts on marrow examination, and extramedullary myeloid tumor (WHO criteria). The median survival of BP is 2 to 6 months (long-term survival is uncommon).[52,1674,1675] The response to high dose chemotherapy with allogeneic stem cell transplantation is poor,[52] due to upregulation of anti-apoptotic signals and MDR. Despite the generally dismal prognosis of CML in the BP when compared to *BCR/ABL*-negative acute leukemia, determination of blast lineage in transformed CML is clinically important. Patients with lymphoblastic transformation have a better response to chemotherapy and longer survival than patients with myeloblastic transformation.[1673,1674] Leukocyte alkaline phosphatase (LAP) is reduced in CML patients at diagnosis and the LAP values increase when CML transforms to more advanced disease (AP or BP). Cytogenetic clonal evolution is a known poor prognostic factor in CML. The lack of cytogenetic response at 3 months appears to be a stronger independent poor prognostic factor for survival than the clonal evolution for both the chronic and accelerated phases. The presence of additional *BCR/ABL* copies (Figure 3.73) suggests disease progression and poor prognosis.

Disease monitoring

Imatinib mesylate (Gleevec; Glivec) can induce complete or nearly complete remission in up to 80% of patients.[50,51,1060,1061] Ninety percent of patients with early-stage (chronic phase) CML and 60% of patients with advanced stage CML (blast crisis) achieve a hematologic response (HR) to imatinib.[1066,1067] The response to treatment is based on the hematologic,

cytogenetic, and molecular parameters (*see* Table 2.6; Chapter 2).[41,42,50,51,1061,1063,1064]

A HR indicates a return of peripheral blood cell counts and bone marrow morphology to normal. A cytogenetic response (CyR) can be divided into complete, major, minor, and minimal (Table 2.6). A complete cytogenetic response (CCyR) indicates the disappearance of the Ph chromosome, and a major cytogenetic response (MCyR) indicates less than 35% Ph^+ cells in bone marrow (Table 2.6). A major molecular response (MMR) is defined as a 3 log reduction in the *BCR/ABL* transcript (≤0.10 *BCR/ABL* ratio according to the International Scale), and a complete molecular response (CMR) is synonymous with undetectable transcripts by qRT-PCR (RQ-PCR). The preferred initial treatment is daily imatinib.[42] A dose increase of imatinib, allogeneic stem cell transplantation, or investigational treatment is recommended for cases of failure, and could be considereds in cases of suboptimal response. The European Leukemia Net suggested the following definitions for failure: 3 months – no hematologic response; 6 months – incomplete HR or no CyR; 12 months – less than partial CyR (Ph chromosome positive); 18 months – less than CCyR; loss of HR or CyR; and appearance of highly imatinib-resistant *BCR/ABL* mutations.[42] The same group defined a suboptimal response at 3 months as an incomplete HR, at 6 months as a less than partial CyR, at 12 months as a less than CCyR, at 18 months as a less than major molecular response, and loss of MMR, other mutations, or additional chromosomal abnormalities.[42]

In a study of 454 patients treated with a daily dose of 400 mg of imatinib, 95% achieved a complete HR, 60% achieved a major CyR, and 89% of patients did not progress to AP or BP CML after 18 months of follow-up.[50] Patients on imatinib who achieved at least some degree of cytogenetic response after 6 months (at least a minor response) had a better survival; those with no CyR or minimal response had significantly worse survival. This suggests that cytogenetic responders obtain benefit from imatinib, but patients who show no cytogenetic response should be given alternative treatment.[1678] Cytogenetic clonal evolution is not an important factor for achieving major or CCyR with imatinib mesylate therapy, but it is an independent poor prognostic factor for survival in both chronic and accelerated phases of CML.[11] Clonal evolution can be identified during treatment with imatinib in patients who are in complete hematologic remission. Patients with *de novo* clonal evolution in the absence of any other sign of disease progression had a significantly higher incidence of progression by 18 months than non-clonal evolution patients.[1679] Acquisition of clonal evolution increases the risk of subsequent disease progression also in CML patients in complete hematologic remission on imatinib.[1676]

Predictors for relapse after hematopoietic stem cell transplantation include advanced disease stage and slow reduction of *BCR/ABL* transcripts at day 28 and day 56 after transplant. Lange et al concluded that a complete clearance of *BCR/ABL* transcripts was achievable within 4 weeks from transplantation even after minimal conditioning and that early kinetics of *BCR/ABL* transcripts significantly correlated with the probability of hematologic relapse.[1677] Sokal and Hasford Scores did not predict survival after hematopoietic stem cell transplantation.[1678] Imatinib has significant activity against CML relapsing after allogeneic stem cell transplantation:[1679] the overall HR rate was 84% (98% for patients relapsing in chronic phase), the CCyR was 58% for patients in the chronic phase, 48% for AP, and 22% for patients in BP. With imatinib treatment, 57% achieved full donor chimerism and 14% mixed chimerism.[1679]

Cytogenetic analysis has been the mainstay of disease monitoring in CML. Response criteria based on the percentage of Ph-positive cells in the bone marrow were established for patients on interferon-α. Since most of the patients treated with imatinib achieve CCyR, monitoring for MRD with more sensitive test(s) became important in recent management of CML patients. Over the past several years

qRT-PCR assays were introduced to measure the transcript levels in the blood and marrow, which correlate with the number of leukemic cells in blood or bone marrow and can be used to monitor response to therapy. The early qPCR studies used titration assays which incorporated competitive targets. Internal controls were introduced for some methods to control the variation in the quality of the samples. The *BCR/ABL* copy number was normalized to the number of the control gene transcripts and the value was used to monitor patients after bone marrow transplantation. The technique was time consuming and expensive and its use was limited to specialized laboratories. The introduction of qRT-PCR techniques in the late 1990s have largely replaced the competitive quantitative procedures. The real-time technique is performed on an analyzer that incorporates a thermal cycler, fluorescence detection, and result calculation, which has greatly simplified quantitative assays. For sensitive and reproducible results high-quality RNA is essential. Normalization to the control gene compensates for variations in the quality of the RNA and for differences in the efficiency between reverse transcription reactions. The control gene must therefore degrade at the same rate as the target for accurate normalization. The two control genes that have been widely assessed for *BCR/ABL* quantitation are *BCR* and *ABL*. The qRT-PCR methods vary in respect to the type of instrument used, the primer and probe location, the real-time chemistry, and the control gene. These differences can lead to a variation in the sensitivity and measurement reliability between methods. It is essential that each laboratory establish these limits for their method to allow accurate interpretation of serial monitoring.

The clinical usefulness of *BCR/ABL* quantitation by qRT-PCR has been demonstrated by several studies. The qRT-PCR analysis of patients treated with imatinib has shown a strong correlation between the percentage of Ph-positive metaphases in the bone marrow and *BCR/ABL* levels in blood measured by qRT-PCR.[1071,1680–1687] Early reduction of *BCR/ABL* transcript levels predicts CyR in chronic phase CML patients treated with imatinib and the reduction of *BCR/ABL* correlates with prognosis.[1681–1683] After 12 months of therapy, the majority of patients treated with imatinib achieve a CCyR (0% Ph-positive cells in the marrow). This is approximately equivalent to a 2-log reduction in leukemia load below a standardized baseline level as measured by qRT-PCR.[1682] To compare, among patients treated with interferon-Ara C (INF-AraC), 93% had not achieved a CCyR after 12 months of therapy.[1071,1072,1073] Among patients with CCyR after 12 months, ~40% achieve a 3-log reduction in transcript levels. Approximately 30% of imatinib treated patients achieve a CCyR but do not achieve a ≥3-log reduction in transcript levels.[1075]

The risk of progression (loss of MCyR, loss of a complete HR, progression to AP or BP, and death from other causes while on imatinib) according to the level of response achieved at 12 months showed that there was a clear difference in progression-free survival (PFS) according to the level of leukemia reduction. The risk of progression in patients who did not achieve a CCyR by 12 months was 20%. In patients who achieved a CCyR but not a 3-log reduction, the risk was 8%. However, in the group who achieved a 3-log reduction at 12 months, not a single patient had disease progression with a 2-year follow-up. Hughes et al proposed that a 3-log reduction be defined as an MMR because it comprised a group of patients with remarkable stability of response and represented a further 1-log reduction below the level of CCyR.[1075] One hundred percent of patients with MMR were alive and free from AP or BP after 60 months' follow-up, compared to 95% of those with CCyR but with less than a 3-log reduction in transcript levels and 88% of those without CCyR.[1066]

Hughes et al suggested that the terms 'PCR negative' and 'complete molecular response' should be used with caution.[1072,1073] Using current technology, a sensitivity of >4.5 logs below baseline can be achieved with good quality RNA samples. The level

of *BCR/ABL* reduction achieved early after the start of imatinib therapy is a good indicator of subsequent response.[1680–1683] Patients who do not achieve a 1-log reduction by 3 months have a very low probability of achieving an MMR, defined as a ≥3-log reduction in *BCR/ABL* level (only 13% at 30 months). This compares to a probability of 100% and 69% in those achieving a >2-log and 1–2-log reduction, respectively. Failure to achieve an MMR in the group with a less that 1 log reduction at 3 months was largely due to a high level of primary and acquired resistance in this group. The estimated risk of resistance in this group was 83% compared to 5% and 0% in the 1–2-log and >2-log groups, respectively.

Imatinib resistance

Imatinib mesylate (Gleevec) is a specific tyrosine kinase inhibitor that blocks the activity of *BCR/ABL*, *ABL*, *ABL*-related gene (*ARG*), platelet-derived growth factor receptor (*PDGFR*) and the Kit receptor. Imatinib is standard treatment for patients with CML with durable responses in most patients. However, a subset of patients eventually develops resistance, particularly those treated in the advanced stages.[44,544,552] Mutations in the *BCR/ABL* kinase domain occur in over 90% of patients who relapse after an initial response.[554] Resistance can be broadly categorized as primary or acquired and as *BCR/ABL*-dependent and *BCR/ABL*-independent.[544,547–549,551,552,1684,1685] Resistance can be additionally subdivided into hematologic, cytogenetic, and molecular resistance, referring to persistence of hematologic parameters, Ph chromosome (lack or loss of MCyR defined by ≤35% Ph^+ metaphases or CCyR defined by 0% Ph^+ metaphases) and residual *BCR/ABL* transcripts (lack or loss of CMR), respectively. Lack or loss of MMR should be confirmed in at least two consecutive samples. Generally, factors contributing to imatinib resistance include mutations within *BCR/ABL*, *BCR* amplification or overexpression, clonal evolution and decreased imatinib bioavailability or cell exposure.[543,545,547–551,553,556,1668,1685–1688]

Primary (intrinsic) resistance means lack of efficacy from the onset of imatinib treatment and can be defined by the failure to achieve complete HR by 3 months or MCR by 6 months. It is not known at this stage what causes primary resistance but it is rarely if ever caused by point mutations in *BCR/ABL* (by contrast, point mutations are the cause of 35–90% of cases of acquired resistance). Overall around 20–25% of newly diagnosed patients appear primarily resistant to imatinib at 400 mg/day (4% of previously untreated patients do not achieve a complete hematologic remission and 23% patients do not achieve a MCR by 6 months with continuing imatinib at 400 mg/day).[547,1072,1073]

Acquired resistance can be defined as progression from CP to BP, progression to AP, loss of HR, loss of MCyR, or loss of CCyR, with a 10-fold rise in *BCR/ABL*.[1689] Mutations in the *BCR/ABL* kinase domain that interfere with imatinib binding and lead to the reactivation of kinase activity are the most common mechanisms of acquired resistance and more than 35 mutations have now been described.[548,549,554,1685] Additionally, amplification of the *BCR/ABL* gene can occasionally be detected in resistant patients.[552,1671,1690] Campbell et al reported two patients who acquired resistance against imatinib due to a *BCR/ABL* amplification presenting as a homogenously staining region (hsr) on either derivative chromosome 22 or 17.[1690] In a series of 72 patients with imatinib resistance reported by Hochhaus et al, one patient showed a point mutation in the *ABL* kinase domain and *BCR/ABL* amplification simultaneously.[542] Other factors considered in imatinib resistance include clonal evolution and decreased imatinib bioavailability or cell exposure, and modulation of drug efflux or influx transporters.[552,553] The acquisition of additional cytogenetic changes (clonal evolution) during treatment of CML with imatinib mesylate is currently regarded as an index of increasing resistance to imatinib. Detection of emerging mutations can be analyzed by relatively rapid PCR methodology with direct sequencing of the *BCR/ABL* kinase domain

(depending on the level of *BCR/ABL* transcripts, a one- or two-step PCR is required to amplify the kinase domain). The frequency of *BCR/ABL* mutations in resistant patients was reported to range from 42[545] to 90%,[554] depending on the methodology of detection, the definition of resistance, and the phase of the disease. Mutations are found more frequently in AP and BP when compared to CP. Acquired imatinib resistance may be associated with *BCR/ABL* independence and mediated in part through overexpression of other tyrosine kinases.[551,1684] The emergence of a mutant clone is highly associated with a rising level of *BCR/ABL* as measured by qRT-PCR.[1691] Even very small rises of just over 2-fold measured by a highly reproducible quantitative assay had biologic significance in most cases, identifying patients with mutations. Therefore, patients with stable or decreasing *BCR/ABL* levels may not require mutation screening. Biochemical and cellular assays have demonstrated different levels of resistance for the various mutations.[544,550,554,1692] Clinical studies have shown that mutations located within the region of the P-loop are associated with a poorer prognosis, with rapid transformation and a median survival of 4.5 months.[549,556] However, Jabbour et al did not confirm a poor prognosis in patients with P-loop mutations compared to those with other or no mutations[1688] (the study included patients still in cytogenetic remission, many of whom received new tyrosine kinase inhibitors, nilotinib and dasatinib, which may have favorably influenced their survival). It is therefore suggested that the prognosis is dependent on several other factors, especially the use of emerging new treatment strategies after failure with imatinib.

The second generation of targeted therapies, which include novel small-molecule inhibitors such as nilotinib (AMN107), dasatinib (BMS-354825), INNO-406 (NS-187), MK-0457 (VX-680), and ON012380 are being developed and successfully tested in patients with imatinib resistance/refractory disease even during BP.[1693–1699] Dasatinib induces hematologic and cytogenetic responses in patients with CML or Ph-positive ALL who cannot tolerate or are resistant to imatinib.[1696]

Table 3.10 presents new definitions of treatment failure and suboptimal response proposed by the European Leukemia Net.[42] To overcome imatinib-resistant disease, the treatment interventions in patients with failure or suboptimal response include: (a) imatinib dose escalations, (b) stem cell transplantation, (c) novel tyrosine kinase inhibitors (dasatinib, nilotinib) with activity against imatinib- resistant mutations and/or inhibition of alternative pathways such as Src activation, (d) combination therapy, (e) treatment interruption to stop clonal selection of resistant cells, and (f) clinical trials.[41,42,543] Clinical strategies to prevent and/or treat resistant disease are presented in the recent reviews by Hochhaus et al and Jabbour et al.[543,1700]

Polycythemia vera

P. vera is a chronic clonal myeloproliferative disorder characterized by an increase in the number of red

Table 3.10 Definitions of failure and suboptimal response by the European Leukemia Net[42]

Time	*Failure*	*Suboptimal response*
3 months after diagnosis	no HR	less than CHR
6 months after diagnosis	less than CHR, no CR	less than PCyR
12 months after diagnosis	less than PCyR	less than CCyR
18 months after diagnosis	less than CCyR	less than MMR
Anytime	loss of CHR, loss of CCyR, mutations	additional chromosomal abnormalities in Ph+ cells, loss of MMR, mutations

CCyR, complete cytogenetic response; CHR, complete hematologic response; HR, hematologic response; MMR, major molecular response; PCyR, partial cytogenetic response

blood cells and the total blood volume, and often leukocytosis, thrombocytosis, and splenomegaly. Thrombosis and bleeding are major causes of morbidity and death in patients with P. vera. Morphologically, P. vera is characterized by megakaryocytosis with cytologic atypia (Figure 3.74), including giant pleomorphic megakaryocytes containing deeply lobulated nuclei and proliferation of erythroid and myeloid precursors (panmyelosis).[1701] The diagnosis of P. vera is based on clinical and biologic parameters defined by WHO and the Polycythemia Vera Study Group (PVSG), and more recently on WHO bone marrow morphology features and European clinical, molecular, and pathological criteria [WHO/ECMP criteria][1,35,1701–1703] (*see* Figure 3.75 and Table 3.11).

Somatic point mutations of the tyrosine kinase *JAK2* (*JAK2*^{V617F}) have been reported in ≥95% of P. vera, but they can be also seen in ET and CIMF.[706,708,710] The presence of the *JAK2* mutation and identification of an abnormal karyotype are very useful in the management of patients with erythrocytosis, since it helps to exclude secondary etiology. Chromosomal abnormalities are found in ~15% of P. vera patients at diagnosis. The most common recurring abnormalities include +8, +9, del(20q), and 1q duplications.[278,279,312,1704–1706] FISH analysis increases the percentage of abnormal cases by 1.5-6% when compared to conventional cytogenetics,[278,1706] but trisomies 8 and 9 and deletions in 13q and 20q are more often detected by classic cytogenetics than FISH.[1707] Similarly to other CMPDs, as the disease progresses, chromosomal abnormalities become more frequent.[279,1705] Patients treated with myelosuppressive agents showed a significantly greater risk of chromosome abnormalities than did patients who had been phlebotomized.[1705]

The risk of disease transformation into marrow fibrosis or myelodysplasia (MDS)/AML increases over time and ranges from 5 to 15% after 10 years of disease.[1705,1708–1717] In a series reported by Fruchtman et al, 2.8% of patients with P. vera developed an acute leukemia/ myelodysplastic syndrome (13/462), with a maximum follow-up of 7 years. ET and PV patients who transformed to acute leukemia had all been previously exposed to other cytotoxics; there were no ET or PV patients in the study who transformed to acute leukemia exposed solely to anagrelide.[1712] Exposure to P32, busulphan, and pipobroman, but not to hydroxyurea, has an independent role in producing an excess risk for progression to

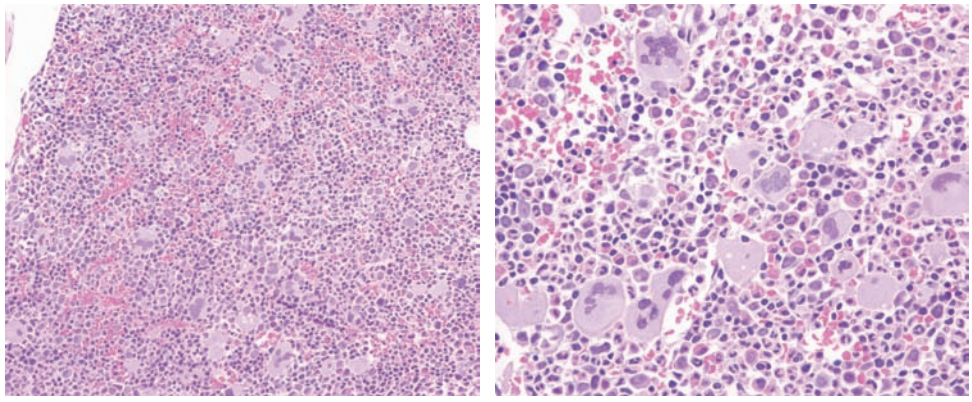


Figure 3.74 Polycythemia vera. A, Bone marrow core biopsy shows hypercellular marrow with increased M:E ratio and megakaryocytosis. B, High magnification displays numerous atypical megakaryocytes

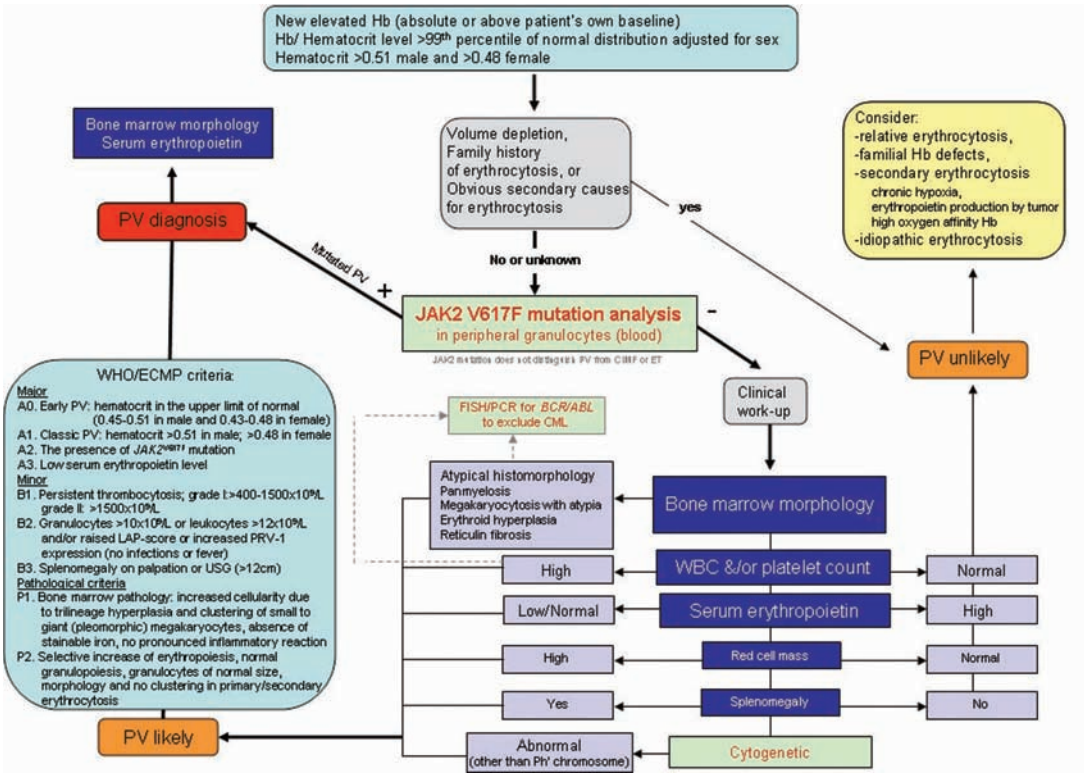


Figure 3.75 Algorithm for the diagnosis of P. vera (PV)

Table 3.11 WHO bone marrow morphology and European clinical, molecular, and pathological (ECMP) criteria for the diagnosis of P. vera (WHO/ECMP criteria)[35–37]

Clinical and molecular criteria
Major
A0. Early PV: hematocrit in the upper limit of normal (Ht: 0.45–0.51 in men and 0.43–0.48 in women)
A1. Classic PV: Ht > 0.51 in men and > 0.48 in women
A2. The presence of *JAK2*^{V617F} mutation
A3. Low serum Epo level
Minor
B1. Persistent increase of platelets count; grade I: 400–1500; grade II:>1500
B2. Granulocytes >10 × 10^9/l or leukocytes >12 × 10^9/l and/or raised LAP score or increased PRV-1 expression in the absence of infection
B3. Splenomegaly on palpation or on USG (>12 cm in diameter)
Pathologic criteria
P1. Bone marrow pathology: increased cellularity due to trilineage hyperplasia and clustering of small to giant (pleomorphic) megakaryocytes with hyperlobulated nuclei (overt PV), absence of stainable iron. No pronounced inflammatory reaction (plasmacytosis, cellular debris)
P2. Selective increase of erythropoiesis, normal granulopoiesis, and megakaryocytes of normal size, morphology, and no clustering in primary/secondary erythrocytosis

By WHO/ECMP criteria, A0, A2, B1, and P1 establish early PV (PV stage 0 or masked PV); A1, A2, P1, and none of B establish polycythemic PV (PV stage 1); A1, A2, P1, and one or more of B establish classic and advanced PV stages 2 and 3. A1 and P2 with normal or increased EPO is consistent with erythrocytosis

AML/MDS compared with treatment with phlebotomy or interferon.[1713]

Essential thrombocythemia

ET is a relatively indolent and often asymptomatic clonal myeloproliferative disorder that involves predominantly megakaryocytic lineage that is characterized primarily by a sustained elevation in platelets ($\geq 450 \times 10^9$/l), megakaryocytosis and minimal to absent bone marrow fibrosis.[1,48,1706,1714–1720] Increased and loose clustering of giant to large megakaryocytes with mature cytoplasm and multilobulated staghorn-like nuclei in a normcellular or only slightly hypercellular bone marrow represent major hallmarks of ET.[1648,1701,1718] The early (prefibrotic) phase of CIMF differs by marrow hypercellularity and cohesive clusters of megakaryocytes with often bulbous, bizarre nuclei with maturation defect.[1721] Diagnosis has to be based on clinical and laboratory features, taking into consideration the dynamics of this disorder. Therefore, ET can be diagnosed with a lower platelet count (a proposed cut-off point is 400×10^9/l), when patients present with signs and symptoms that are not usually encountered in reactive thrombocytosis (e.g. relevant thromboembolic or hemorrhagic complications).[1701,1715,1722–1724] It was recently suggested that the platelet count threshold for ET diagnosis can be lowered from 600 to 450×10^9/l.[35,1703] Currently proposed WHO bone marrow and ECMP criteria for the diagnosis of ET include: (C1) sustained platelet count above the upper limit of normal ($>400 \times 10^9$/l); (C2) presence of large or giant platelets in blood smear; (C3) normal values of hemoglobin, hematocrit, erythrocytes, white blood cell differential count; (C4) presence of *JAK2* mutation or *MPL* mutation; (C5) absence of Ph chromosome or any other cytogenetic fusion-gene abnormality; (P1) increase of dispersed or loosely clustered, predominantly enlarged megakaryocytes with mature cytoplasm and hyperlobulated nuclei; (P2) no proliferation or immaturity of erythropoiesis and granulopoiesis and no or borderline increase of reticulin (myelofibrosis grade 0). According to WHO/ECMP criteria C1 + P1 and P2 establishes the diagnosis of true ET.[35,37]

A mutation in the *JAK2* gene can be detected in a significant subset of patients with ET (up to 57%).[1718,1725] Patients with *JAK2*-V617F$^-$ ET do not commonly progress to become *JAK2*-V617F$^+$.[1726] Consistent with the concept of distinct pathogenetic mechanisms, Campbell et al showed that patients with and without the *JAK2* mutation have different patterns of cytogenetic abnormality, with virtually all patients carrying the 20q deletion or trisomy 9 being *JAK2*^{V617F}-positive.[1726] The presence of the *JAK2* mutation does not appear to be a predictor of inferior survival in ET patients or risk of transformation to acute leukemia.[1725,1727] Chromosomal abnormalities in ET are very rare and most of the patients studied were either in leukemic transformation or they had received treatment with cytotoxic agents. In the study by Panani et al (67 cases with ET) 4 cases presented chromosomal abnormalities, which included del(5)(q13q33) (accompanied by trisomy 20 in one case), monosomy 17, trisomy 13, monosomy 14, and a small marker chromosome.[1728] FISH studies detect chromosomal abnormalities more often than conventional cytogenetics, with trisomy 8 and trisomy 9 being often reported (the trisomies are detected by FISH usually in a small subset of analyzed cells).[1729]

A transformation of ET to AML, MDS or myelofibrosis is a relatively rare event and occurs in 1–5% of all patients.[48,1727,1730–1733] The risk of leukemic or any myeloid disease transformation is low in the first 10 years (1.4% and 9.1%, respectively) but increases substantially in the second (8.1% and 28.3%, respectively) and third (24.0% and 58.5%, respectively) decades of the disease.[1730] Cytogenetic changes that may be associated with the transformation to AML are common and include: t(2;3), der(1;7)(q10;p10), t(2;17), del(17p), trisomy 8, trisomy 8, trisomy 21, and chromosome 7 and 13q abnormalities.[1734–1740] Mutations of the *TP53* the gene are commonly detected in the blast phase of ET

(the CP usually lack the alterations involving the *TP53*, *NRAS*, *KRAS*, and *MDM2* genes).[1741]

Chronic idiopathic myelofibrosis

CIMF (Figure 3.76) is a myeloproliferative disorder of the multipotent hematopoietic progenitor cells characterized by the proliferation of megakaryocytes and granulocytic elements and inappropriate release of fibrogenic cytokines or growth factors in the bone marrow leading to deposition of excess collagen in the bone marrow stroma, and extramedullary hematopoiesis.[1,1742–1748] The initial prefibrotic and the overt and more advanced myelofibrotic stages of CIMF show a pronounced proliferation of an abnormal megakaryopoiesis and granulopoiesis dominated by clustered atypical medium-sized to giant megakaryocytes with cloud-like, bulbous, and often hyperchromatic nuclei, which are not seen in other subtypes of MPDs including CML or MDS.[1701] The amount of marrow fibrosis can be graded into several stages: stage 0 (prefibrotic CIMF-0) shows scattered linear reticulin with no intersections (cross-over) corresponding to normal marrow; stage 1 (early fibrotic CIMF-1) shows a loose network of reticulin with many intersections but no collagenization; stage 2 (fibrotic CIMF-2) shows a diffuse and dense increase in reticulin with extensive intersections with only occasional bundles of collagen and/or focal osteosclerosis; stage 3 (classic CIMF-3) shows a diffuse and dense increase in reticulin with bundles of collagen and osteosclerosis; and stage >3 represents end-stage CIMF with hypocellular marrow showing extensive osteomyelosclerosis.[35–37]

CIMF has a poor prognosis. Allogeneic stem cell transplantation is the only curative approach for patients with myelofibrosis.[1749–1752] The median survival of patients with CIMF is about 5 years, but there is wide variability. Hemoglobin level at diagnosis, age, constitutional symptoms, low or high leukocyte counts, cytogenetic abnormalities, and number of circulating $CD34^+$ blasts all influence the prognosis in patients with CIMF.[281,283,1753,1754]

The cytogenetic data on CIMF are scanty with few distinct recurrent cytogenetic aberrations being identified, most often partial trisomy 1q, 13q−, 20q−, trisomy 8, and abnormalities of chromosomes 1, 7, and 9. Chromosomal abnormalities are reported in ~30–56% of patients with CIMF.[280,282,283,1754,1755] Three characteristic defects, namely del(13q) (9 cases), del(20q) (8 cases), and partial trisomy 1q (7 cases) are present in ~65% (24/37) of patients with clonal abnormalities.[283] Djordjevic et al found an abnormal clone in 41% of patients, whereas 59% of patients had a normal karyotype.[280] Most frequent pathologic findings in that series included trisomy 8 (either alone or within a complex karyotype; 5 patients), aberrations of chromosome 12 (translocation in 2, monosomy in 2, and trisomy in 1 patient), aberrations of chromosome 20 (interstitial deletion in 2, monosomy in 2, and trisomy in 1 patient), aberrations of chromosome 13 (translocation in 2 and an interstitial deletion and trisomy in 1 patient each), and chromosome 18 (derivative 18 in 2 patients and a monosomy; and deletion in 1 patient each).[280] Three patients exhibited complex aberrations involving several chromosomes, sometimes with a mosaicism; a near-tetraploid karyotype was observed in a single patient and balanced translocations [t(2;16) (q31;q24), t(5;13)(q13;q32), t(12;13)(p12;q13), and t(12;16)(q24;q24)] were present in four patients.[280] Using FISH analysis, Strasser-Weippl et al identified chromosomal aberrations in 56% of patients with CIMF, with 20q− (~24%) and 13q− (~17%) being the most frequent.[1755] Tefferi et al reported a worse prognosis for patients with chromosomal abnormalities (other than interstitial deletions involving the long arm of chromosome 13 or 20) when compared to patients with normal karyotype or interstitial deletions involving chromosome 13 or 20.[1754] Mark et al reported a case of CIMF associated with a reciprocal t(3;9) translocation with the 3q21 and 9p24 breakpoints disrupting the *JAK2* gene.[1756]

The $JAK2^{V617F}$ mutation has been found in ~50% of patients with CIMF.[708,711] Kroger et al described a

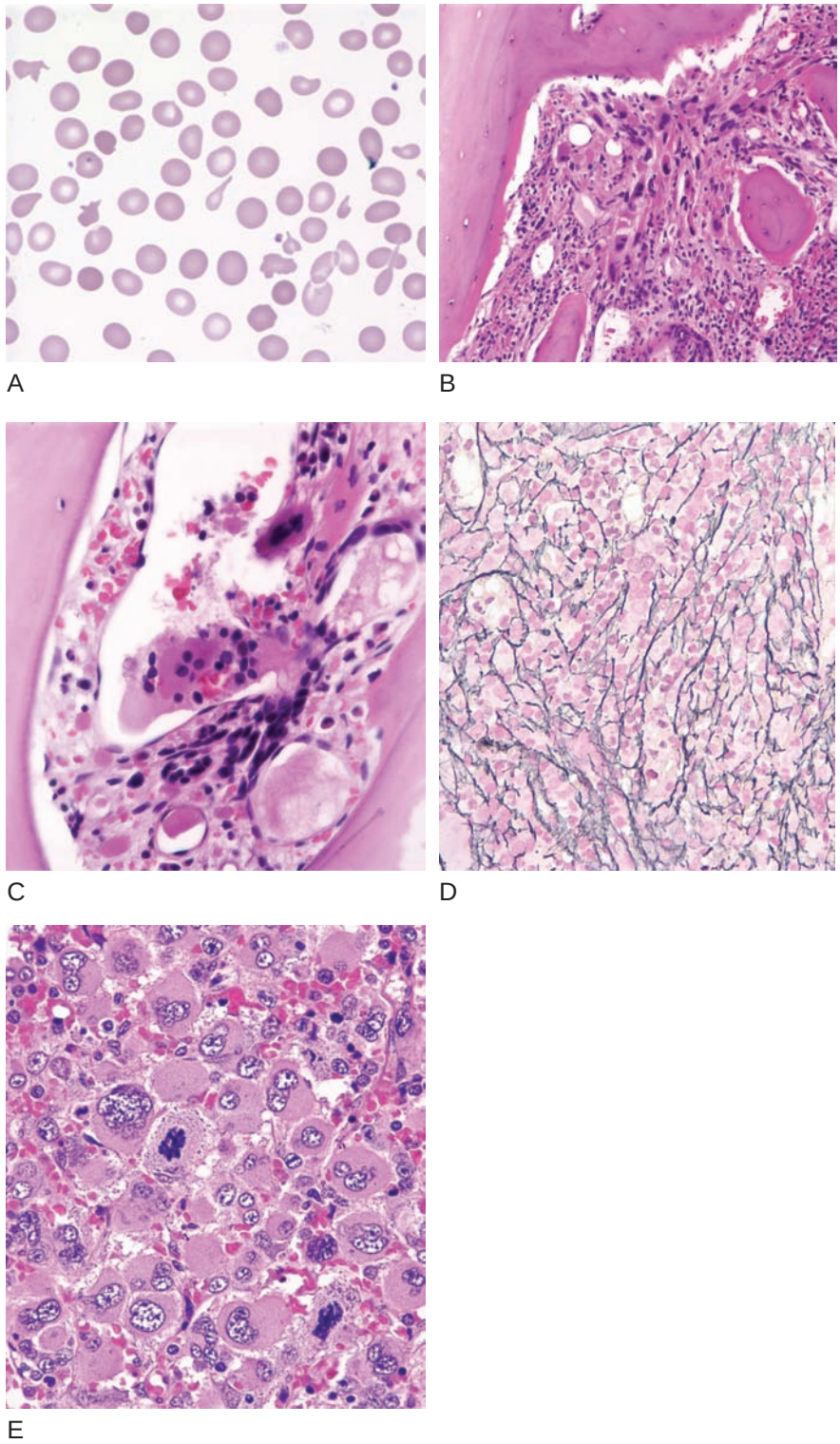


Figure 3.76 Chronic idiopathic myelofibrosis (CIMF). A, blood smear with irregular red cells (including teardrop forms); B, histology with clusters of atypical megakaryocytes; C, high magnification showing intravascular hematopoiesis; D, diffuse reticulin fibrosis; E, myeloid metaplasia, spleen

new, highly sensitive (≥0.01%) RT-PCR to monitor and quantify *JAK2*^{V617F}-positive cells after dose-reduced allogeneic stem cell transplantation.[1752] After 22 allogeneic stem cell transplantation procedures in 21 *JAK2*^{V617F}-positive patients with myelofibrosis, 78% became PCR negative with a significant inverse correlation between *JAK2*^{V617F} positivity and donor-cell chimerism.[1752]

Chronic neutrophilic leukemia

Chronic neutrophilic leukemia (CNL) is a rare CMPD that is recognized as a distinct *BCR-ABL*-negative CMPD defined by sustained (mature) neutrophilia.[1,1757,1758] Apart from lack of *BCR/ABL* fusion/t(9;22), CNL is characterized by neutrophilic leukocytosis (≥25 × 10^9/L), absence of basophilia, normal platelet count, increased alkaline phosphatase levels, a normal granulocytic maturation pattern in the bone marrow with less than 5% blasts (<1% blasts in blood), raised vitamin B12 level, and hepatosplenomegaly. CNL has a heterogeneous clinical course with a definite risk of death from either blastic transformation, brain hemorrhage, or progressive neutrophilic leukocytosis, and therefore the prognosis is generally poor.[1759,1762] Cytogenetic and molecular studies have demonstrated the clonal nature of the disease in individual cases, although most patients have a normal karyotype by conventional cytogenetics at the time of diagnosis.[1758,1760,1761] The exclusion of underlying *BCR/ABL*- driven oncogenesis is an essential component in the diagnosis of this chronic leukemic process. The differential diagnosis with reactive neutrophilia/leukemoid reaction may be difficult and requires careful correlation with clinical history and presentation. The major causes of leukemoid reactions are severe infections, intoxications, malignancies, severe hemorrhage, or acute hemolysis. Some patients with CNL may harbor the *JAK2* mutations.[1762,1763] Reported isolated chromosomal abnormalities include +8, +9/del (20q), 20q−, del(11q14), +21, and complex karyotypes.[321,1758,1760,1764]

Eosinophilic disorders

Eosinophilia accompanies a number of pathologic conditions, including allergic reactions, hypersensitivity reactions, parasitic infections, connective tissue disorders, and hematologic malignancies (e.g. Hodgkin's lymphoma and certain types of AML, like AML-M4Eo). Chronic eosinophilic leukemia (CEL) and hypereosinophilic syndrome (HES) are primary eosinophilic disorders characterized by marked and persistent blood eosinophilia (>1500/μl) in the absence of reactive conditions and specific organ damage (lungs, skin, heart, and/or GI-tract). Differentiating idiopathic HES from CEL is problematic, and based on WHO classification only demonstration of the clonal origin of eosinophils or an increased number of blasts confirms the diagnosis of CEL.[1,801,1765,1766] The clinical presentation of HES/CEL is variable, with cardiovascular, cutaneous, and/or neurologic systems being most often involved.[1,1766] Recent publications recognize three subtypes of HES: myeloproliferative, lymphocytic, and idiopathic.[1766–1769] The myeloproliferative HES is characterized by marrow fibrosis and mastocytosis (and therefore may be also designated eosinophilia-associated systemic mast cell disease; SM-HES). This type often shows chromosomal abnormalities or tyrosine kinase involvement due to *FIP1L1/PDGFRA* fusion [as the consequence of a del(4)(q12q12) that includes the cysteine-rich hydrophobic domain 2 (*CHIC2*) locus, with the centromeric breakpoint on *FIP1L1* and the telomeric breakpoint in *PDGFRA*], and therefore fulfils the WHO criteria for CEL diagnosis. Lymphocytic HES is characterized by the presence of clonal T-cells (usually $CD4^+$) with aberrant expression of pan-T antigens (most often lack of surface CD3). Idiopathic HES is defined by lack of specific chromosomal or molecular abnormalities, and is diagnosed by excluding other types of HES.

Demonstration of the clonal origin of eosinophils is required for establishing the diagnosis of CEL. Most patients with hepereosinophilia exhibit normal

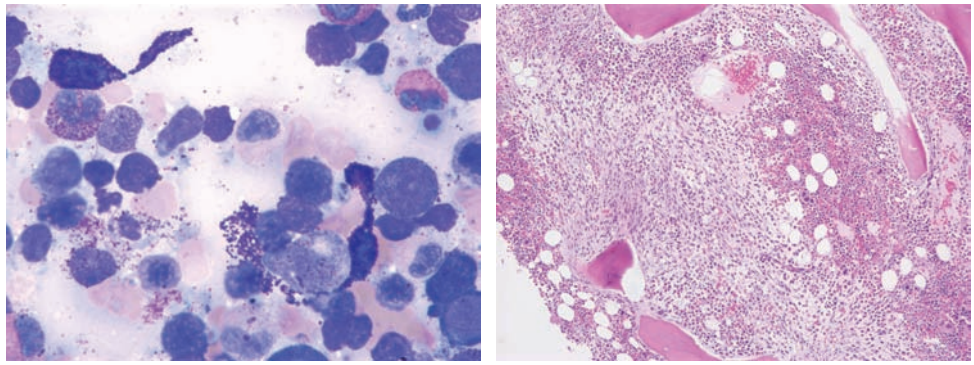
karyotype by conventional cytogenetics. Trisomy 8 is the most common abnormality, but is not specific, since it is often observed in other hematologic malignancies. Other chromosomal abnormalities include complex karyotypes (>3 abnormalities), –Y, t(8;9)(p21;p24), del(6)(q24), and ins(9;4)(q34;q12q31). Some patients with CEL harbor chromosomal translocations involving the *PDGFRB* gene at 5q33 or the *FGFR1* gene at 8p11 (the most common fusions are *ETV6/PDGFRB* and *ZANF198/FGFR1*). Other chromosomal abnormalities observed in CEL include t(5;12)(q33;p13) [*ETV6/PDGFRB*], t(5;7)(q33;q11) [*HIP1/PDGFRB*],, t(4;22)(q12;q11) [*BCR/PDGFRA*], t(8;13)(p11;q12) [*ZNF198/FGFR1*], t(7;8)(q32;p11) [*TRIM24/ FGFR*1], t(6;8)(q27;p11) [*FGFR1OP/FGFR1*], ins(12;8)(p11;p11p22) [*FGFR*1*OP2/FGFR*1], t(8;17)(p11;q11) [*MYO18A/FGFR1*], t(8;22) [*BCR/ FGFR1*], and t(8;9)(p12;p11).

Apart from translocations involving *PDGFRB* or *FGFR1*, or other rare translocations mentioned above, many cases of HES/CEL showed a normal karyotype by conventional cytogenetics, and therefore they were usually classified as 'idiopathic' HES. The discovery of a cryptic deletion on 4q12 [del(4)(q12q12)] associated with the *FIP1L1/PDGFRA* fusion gene makes a correct diagnosis of CEL possible using either FISH (to detect deletion of the CHIC2 locus at 4q12 as a surrogate for the *FIP1L1/PDGFRA* fusion) or RT-PCR for fusion transcript.[46,799,1770,1771] The *FIP1L1/PDGFRA* fusion gene is identified in 14–60% of patients with HES/CEL.[46,798,1772–1774] As mentioned above, many patients with *FIP1L1/PDGFRA* fusion present with features of marrow fibrosis, an elevated serum tryptase level, eosinophilia and mastocytosis and therefore are labeled as eosinophilia associated with systemic mast cell disease (SM-HES). The presence of the *FIP1L1/PDGFRA* fusion gene predicts a response to low dose imatinib (~100 mg/day) (the tyrosine kinase encoded by *FIP1L1/PDGFRA* is more sensitive to imatinib that in CML with *BCR/ABL* fusion which usually requires at least 400 mg/day). In the study by Pardanani et al, none of 57 patients with HES but 10 (56%) of 19 patients with systemic mast cell disease associated with eosinophilia carried the specific mutation (*FIP1L1/PDGFRA*).[798]

MASTOCYTOSIS

Mastocytosis is a heterogeneous group of disorders characterized by proliferation of mast cells in one or more organ systems.[1775–1781] The symptoms observed in mastocytosis are related to the spontaneous or triggered release of mast cell mediators, or are due to consequences of the clonal proliferation and pathologic accumulation of mast cells. They range from urticaria pigmentosa characterized by a rash of hyperpigmented and teleangiectatic papules to disseminated diseases with an aggressive clinical course. The WHO has specified criteria for classification of systemic mastocytosis (SM) into six major subtypes: cutaneous mastocytosis, indolent systemic mastocytosis, systemic mastocytosis with an associated clonal hematologic non-mast-cell disorder, aggressive systemic mastocytosis, mast cell leukemia, and mast cell sarcoma.[1,1777,1782,1783] Mast cell leukemia is an extremely rare subtype of mastocytosis with a leukemic spread of mast cells and a rapid progression of disease.

Mast cells in the bone marrow aspirate have round to oval nuclei and dark cytoplasm, packed with small basophilic granules (Figure 3.77). The cytoplasmic granules are usually so abundant that they obscure the nucleus. Mast cells may be round to polygonal, but atypical forms, including spindle cells, multilobed cells, and metachromatically granulated blast-like cells, may be present. Systemic and more aggressive variants of mast cell disease more often display atypical cytomorphologic features. A bone marrow core biopsy shows multiple, well-demarcated foci of mast cells within a fibrohistiocytic matrix, with a tendency to perivascular and/or paratrabecular locations. Systemic mastocytosis may be associated with other hematopoietic neoplasms,



A B

Figure 3.77 Systemic mastocytosis. A, aspirate smear shows elongated atypical mast cells with numerous dark basophilic granules (arrows). B, histologic section shows atypical large aggregate of mast cells in the bone marrow

such as acute leukemia, MDS, malignant lymphoma, or CMPD. The majority of patients with systemic mastocytosis with associated clonal, hematologic non-mast cell lineage disease (SM-AHNMD) have a myeloid stem cell malignancy including MDS, myelodysplastic/myeloproliferative disorders, AML or CMPD. Immunophenotypic studies of neoplastic mast cells reveal expression of CD2, CD25, CD43, CD45, CD68, CD117, and mast cell tryptase. Normal (benign) mast cells lack the expression of CD2 and CD25.

With conventional cytogenetics about 35–40% of the patients have chromosomally abnormal clones in bone marrow cells which are similar to those observed in other hematologic neoplasms.[1784,1785] The following chromosomal abnormalities have been described: del(5q), del(7q), del(11q), del(20q), −Y, +8, +14.[1784,1785] Activating codon 816 mutations (D816V) in the c-*KIT* receptor tyrosine kinase gene resulting in a deregulation of the c-kit receptor are present in the majority of patients.[1783,1786–1788] Activating mutations of platelet-derived growth factor receptor-alpha (*PDGFRA*) are identified in a significant number of SM cases that have associated eosinophilia. Detection of the codon 816 c-*KIT* mutation is a minor diagnostic criterion of SM.[1786] Demonstration of one minor criterion the (the D816V mutation) in the presence of a major criterion (presence of multifocal mast cell clusters of 15 or more cells in the bone marrow biopsy) is required for the diagnosis of SM. In most cases, the diagnosis of SM can be established by morphologic evaluation of a bone marrow biopsy. Analysis of the bone marrow in conjunction with cytogenetics, FC immunophenotyping and other relevant clinical information is required to exclude accompanying hematologic malignancy.

MYELOPROLIFERATIVE/ MYELODYSPLASTIC DISEASES

Refractory anemia with ringed sideroblasts associated with marked thrombocytosis

Certain conditions share clinical and/or morphologic features associated with either the MDS or CMPD and are recognized in the WHO classification as mixed myelodysplastic/myeloproliferative disease (MDS/MPD). Such overlap syndromes include juvenile myelomonocytic leukemia (JMML), chronic myelomonocytic leukemia (CMML), atypical chronic myeloid leukemia (aCML), and MDS/MPD-unclassifiable (MDS/MPD-U). The MDS/MPD-U group includes a provisional entity

called refractory anemia with ringed sideroblasts with marked thrombocytosis (RARS-T). Patients with this condition present with some signs of ET, including a marked thrombocytosis, hypercellular marrow, and increased megakaryocytes, but also have ringed sideroblasts, a feature usually associated with MDS.[1,376,1789] In a study by Szpurka et al, on 9 RARS-T patients, 6 showed the presence of the *JAK2*^{V617F} mutation.[1790] *JAK2* mutants have been found only rarely in patients with MDS.[1762,1791,1792] Szpurka et al found the *JAK2* mutation in only 2 (out of 89) patients with MDS. The presence of the JAK2 mutation in RARS-T puts this disorder closer to a category of *JAK2* mutation-associated CMPD, rather than MDS.

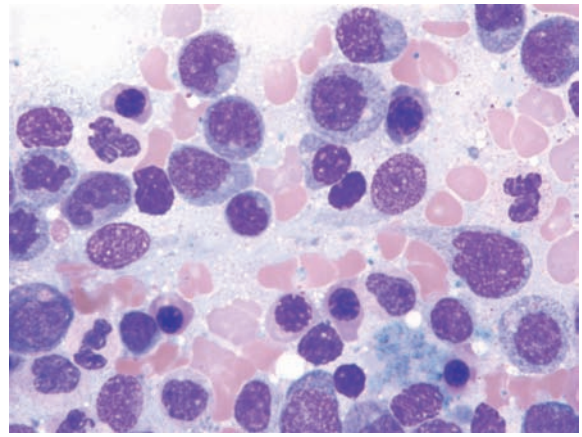


Figure 3.78 Chronic myelomonocytic leukemia (CMML), bone marrow aspirate smear

Chronic myelomonocytic leukemia

CMML is a myeloid stem cell disorder characterized by persistent monocytosis in the bone marrow (Figure 3.78) and blood (>1 × 10^9/l), a variable degree of marrow dyspoiesis, lack of Ph chromosome, <20% blasts in the blood or bone marrow, and a wide heterogeneity of clinical presentation. A large fraction of patients with CMML show unequivocal predominance of proliferative features with marked leukocytosis (WBC count >13 × 10^9/l) and hepatosplenomegaly ('proliferative-type' CMML). Other patients show a predominance of dysplastic features with only modest leukocytosis (WBC count ≤13 × 10^9/l) and often cytogenetic abnormalities ('dysplastic-type' CMML). The current WHO classification includes CMML in a new category of mixed chronic myeloproliferative/myelodysplastic disorder.[1] The natural course of the disease is variable, with survival ranging from a few months to several years. The survival for patients with CMML is usually between 12 and 40 months (median ~20 months).[1793–1799]

No specific cytogenetic abnormalities have been reported in patients with CMML. Clonal chromosomal changes are present in 20–40%, with +8, –7/del(7q), and 12p rearrangements being the most common.[13,1796–1798,1800–1802] Oo et al reported therapy-related CMML with monosomy 7 and t(12;17) (p13;q11.2).[1803] A subgroup of CMML has at(5;12) (q33;p13) balanced translocation leading to *PDGFRB* and *ETV6* fusion. Abnormalities of *PDGFRB* and *ETV6* genes are most often seen in CMML associated with eosinophilia.[1804–1807]

MYELODYSPLASTIC SYNDROMES

MDSs are oligoclonal hematopoietic stem cell disorders characterized clinically and morphologically by ineffective hematopoiesis and dyspoiesis (Figure 3.79), leading to bone marrow failure, cytopenia(s) (which often results in transfusion-dependent anemia and/or an increased risk of infection or hemorrhage), and the potential to progress to acute leukemia.[1,1808] The incidence of MDS varies from 2.1 to 12.6 cases per 100 000 population per year, but approaches 50 cases per 100 000 per year in persons over age 70.[1809–1811] MDS disorders may arise *de novo* or following chemotherapy or irradiation. Ineffective hematopoiesis in MDS has been attributed in part to a complex interaction between progenitor cells and the microenvironment that results in the premature apoptotic death of blood cell precursors. An increased frequency of apoptosis in the bone marrow cells of patients with MDS has

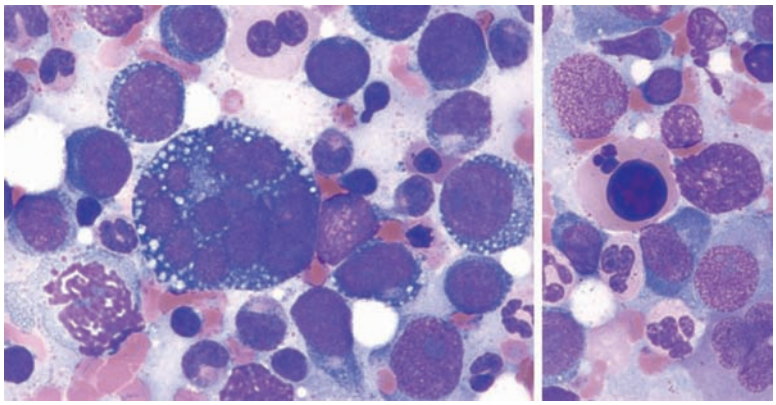


Figure 3.79 Myelodysplastic syndrome; Cytomorphologic features showing dyserythropoiesis

been confirmed by many studies.[1815–1817] The initial stages of MDS are defined by excessive apoptosis of progenitor cells that leads to ineffective hematopoiesis and is counterbalanced by increased proliferation of hematopoietic elements.

Based on clinical symptoms, morphology, number of blasts and chromosomal changes, the WHO classification recognizes several categories of MDS: RA, RARS, RCMD, RCMD and ringed sideroblasts, RA with excess blasts (RAEB), and MDS associated with isolated deletion of 5q (5q$^-$ syndrome; Figure 3.80).[1] RAEB is further subdivided into type I (5–9% blasts in the bone marrow) and type II (10–19% blasts in the bone marrow). Based on the duration of survival and incidence of progression into acute leukemia, MDS can be categorized into two risk groups: low risk (MDS with isolated 5q deletion; RA, RARS) and high risk (RCMD and RAEB). The median survival for RA

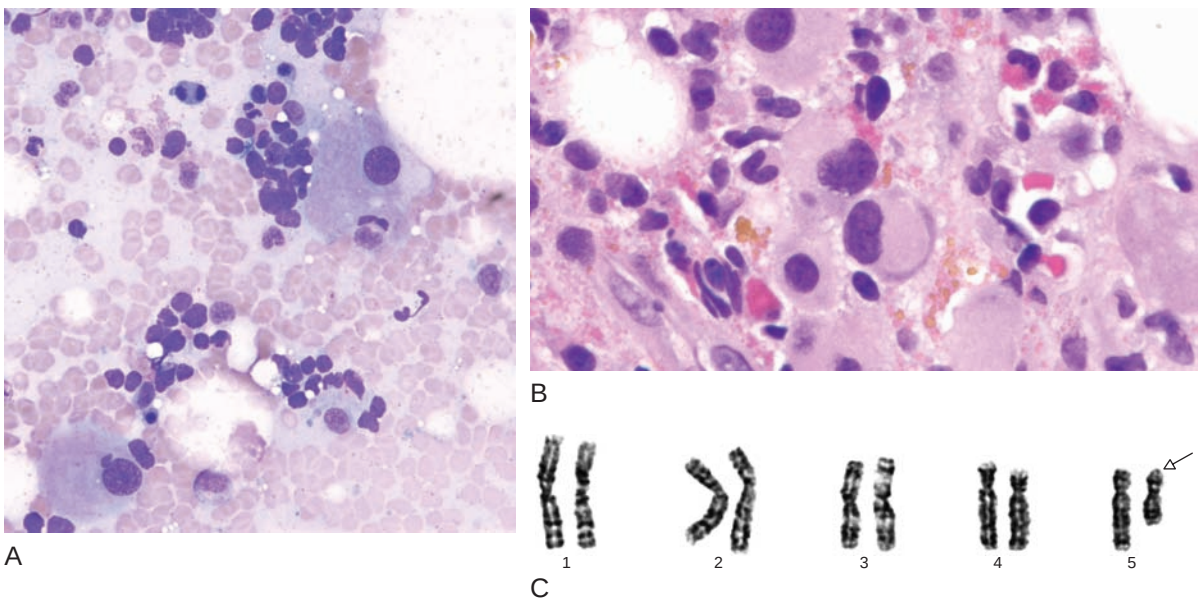


Figure 3.80 Myelodysplastic syndrome with isolated deletion of 5q (5q– syndrome). Note typical hypolobated micromegakaryocytes (A, cytology; B, histology; C, conventional cytogenetics; partial karyotype)

patients is 66 months, for RARS patients 6 years, for RCMD patients 24–33 months, for RAEB-I patients 18 months, and RAEB-II patients for 10 months.[1815,1816] In untreated MDS patients, the median survival time for patients with a complex karyotype is 8 months, and for those with one or two chromosomal abnormalities, 40 months.[950]

Cytogenetics

Cytogenetic analysis of MDS allows confirmation of clonality (and therefore establishes the definite diagnosis of MDS) and further subclassifies the disease. Recurring chromosomal abnormalities occur in 40–70% of patients with primary MDS and in 95% of tMDS patients, and are strong and independent prognostic indicators (Table 3.12). [950,1817–1823] Specific chromosomal abnormalities may be difficult to define by conventional cytogenetics alone. Trost et al using spectral karyotyping (SKY) and FISH in 17 patients with MDS and 3 patients with AML, revealed chromosome 5 abnormalities in all cases: unbalanced translocations and interstitial deletions, or del(5q), each occurring in 10 patients.[286] The most frequent additional chromosome aberrations in the same report included –3/–3p/–3q, –7/7q–, +8, 13q–, –16, 17p–, –18/18p–, –20/20q–, and +21q, each occurring in 25%.[286] Unbalanced 5q translocations occurred more often in combination with monosomy 3 and 7 and with gain of 21q, whereas del(5q) was associated more often with -1p and trisomy 8. [286] In a series of 491 patients with primary MDS reported by Bernasconi et al, most common single abnormalities included +1q (1.2%), 3q rearrangements (2.6%), del(5q) (8.7%), –7 (2.7%), del(7q)4.1%), +8 (4.5%), del(11)(q14q23) (1%), del(12p) (3.4%), del(17p) (1.2%), del(20q) (3%), and –Y (1%).[1821]

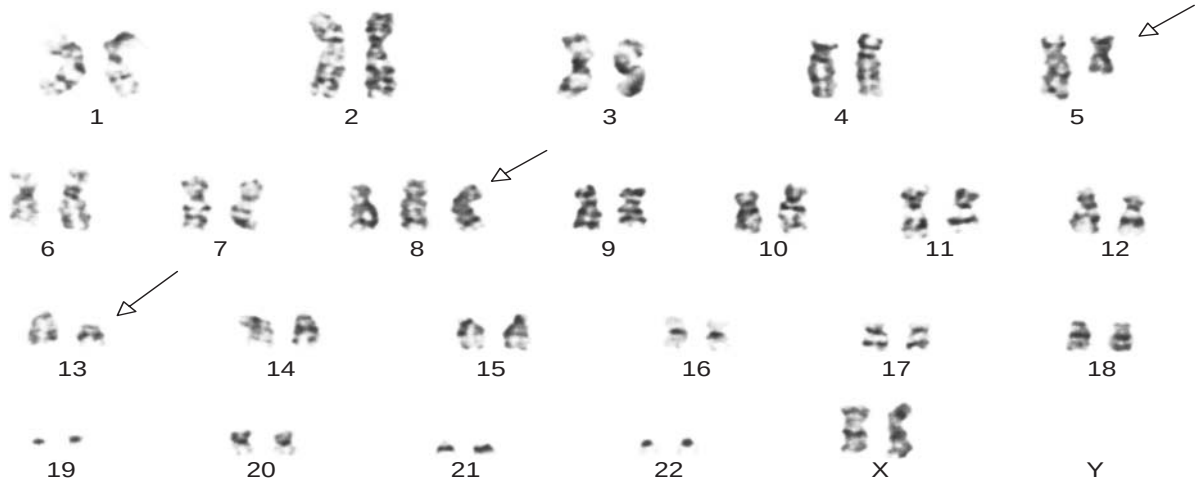
Deletions are the most common aberrations in *de novo* MDS, with del(5q)/–5 and del(7q) being the most frequent.[950,955,1821–1824] In a large series of patients with MDS reported by Heim et al, the most common aberrations in primary MDS are del(5q) (27%), trisomy 8 (19%), monosomy 7 (15%), der(11q) (7%), –5, der(12p) and –Y (5%), del(7q) (4%), and t(1;7), der(3q), del(13q), i(17q), and del(20q) in 2% or less.[21] The 5q– is mostly, but not always, a del(5)(q13q33); it is the cytogenetic hall-mark of the '5q– syndrome' and is frequently found as the sole abnormality.[21] The frequency of the aberrations varies among MDS subgroups: 5q– is most frequent in RA; –5, –7, and der(12p) are more common in CMML and especially in RAEB; and +8 and der(11q) are more often found in RARS.[21] The most common aberrations in secondary MDS are –7 (41%), del(5q) (28%), –5 (11%), der(21q) (9%), 7q–, +8 and der(12p) (8%), t(1;7) and –12 (7%), der(17p) (6%), der(3p) and der(6p) (5%), and der(3q), der(11q), –17, –18, and der(19q) (4%).[21] MDS patients with del(5q) respond well to lenalidomide treatment.

Three risk-based cytogenetic groups (good, intermediate, and poor) can be distinguished. The cytogenetic subgroup with a good outcome includes normal karyotype, –Y alone (monosomy Y), del(5q) alone (Figure 3.80), del(20q) alone; poor outcome

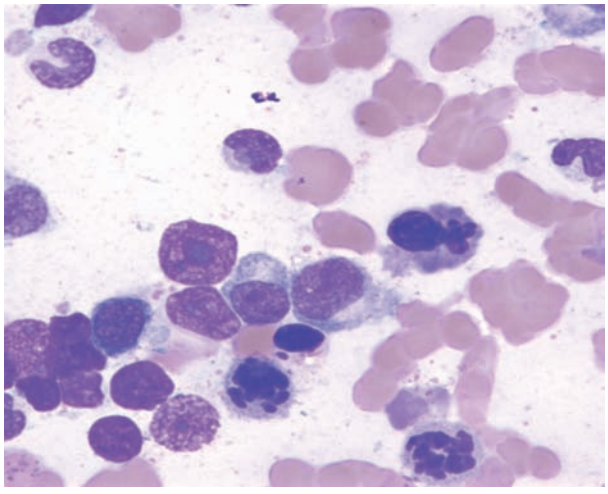
Table 3.12 Chromosomal abnormalities in MDS

Cytogenetic abnormality	*Percent of MDS*	*Risk of progression to acute leukemia*	*Prognosis*
del(20q)	5	low	good
monosomy 5/del(5q)	10–20	high	poor
5q(–) syndrome		low	good
monosomy 7/del(7q)	5	high	poor
17p-syndrome	7	high	poor
11q23	6	intermediate	medium
trisomy 8	10	intermediate	medium
complex karyotype	10–20	high	poor

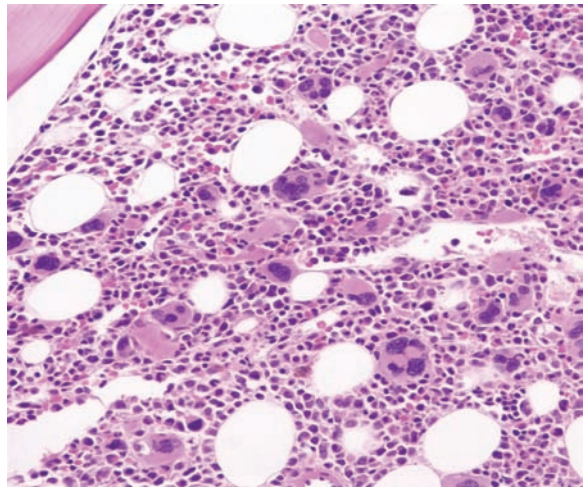
includes complex karyotype (≥3 abnormalities; Figure 3.81) or chromosome 7 abnormalities (Figure 3.82); and intermediate outcome includes all other abnormalities.[956] Abnormalities of chromosomes 5 and 7 or complex aberrations are seen only in RCMD and RAEB. Patients with a normal karyotype, del(5q) alone, or del(20q) alone have a median survival >3 years, whereas patients with high risk cytogenetic changes (complex karyotype, chromosome 3 abnormalities, or chromosome 7 abnormalities) have a median survival <12 months.[103,951,1822,1825] MDS patients with complex karyotype and –7/del(7q) have a greater risk of progression to AML. The International Prognostic Scoring System of MDS has defined patients with a normal karyotype as a good-risk cytogenetic subgroup. It is suggested that the



A



B



C

Figure 3.81 MDS (RAEB) with complex chromosomal abnormalities: 46,XX,del5(q13;q33),del(13)(q12q14)[1]/47, idem,+8[15]; A, cytogenetics; B, aspirate smear with prominent dyserythropoiesis; C, histomorphology (note prominent megakaryocytic atypia)

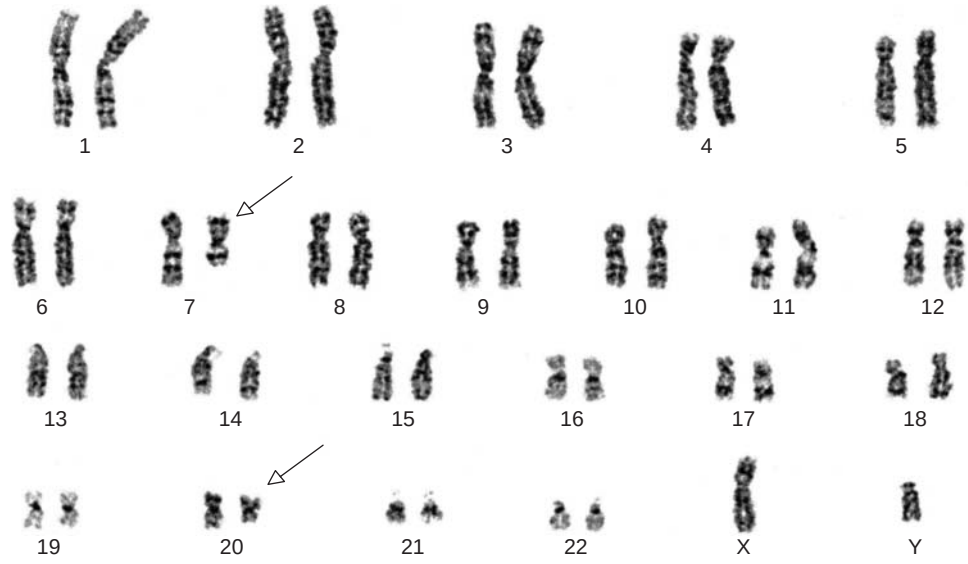


Figure 3.82 MDS with partial deletion of chromosomes 7 and 20; cytogenetics

prognostic impact of karyotypic subgroups in MDS is modified by sex.[1826] Shorter survival was detected for men in the favorable and the intermediate subgroups, but not in the poor prognosis subgroup, when compared to women. The better outcome for women in the favorable subgroup was mainly the result of the 5(q)$^-$ syndrome.[1826] The cytogenetic profile of MDS, particularly of RAEB, is nearly identical to that of elderly patients with AML, both in frequency and in the type of chromosomal abnormalities.[1827]

Patients with aplastic anemia and clonal karyotypic evolution most often show numeric and structural abnormalities of chromosome 7 (40%), followed by trisomy 8, aberrations of chromosome 13, deletion of Y chromosome, and complex cytogenetic abnormalities.[1828] Unlike primary MDS, aberrations of chromosomes 5 and 20 are infrequent. Most deaths related to leukemic transformation occurred in patients with abnormalities of chromosome 7 or complex cytogenetic alterations or both. Evolution of chromosome 7 abnormalities is seen most often in refractory patients who had failed to respond to therapy. In contrast, trisomy 8 developed in patients with good hematologic responses who often required chronic immunosuppression with cyclosporine A (CsA), and survival was excellent.[1828] Although aplastic anemia patients with monosomy 7 showed a similar prognosis to those with primary MDS, trisomy 8 in aplastic anemia appears to have a more favorable prognosis than in MDS.[1828]

Molecular markers

Many genetic defects underlying MDS and AML have been identified and allow for the new molecular-targeted therapies: farnesyltransferase inhibitors and receptor tyrosine kinase inhibitors (including *FLT3* and *VEGF* inhibitors), the DNA hypomethylating compounds (azacytidine and decitabine), and immunomodulating drugs (e.g. lenalidomide used in patients with 5q deletion). The t(5;12)(q33;p13), which disrupts a gene coding for a tyrosine kinase, and the platelet-derived growth factor receptor-β (*PDGFRB*) lead to deregulation of cell growth. This translocation is seen in both myeloproliferative and myeloproliferative/myelodysplastic disorders (e.g. CMML), especially those accompanied by

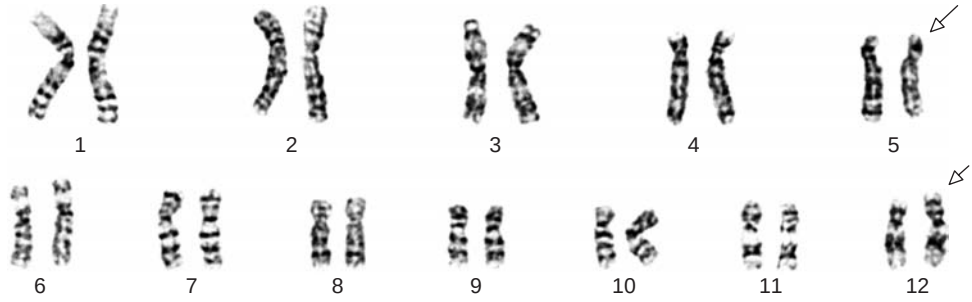


Figure 3.83 MDS with t(5;12); cytogenetics

eosinophilia. Patients with the *PDGFRB* abnormalities respond well to treatment with the tyrosine kinase inhibitors (e.g. imatinib mesylate) and experience resolution of the cytogenetic abnormalities.[1634] The t(5;12) translocation is shown in Figure 3-83. Apart from *PDGFRB*, activating mutations of other genes encoding tyrosine kinases receptors (*EGFR*, *ErbB2/HER2*, *PDGFRB*, c-*KIT,* and *FLT3*) are involved in pathogenesis of myeloid neoplasms including MDS.[1784] The mutations of c-*fms* in MDS are associated with a poor outcome.[1829] The *FLT3* mutations (ITDs) in MDS are associated with more common and rapid progression to AML.[1502,1830] Patients with *FLT3*-ITD$^+$ disease have a significantly shorter survival compared with patients without *FLT3*-ITD. On multivariate analysis, *FLT3*-ITD was identified as an independent predictor of reduced time to AML progression and reduced overall survival.[674] Point mutations in the *RAS* gene family are identified in approximately 30% of patients with advanced MDS.[1817] Mutations of *p53/TP53* are infrequent and are generally associated with high-risk MDS and other cytogenetic abnormalities.[1817] Similarly to cytogenetics used today to predict prognosis in MDS patients, it appears that molecular profiling in the near future will help to identify patients who might benefit from specific treatment (e.g. the erythroid gene expression signature may stratify patients for lenalidomide regardless of 5q status).

Abbreviations/terminology

add = additional material of unknown origin

AILT = angioimmunoblastic T-cell lymphoma

ALCL = anaplastic large cell lymphoma

ALL = acute lymphoblastic leukemia

AML = acute myeloid leukemia

aneuploidy = abnormal number of chromosomes

AP = accelerated phase

APL = acute promyelocytic leukemia

banding = the staining of chromosomes to produce a pattern which allows their classification

BL = Burkitt lymphoma

BP = blast phase

CCyR = complete cytogenetic response

CEL = chronic eosinophilic leukemia

cen = centromere

CGH = comparative genomic hybridization

CIMF = chronic idiopathic myelofibrosis

CLL = chronic lymphocytic leukemia

CML = chronic myeloid leukemia

CMML = chronic myelomonocytic leukemia

CMPD = chronic myeloproliferative disorder

CMR = complete molecular response

CNL = chronic neutrophilic leukemia

CP = chronic phase

CR = complete remission

CyR = cytogenetic response

del = deletion (loss of part of chromosome)

der = derivative chromosome

dic = dicentric

diploid(number) = number of chromosomes in the somatic cells (46)

DLBCL = diffuse large B-cell lymphoma

dup = duplication

ET = essential thrombocythemia

euploidy = normal (balanced) set of chromosomes (46)

FC = flow cytometry

FISH = fluorescence in situ hybridization

FL = follicular lymphoma

fra = fragile site

GDB = Genome Data Base designations for genetic loci (the locus designation is given in capital letters); when GDB designation is not available, the commercial probe name is used

HL = Hodgkin lymphoma

HR = hematologic remission

hyploid (number) = number of chromosomes in germ cells (egg or sperm; 23)

i (or **iso**) = isochromosome

idic = isodicentric chromosome

ins = insertion

interphase = the part of the cell cycle between mitoses, which includes synthesis of DNA (S phase), interval between cell divison and S phase (gap 1; G1), and interval between S phase and cell division (gap 2; G2)

inv = inversion

JT = jumping translocation; nonreciprocal trnslocations involving chromosome arem (or segment) onto two or more recipient chromosomes in different cell lines in the same patient/extra copies of same donor segment on different recipient chromosomes in a single clone

MALT = mucosa-associated lymphoid tissue

mar (marker chromosome) = a structurally abnormal chromosome that cannot be identified with standard cytogenetics

MCL = mantle cell lymphoma

MCyR = major cytogenetic response

MDS = myelodysplastic syndrome

methaphase = the stage of cell cycle (between prophase and anaphase) in which chromosomes contract completely and move to the center of the cell

MM = multiple myeloma (plasma cell myeloma)

MMR = major molecular response

mos (mosaic) = two or more cell lines present in one individual (two or more cell types are present, which differ in the number of chromosomes or their structure)

MRD = minimal residual disease

MZL = marginal zone lymphoma

NHL = non-Hodgkin's lymphoma

NLPHL = nodular lymphocyte predominant Hodgkin's lymphoma

p = short arm of chromosome

PCR = polymerase chain reaction

Ph = Philadelphia chromosome [result of t(9;22) translocation]

PLL = prolymphocytic leukemia

PTCL = peripheral T-cell lymphoma, unspecified

P. vera = Plycythemia vera

PV = P. vera

q = long arm of chromosome

r = ring chromosome

rcp = reciprocal

rea = rearrangement

rec = recombinant chromosome

rob = Robertsonian translocation

RQ-PCR = real-time quantitative **RT-PCR** (**qRT-PCR**)

RT-PCR = reverse transcriptase PCR

SKY = spectral karyotyping

SLL = small lymphocytic lymphoma

SS = Sézary's syndrome

t = translocation

tel = telomere (end of chromosome arm)

ter = terminal end of chromosome

TMP = transient myeloproliferative disorder

WCP = whole chromosome probe

+(plus sign in front of chromosome number) = gain of chromosome (e.g. +21)

–(minus sign in front of chromosome number) = loss of chromosome (e.g. –7)

minus sign (–; after chromosome) = loss of fragment of chromosome(e.g.5q–)

[](square brackets) = number of cells in each clone

References

1. Jaffe ES, Harris NL, Stein H, Vardiman JW. World Health Organization Classification of Tumors. Pathology and Genetics of Tumors of Hematopoietic and Lymphoid Tissues. Lyon: IARC Press, 2001.
2. Tibiletti MG, Bernasconi B, Dionigi A, Riva C. The applications of FISH in tumor pathology. Adv Clin Pathol. 1999;3:111–18.
3. Cook JR. Paraffin section interphase fluorescence in situ hybridization in the diagnosis and classification of non-Hodgkin lymphomas. Diagn Mol Pathol 2004;13:197–206.
4. Wolfe KQ, Herrington CS. Interphase cytogenetics and pathology: a tool for diagnosis and research. J Pathol 1997; 181:359–61.
5. Campbell LJ. Cytogenetics of lymphomas. Pathology. 2005;37:493–507.
6. Gorczyca W. Prognostic markers in hematologic oncology. London, New York: Taylor and Francis; 2005.
7. Andreasson P, Hoglund M, Bekassy AN et al. Cytogenetic and FISH studies of a single center consecutive series of 152 childhood acute lymphoblastic leukemias. Eur J Haematol 2000;65:40–51.
8. Anonymous. Cytogenetic abnormalities in adult acute lymphoblastic leukemia: correlations with hematologic findings outcome. A Collaborative Study of the Groupe Francais de Cytogenetique Hematologique. Blood. 1996;87:3135–42.
9. Arber DA, Stein AS, Carter NH, Ikle D, Forman SJ, Slovak ML. Prognostic impact of acute myeloid leukemia classification. Importance of detection of recurring cytogenetic abnormalities and multilineage dysplasia on survival. Am J Clin Pathol. 2003;119:672–80.
10. Bain BJ. Classification of acute leukaemia: the need to incorporate cytogenetic and molecular genetic information. J Clin Pathol. 1998;51:420–3.
11. Cortes JE, Talpaz M, Giles F et al. Prognostic significance of cytogenetic clonal evolution in patients with chronic myelogenous leukemia on imatinib mesylate therapy. Blood. 2003;101:3794–800.
12. Fan YS, Rizkalla K. Comprehensive cytogenetic analysis including multicolor spectral karyotyping and interphase fluorescence in situ hybridization in lymphoma diagnosis. A summary of 154 cases. Cancer Genet Cytogenet 2003;143:73–9.
13. Fenaux P, Morel P, Lai JL. Cytogenetics of myelodysplastic syndromes. Semin Hematol. 1996;33:127–38.
14. Fonseca R, Barlogie B, Bataille R et al. Genetics and cytogenetics of multiple myeloma: a workshop report. Cancer Res. 2004;64:1546–58.
15. Grimwade D. The clinical significance of cytogenetic abnormalities in acute myeloid leukaemia. Best Pract Res Clin Haematol. 2001;14:497–529.
16. Grimwade D, Biondi A, Mozziconacci MJ et al. Characterization of acute promyelocytic leukemia cases lacking the classic t(15;17): results of the European Working Party. Groupe Francais de Cytogenetique Hematologique, Groupe de Francais d'Hematologie Cellulaire, UK Cancer Cytogenetics Group and BIOMED 1 European Community-Concerted Action 'Molecular Cytogenetic Diagnosis in Haematological Malignancies'. Blood. 2000;96:1297–308.
17. Grimwade D, Walker H, Harrison G et al. The predictive value of hierarchical cytogenetic classification in older adults with acute myeloid leukemia (AML): analysis of 1065 patients entered into the United Kingdom Medical Research Council AML11 trial. Blood. 2001;98:1312–20.
18. Grimwade D, Walker H, Oliver F et al. The importance of diagnostic cytogenetics on outcome in AML: analysis of 1,612 patients entered into the MRC AML 10 trial.

The Medical Research Council Adult and Children's Leukaemia Working Parties. Blood. 1998;92:2322–33.

19. Han T, Ozer H, Sadamori N et al. Prognostic importance of cytogenetic abnormalities in patients with chronic lymphocytic leukemia. N Engl J Med. 1984;310:288–92.
20. Harrison CJ, Mazzullo H, Cheung KL et al. Cytogenetics of multiple myeloma: interpretation of fluorescence in situ hybridization results. Br J Haematol. 2003;120:944–52.
21. Heim S. Cytogenetic findings in primary and secondary MDS. Leuk Res. 1992;16:43–6.
22. Levine EG, Bloomfield CD. Cytogenetics of non-Hodgkin's lymphoma. J Natl Cancer Inst Monogr. 1990:7–12.
23. Colomo L, Lopez-Guillermo A, Perales M et al. Clinical impact of the differentiation profile assessed by immunophenotyping in patients with diffuse large B–cell lymphoma. Blood. 2003;101:78–84.
24. Mrozek K, Heerema NA, Bloomfield CD. Cytogenetics in acute leukemia. Blood Rev. 2004;18:115–36.
25. Mrozek K, Marcucci G, Paschka P, Whitman SP, Bloomfield CD. Clinical relevance of mutations and gene-expression changes in adult acute myeloid leukemia with normal cytogenetics: are we ready for a prognostically prioritized molecular classification? Blood. 2007;109:431–48.
26. Panani AD, Roussos C. Cytogenetic aspects of adult primary myelodysplastic syndromes: clinical implications. Cancer Lett. 2006;235:177–90.
27. Pinkel D, Gray JW, Trask B,et al. Cytogenetic analysis by in situ hybridization with fluorescently labeled nucleic acid probes. Cold Spring Harb Symp Quant Biol. 1986;51 Pt 1:151–7.
28. Raimondi SC. Current status of cytogenetic research in childhood acute lymphoblastic leukemia. Blood. 1993;81:2237–51.
29. Streubel B, Sauerland C, Heil G et al. Correlation of cytogenetic, molecular cytogenetic, and clinical findings in 59 patients with ANLL or MDS and abnormalities of the short arm of chromosome 12. Br J Haematol. 1998;100:521–33.
30. Vallespi T, Imbert M, Mecucci C, Preudhomme C, Fenaux P. Diagnosis, classification, and cytogenetics of myelodysplastic syndromes. Haematologica. 1998;83:258–75.
31. Walters R, Kantarjian HM, Keating MJ et al. The importance of cytogenetic studies in adult acute lymphocytic leukemia. Am J Med. 1990;89:579–87.
32. Zhan F, Sawyer J, Tricot G. The role of cytogenetics in myeloma. Leukemia. 2006;20:1484–86.
33. Mitelman F, Mertens F, Johansson B. Prevalence estimates of recurrent balanced cytogenetic aberrations and gene fusions in unselected patients with neoplastic disorders. Genes Chromosomes Cancer. 2005;43:350–66.
34. Heim S, Mitelman F. Numerical chromosome aberrations in human neoplasia. Cancer Genet Cytogenet. 1986;22:99–108.
35. Michiels JJ, Bernema Z, Van Bockstaele D, De Raeve H, Schroyens W. Current diagnostic criteria for the chronic myeloproliferative disorders (MPD) essential thrombocythemia (ET), polycythemia vera (PV) and chronic idiopathic myelofibrosis (CIMF). Pathol Biol (Paris). 2007;55:92–104.
36. Michiels JJ, De Raeve H, Berneman Z et al. The 2001 World Health Organization and updated European clinical and pathological criteria for the diagnosis, classification, and staging of the Philadelphia chromosome-negative chronic myeloproliferative disorders. Semin Thromb Hemost. 2006;32:307–40.
37. Michiels JJ, De Raeve H, Hebeda K et al. WHO bone marrow features and European clinical, molecular, and pathological (ECMP) criteria for the diagnosis of myeloproliferative disorders. Leuk Res. 2007; 31(8):1031–8.
38. Gorczyca W. Flow cytometry in neoplastic hematopathology. London, New York: Taylor and Francis; 2006.
39. Rosenwald A, Staudt LM. Gene expression profiling of diffuse large B-cell lymphoma. Leuk Lymphoma. 2003;44(Suppl 3):S41–7.
40. Staudt LM. Molecular diagnosis of the hematologic cancers. N Engl J Med. 2003;348:1777–85.
41. Alvarez RH, Kantarjian H, Cortes JE. The biology of chronic myelogenous leukemia: implications for imatinib therapy. Semin Hematol. 2007;44:S4–14.
42. Baccarani M, Saglio G, Goldman J et al. Evolving concepts in the management of chronic myeloid leukemia: recommendations from an expert panel on behalf of the European LeukemiaNet. Blood. 2006;108:1809–20.
43. Cortes JE, Talpaz M, Kantarjian H. Chronic myelogenous leukemia: a review. Am J Med. 1996;100:555–70.
44. Kantarjian HM, Talpaz M, Giles F, O'Brien S, Cortes J. New insights into the pathophysiology of chronic myeloid leukemia and imatinib resistance. Ann Intern Med. 2006;145:913–23.
45. Bible KC, Tefferi A. Cyclosporine A alleviates severe anaemia associated with refractory large granular lymphocytic leukaemia and chronic natural killer cell lymphocytosis. Br J Haematol. 1996;93:406–8.
46. Cools J, DeAngelo DJ, Gotlib J et al. A tyrosine kinase created by fusion of the PDGFRA and FIP1L1 genes

as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome. N Engl J Med. 2003;348:1201–14.

47. Tefferi A. A contemporary approach to the diagnosis and management of polycythemia vera. Curr Hematol Rep. 2003;2:237–41.

48. Tefferi A. Essential thrombocythemia: scientific advances and current practice. Curr Opin Hematol. 2006;13:93–8.

49. Ren R. Mechanisms of BCR-ABL in the pathogenesis of chronic myelogenous leukaemia. Nat Rev Cancer. 2005;5:172–83.

50. Kantarjian H, Sawyers C, Hochhaus A et al. Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. N Engl J Med. 2002;346:645–52.

51. O'Brien SG, Guilhot F, Larson RA et al. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med. 2003;348:994–1004.

52. Faderl S, Talpaz M, Estrov Z et al. The biology of chronic myeloid leukemia. N Engl J Med. 1999;341:164–72.

53. Goldman JM, Melo JV. Chronic myeloid leukemia – advances in biology and new approaches to treatment. N Engl J Med. 2003;349:1451–64.

54. Jones RJ. Biology and treatment of chronic myeloid leukemia. Curr Opin Oncol. 1997;9:3–7.

55. Schnittger S, Schoch C, Dugas M et al. Analysis of FLT3 length mutations in 1003 patients with acute myeloid leukemia: correlation to cytogenetics, FAB subtype, and prognosis in the AMLCG study and usefulness as a marker for the detection of minimal residual disease. Blood. 2002;100:59–66.

56. Chillon MC, Fernandez C, Garcia-Sanz R et al. FLT3-activating mutations are associated with poor prognostic features in AML at diagnosis but they are not an independent prognostic factor. Hematol J. 2004;5:239–46.

57. Frohling S, Schlenk RF, Breitruck J et al. Prognostic significance of activating FLT3 mutations in younger adults (16 to 60 years) with acute myeloid leukemia and normal cytogenetics: a study of the AML Study Group Ulm. Blood. 2002;100:4372–80.

58. Gilliland DG, Griffin JD. The roles of FLT3 in hematopoiesis and leukemia. Blood. 2002;100:1532–42.

59. Kondo M, Horibe K, Takahashi Y et al. Prognostic value of internal tandem duplication of the FLT3 gene in childhood acute myelogenous leukemia. Med Pediatr Oncol. 1999;33:525–29.

60. Kottaridis PD, Gale RE, Linch DC. FLT3 mutations and leukaemia. Br J Haematol. 2003;122:523–38.

61. Stirewalt DL, Kopecky KJ, Meshinchi S et al. FLT3, RAS, and TP53 mutations in elderly patients with acute myeloid leukemia. Blood. 2001;97:3589–95.

62. Thiede C, Steudel C, Mohr B et al. Analysis of FLT3-activating mutations in 979 patients with acute myelogenous leukemia: association with FAB subtypes and identification of subgroups with poor prognosis. Blood. 2002;99:4326–35.

63. Armstrong SA, Staunton JE, Silverman LB et al. MLL translocations specify a distinct gene expression profile that distinguishes a unique leukemia. Nat Genet. 2002;30 :41–7.

64. Behm FG, Raimondi SC, Frestedt JL et al. Rearrangement of the MLL gene confers a poor prognosis in childhood acute lymphoblastic leukemia, regardless of presenting age. Blood. 1996;87:2870–7.

65. Dimartino JF, Cleary ML. MLL rearrangements in haematological malignancies: lessons from clinical and biological studies. Br J Haematol. 1999;106:614–26.

66. Dohner K, Tobis K, Ulrich R et al. Prognostic significance of partial tandem duplications of the MLL gene in adult patients 16 to 60 years old with acute myeloid leukemia and normal cytogenetics: a study of the Acute Myeloid Leukemia Study Group Ulm. J Clin Oncol. 2002;20:3254–61.

67. Munoz L, Nomdedeu JF, Villamor N et al. Acute myeloid leukemia with MLL rearrangements: clinicobiological features, prognostic impact and value of flow cytometry in the detection of residual leukemic cells. Leukemia. 2003;17:76–82.

68. Schnittger S, Kinkelin U, Schoch C et al. Screenings for MLL tandem duplication in 387 unselected patients with AML identify a prognostically unfavorable subset of AML. Leukemia. 2000;14:796–804.

69. Nowell PC, Hungerford DA. Chromosome studies on normal and leukemic human leukocytes. J Natl Cancer Inst. 1960;25:85–109.

70. Rowley JD. Letter: A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. Nature. 1973;243:290–3.

71. Heisterkamp N, Stam K, Groffen J, de Klein A, Grosveld G. Structural organization of the bcr gene and its role in the Ph' translocation. Nature. 1985;315:758–61.

72. Bloomfield CD, Arthur DC, Frizzera G, Levine EG, Peterson BA, Gajl-Peczalska KJ. Nonrandom chromosome abnormalities in lymphoma. Cancer Res. 1983;43:2975–84.

73. Bloomfield CD, Goldman AI, Alimena G et al. Chromosomal abnormalities identify high-risk and low-risk

patients with acute lymphoblastic leukemia. Blood. 1986;67:415–20.

74. Rowley JD, Testa JR. Chromosome abnormalities in malignant hematologic diseases. Adv Cancer Res. 1982;36: 103–48.

75. Erickson P, Gao J, Chang KS et al. Identification of breakpoints in t(8;21) acute myelogenous leukemia and isolation of a fusion transcript, AML1/ETO, with similarity to Drosophila segmentation gene, runt. Blood. 1992;80:1825–31.

76. Liu PP, Hajra A, Wijmenga C, Collins FS. Molecular pathogenesis of the chromosome 16 inversion in the M4Eo subtype of acute myeloid leukemia. Blood. 1995;85:2289–302.

77. Larson RA, Kondo K, Vardiman JW, Butler AE, Golomb HM, Rowley JD. Evidence for a 15;17 translocation in every patient with acute promyelocytic leukemia. Am J Med. 1984;76:827–41.

78. Bitter MA, Le Beau MM, Larson RA et al. A morphologic and cytochemical study of acute myelomonocytic leukemia with abnormal marrow eosinophils associated with inv(16)(p13q22). Am J Clin Pathol. 1984;81: 733–41.

79. Larson RA, Williams SF, Le Beau MM, Bitter MA, Vardiman JW, Rowley JD. Acute myelomonocytic leukemia with abnormal eosinophils and inv(16) or t(16;16) has a favorable prognosis. Blood. 1986;68:1242–9.

80. Mrozek K, Heinonen K, de la Chapelle A, Bloomfield CD. Clinical significance of cytogenetics in acute myeloid leukemia. Semin Oncol. 1997;24:17–31.

81. Berger R, Bernheim A. Cytogenetics of Burkitt's lymphoma-leukaemia: a review. IARC Sci Publ. 1985: 65–80.

82. Berger R, Bernheim A, Weh HJ, Daniel MT, Flandrin G. Cytogenetic studies on acute monocytic leukemia. Leuk Res. 1980;4:119–27.

83. Rowley JD. Rearrangements involving chromosome band 11Q23 in acute leukaemia. Semin Cancer Biol. 1993;4:377–85.

84. Thirman MJ, Gill HJ, Burnett RC et al. Rearrangement of the MLL gene in acute lymphoblastic and acute myeloid leukemias with 11q23 chromosomal translocations. N Engl J Med. 1993;329:909–14.

85. Schichman SA, Caligiuri MA, Gu Y et al. ALL-1 partial duplication in acute leukemia. Proc Natl Acad Sci USA. 1994;91:6236–9.

86. Johnson E, Cotter FE. Monosomy 7 and 7q− associated with myeloid malignancy. Blood Rev. 1997;11:46–55.

87. Tchinda J, Neumann TE, Volpert S, Berdel WE, Buchner T, Horst J. Characterization of chromosomal rearrangements in hematological diseases using spectral karyotyping. Diagn Mol Pathol. 2004;13:190–5.

88. Neuman WL, Rubin CM, Rios RB et al. Chromosomal loss and deletion are the most common mechanisms for loss of heterozygosity from chromosomes 5 and 7 in malignant myeloid disorders. Blood. 1992;79:1501–10.

89. Van den Berghe H, Cassiman JJ, David G, Fryns JP, Michaux JL, Sokal G. Distinct haematological disorder with deletion of long arm of no. 5 chromosome. Nature. 1974;251:437–8.

90. Testa JR, Kinnealey A, Rowley JD, Golde DW, Potter D. Deletion of the long arm of chromosome 20 [del(20)(q11)] in myeloid disorders. Blood. 1978;52:868–77.

91. Rowley JD. Nonrandom chromosomal abnormalities in hematologic disorders of man. Proc Natl Acad Sci USA. 1975;72:152–6.

92. Toyama K, Ohyashiki K, Yoshida Y et al. Clinical implications of chromosomal abnormalities in 401 patients with myelodysplastic syndromes: a multicentric study in Japan. Leukemia. 1993;7:499–508.

93. Secker-Walker LM, Lawler SD, Hardisty RM. Prognostic implications of chromosomal findings in acute lymphoblastic leukaemia at diagnosis. BMJ. 1978;2:1529–30.

94. Pui CH, Carroll AJ, Raimondi SC et al. Clinical presentation, karyotypic characterization, and treatment outcome of childhood acute lymphoblastic leukemia with a near-haploid or hypodiploid less than 45 line. Blood. 1990;75:1170–7.

95. Pui CH, Williams DL, Kalwinsky DK et al. Cytogenetic features and serum lactic dehydrogenase level predict a poor treatment outcome for children with pre-B-cell leukemia. Blood. 1986;67:1688–92.

96. Williams DL, Raimondi S, Rivera G, George S, Berard CW, Murphy SB. Presence of clonal chromosome abnormalities in virtually all cases of acute lymphoblastic leukemia. N Engl J Med. 1985;313:640–1.

97. Raimondi SC, Behm FG, Roberson PK et al. Cytogenetics of pre-B-cell acute lymphoblastic leukemia with emphasis on prognostic implications of the t(1;19). J Clin Oncol. 1990;8:1380–8.

98. Manolov G, Manolova Y. Marker band in one chromosome 14 from Burkitt lymphomas. Nature. 1972;237:33–4.

99. Dierlamm J, Michaux L, Criel A, Wlodarska I, Van den Berghe H, Hossfeld DK. Genetic abnormalities in chronic lymphocytic leukemia and their clinical and prognostic implications. Cancer Genet Cytogenet. 1997;94:27–35.

100. Yunis JJ, Oken MM, Kaplan ME, Ensrud KM, Howe RR, Theologides A. Distinctive chromosomal abnormalities in histologic subtypes of non-Hodgkin's lymphoma. N Engl J Med. 1982;307:1231–6.

101. Offit K, Jhanwar SC, Ladanyi M, Filippa DA, Chaganti RS. Cytogenetic analysis of 434 consecutively ascertained specimens of non-Hodgkin's lymphoma: correlations between recurrent aberrations, histology, and exposure to cytotoxic treatment. Genes Chromosomes Cancer. 1991;3:189–201.

102. Offit K, Richardson ME, Ceng QQ et al. Nonrandom chromosomal aberrations are associated with sites of tissue involvement in non-Hodgkin's lymphoma. Cancer Genet Cytogenet. 1989;37:85–93.

103. Bernasconi P, Boni M, Cavigliano PM et al. Clinical relevance of cytogenetics in myelodysplastic syndromes. Ann NY Acad Sci. 2006;1089:395–410.

104. Cigudosa JC, Parsa NZ, Louie DC et al. Cytogenetic analysis of 363 consecutively ascertained diffuse large B-cell lymphomas. Genes Chromosomes Cancer. 1999;25:123–33.

105. Cuneo A, Bigoni R, Rigolin GM et al. Cytogenetic profile of lymphoma of follicle mantle lineage: correlation with clinicobiologic features. Blood. 1999;93:1372–80.

106. Fonseca R, Coignet LJ, Dewald GW. Cytogenetic abnormalities in multiple myeloma. Hematol Oncol Clin North Am. 1999;13:1169–80, viii.

107. Heim S, Mitelman F. Cytogenetic analysis in the diagnosis of acute leukemia. Cancer. 1992;70:1701–9.

108. Elliott MA, Letendre L, Hanson CA, Tefferi A, Dewald GW. The prognostic significance of trisomy 8 in patients with acute myeloid leukemia. Leuk Lymphoma. 2002;43:583–6.

109. Wotherspoon AC, Pan LX, Diss TC, Isaacson PG. Cytogenetic study of B-cell lymphoma of mucosa-associated lymphoid tissue. Cancer Genet Cytogenet. 1992;58:35–8.

110. Perry A. Fluorescence in situ hybridization. In: Pfeifer JD, ed. Molecular Genetics Testing in Surgical Pathology. Philadelphia: Lippincott Wiliams & WIlkins; 2006: 72–85.

111. Dewald GW. Cytogenetic and FISH studies in myelodysplasia, acute myeloid leukemia, chronic lymphocytic leukemia and lymphoma. Int J Hematol. 2002;76(Suppl 2): 65–74.

112. Perez-Simon JA, Garcia-Sanz R, Tabernero MD et al. Prognostic value of numerical chromosome aberrations in multiple myeloma: a FISH analysis of 15 different chromosomes. Blood. 1998;91:3366–71.

113. Speicher MR, Gwyn Ballard S, Ward DC. Karyotyping human chromosomes by combinatorial multi-fluor FISH. Nat Genet. 1996;12:368–75.

114. Wilkens L, Tchinda J, Burkhardt D, Nolte M, Werner M, Georgii A. Analysis of hematologic diseases using conventional karyotyping, fluorescence in situ hybridization (FISH), and comparative genomic hybridization (CGH). Hum Pathol. 1998;29:833–9.

115. Andreasson P, Johansson B, Billstrom R, Garwicz S, Mitelman F, Hoglund M. Fluorescence in situ hybridization analyses of hematologic malignancies reveal frequent cytogenetically unrecognized 12p rearrangements. Leukemia. 1998;12:390–400.

116. Belaud-Rotureau MA, Parrens M, Carrere N et al. Interphase fluorescence in situ hybridization is more sensitive than BIOMED-2 polymerase chain reaction protocol in detecting IGH-BCL2 rearrangement in both fixed and frozen lymph node with follicular lymphoma. Hum Pathol. 2007;38(2):365–72.

117. Dave BJ, Nelson M, Pickering DL et al. Cytogenetic characterization of diffuse large cell lymphoma using multicolor fluorescence in situ hybridization. Cancer Genet Cytogenet. 2002;132:125–32.

118. Dewald GW, Brockman SR, Paternoster SF et al. Chromosome anomalies detected by interphase fluorescence in situ hybridization: correlation with significant biological features of B-cell chronic lymphocytic leukaemia. Br J Haematol. 2003;121:287–95.

119. Lee DY, Cho HI, Kang YH et al. The role of fluorescence in situ hybridization (FISH) for monitoring hematologic malignancies with BCR/ABL or ETO/AML1 rearrangement: a comparative study with FISH and G-banding on 919 consecutive specimens of hematologic malignancies. Cancer Genet Cytogenet. 2004;152:1–7.

120. Schrock E, Padilla-Nash H. Spectral karyotyping and multicolor fluorescence in situ hybridization reveal new tumor-specific chromosomal aberrations. Semin Hematol. 2000;37:334–47.

121. Remstein ED, Kurtin PJ, Buno I et al. Diagnostic utility of fluorescence in situ hybridization in mantle-cell lymphoma. Br J Haematol. 2000;110:856–62.

122. Paternoster SF, Brockman SR, McClure RF, Remstein ED, Kurtin PJ, Dewald GW. A new method to extract nuclei from paraffin-embedded tissue to study lymphomas using interphase fluorescence in situ hybridization. Am J Pathol. 2002;160:1967–72.

123. Godon A, Genevieve F, Valo I et al. Formalin-fixed and paraffin-embedded nodal non-Hodgkin's lymphomas

demonstrate the same chromosome changes as those found in frozen samples: a comparative study using interphase fluorescence in situ hybridization. Diagn Mol Pathol. 2004;13:97–104.

124. Rack KA, Delabesse E, Radford-Weiss I et al. Simultaneous detection of MYC, BVR1, and PVT1 translocations in lymphoid malignancies by fluorescence in situ hybridization. Genes Chromosomes Cancer. 1998;23:220–6.

125. Rack KA, Salomon-Nguyen F, Radford-Weiss I et al. FISH detection of chromosome 14q32/IgH translocations: evaluation in follicular lymphoma. Br J Haematol. 1998;103:495–504.

126. Coignet LJ, Schuuring E, Kibbelaar RE et al. Detection of 11q13 rearrangements in hematologic neoplasias by double-color fluorescence in situ hybridization. Blood. 1996;87:1512–19.

127. Wlodarska I, Mecucci C, Stul M et al. Fluorescence in situ hybridization identifies new chromosomal changes involving 3q27 in non-Hodgkin's lymphomas with BCL6/LAZ3 rearrangement. Genes Chromosomes Cancer. 1995;14:1–7.

128. Macville M, Veldman T, Padilla-Nash H et al. Spectral karyotyping, a 24-colour FISH technique for the identification of chromosomal rearrangements. Histochem Cell Biol. 1997;108:299–305.

129. Langer S, Kraus J, Jentsch I, Speicher MR. Multicolor chromosome painting in diagnostic and research applications. Chromosome Res. 2004;12:15–23.

130. Tchinda J, Volpert S, McNeil N et al. Multicolor karyotyping in acute myeloid leukemia. Leuk Lymphoma. 2003;44:1843–53.

131. Veldman T, Vignon C, Schrock E, Rowley JD, Ried T. Hidden chromosome abnormalities in haematological malignancies detected by multicolour spectral karyotyping. Nat Genet. 1997;15:406–10.

132. Kallioniemi A, Kallioniemi OP, Sudar D et al. Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. Science. 1992;258:818–21.

133. Mullis K, Faloona F, Scharf S, Saiki R, Horn G, Erlich H. Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. Cold Spring Harb Symp Quant Biol. 1986;51(Pt 1):263–73.

134. Mullis K, Faloona F, Scharf S, Saiki R, Horn G, Erlich H. Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. 1986. Biotechnology. 1992;24:17–27.

135. Mullis KB, Faloona FA. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. Methods Enzymol. 1987;155:335–50.

136. Zhao X, Li C, Paez JG et al. An integrated view of copy number and allelic alterations in the cancer genome using single nucleotide polymorphism arrays. Cancer Res. 2004;64:3060–71.

137. Bignell GR, Huang J, Greshock J et al. High-resolution analysis of DNA copy number using oligonucleotide microarrays. Genome Res. 2004;14:287–95.

138. Dutt A, Beroukhim R. Single nucleotide polymorphism array analysis of cancer. Curr Opin Oncol. 2007;19:43–9.

139. Dunphy CH. Gene expression profiling data in lymphoma and leukemia: review of the literature and extrapolation of pertinent clinical applications. Arch Pathol Lab Med. 2006;130:483–520.

140. Golub TR, Slonim DK, Tamayo P et al. Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. Science. 1999;286:531–7.

141. Haferlach T, Kern W, Schnittger S et al. Gene expression profiling in leukemias using microarrays. In: Degos L, Linch DC, Lowenberg, B, eds. Textbook of Malignant Hematology, (2nd edn). London: Taylor & Francis, 2005:254–65.

142. Baumgarth N, Roederer M. A practical approach to multicolor flow cytometry for immunophenotyping. J Immunol Methods. 2000;243:77–97.

143. Borowitz MJ, Bray R, Gascoyne R et al. U.S.–Canadian Consensus recommendations on the immunophenotypic analysis of hematologic neoplasia by flow cytometry: data analysis and interpretation. Cytometry. 1997;30:236–44.

144. Braylan RC. Flow cytometry is becoming an indispensable tool in leukemia diagnosis and classification. Cancer Invest. 1997;15:382–3.

145. Braylan RC, Atwater SK, Diamond L et al. U.S.–Canadian Consensus recommendations on the immunophenotypic analysis of hematologic neoplasia by flow cytometry: data reporting. Cytometry. 1997;30:245–8.

146. Gorczyca W. Flow cytometry immunophenotypic characteristics of monocytic population in acute monocytic leukemia (AML-M5), acute myelomonocytic leukemia (AML-M4), and chronic myelomonocytic leukemia (CMML). Methods Cell Biol. 2004;75:665–77.

147. Gorczyca W. Differential diagnosis of T-cell lymphoproliferative disorders by flow cytometry multicolor immunophenotyping. Correlation with morphology. Methods Cell Biol. 2004;75:595–21.

148. Gorczyca W, Tugulea S, Liu Z, Li X, Wong JY, Weisberger J. Flow cytometry in the diagnosis of mediastinal tumors with emphasis on differentiating thymocytes from precursor T-lymphoblastic lymphoma/leukemia. Leuk Lymphoma. 2004;45:529–38.

149. Jennings CD, Foon KA. Recent advances in flow cytometry: application to the diagnosis of hematologic malignancy. Blood. 1997;90:2863–92.

150. Vidriales MB, San-Miguel JF, Orfao A, Coustan-Smith E, Campana D. Minimal residual disease monitoring by flow cytometry. Best Pract Res Clin Haematol. 2003;16:599–612.

151. Weir EG, Borowitz MJ. Flow cytometry in the diagnosis of acute leukemia. Semin Hematol. 2001;38:124–38.

152. Le Beau MM. Deletions of chromosome 5 in malignant myeloid disorders. Cancer Surv. 1992;15:143–59.

153. Giagounidis AA, Germing U, Aul C. Biological and prognostic significance of chromosome 5q deletions in myeloid malignancies. Clin Cancer Res. 2006;12:5–10.

154. Strupp C, Gattermann N, Giagounidis A et al. Refactory anemia with excess of blasts in trasformation: analysis of reclassification according to the WHO proposal. Leuk Res 2003:27:397–404.

155. Giagounidis AA, Germing U, Haase S et al. Clinical, morphological, cytogenetic, and prognostic features of patients with myelodysplastic syndromes and del(5q) including band q31. Leukemia. 2004;18:113–19.

156. Giagounidis AA, Germing U, Wainscoat JS, Boultwood J, Aul C. The 5q− syndrome. Hematology. 2004;9: 271–7.

157. Boultwood J, Lewis S, Wainscoat JS. The 5q − syndrome. Blood. 1994;84:3253–60.

158. Mathew P, Tefferi A, Dewald GW et al. The 5q− syndrome: a single-institution study of 43 consecutive patients. Blood. 1993;81:1040–5.

159. Tefferi A, Mathew P, Noel P. The 5q- syndrome: a scientific and clinical update. Leuk Lymphoma. 1994;14:375–8.

160. Cermak J, Michalova K, Brezinova J, Zemanova Z. A prognostic impact of separation of refractory cytopenia with multilineage dysplasia and 5q- syndrome from refractory anemia in primary myelodysplastic syndrome. Leuk Res. 2003;27:221–9.

161. van der Holt B, Breems DA, Berna Beverloo H et al. Various distinctive cytogenetic abnormalities in patients with acute myeloid leukaemia aged 60 years and older express adverse prognostic value: results from a prospective clinical trial. Br J Haematol. 2007;136:96–105.

162. Larripa I, Acevedo S, Palau Nagore MV, Bengio R, Mondini N, Slavutsky I. Leukemic transformation in patients with 5q− and additional abnormalities. Haematologica. 1991;76:363–7.

163. Side LE, Curtiss NP, Teel K et al. RAS, FLT3, and TP53 mutations in therapy-related myeloid malignancies with abnormalities of chromosomes 5 and 7. Genes Chromosomes Cancer. 2004;39:217–23.

164. Grimwade D, Moorman A, Hills R et al. Impact of karyotype on treatment outcome in acute myeloid leukemia. Ann Hematol. 2004;83(Suppl 1):S45–8.

165. Fidler C, Watkins F, Bowen DT, Littlewood TJ, Wainscoat JS, Boultwood J. NRAS, FLT3 and TP53 mutations in patients with myelodysplastic syndrome and a del(5q). Haematologica. 2004;89:865–6.

166. Petrella T, Dalac S, Maynadie M et al. CD4+ CD56+ cutaneous neoplasms: a distinct hematological entity? Groupe Francais d'Etude des Lymphomes Cutanes (GFELC). Am J Surg Pathol. 1999;23:137–46.

167. Touahri T, Belaouni H, Mossafa H et al. Agranular CD4+/CD56+ cutaneous neoplasm. Leuk Lymphoma. 2002;43:1475–9.

168. Jacob MC, Chaperot L, Mossuz P et al. CD4+ CD56+ lineage negative malignancies: a new entity developed from malignant early plasmacytoid dendritic cells. Haematologica. 2003;88:941–55.

169. Kamada N, Sakurai M, Miyamoto K et al. Chromosome abnormalities in adult T-cell leukemia/lymphoma: a karyotype review committee report. Cancer Res. 1992;52:1481–93.

170. Nakase K, Hasegawa M, Tsuji K et al. HTLV-1 unrelated adult T-cell leukemia/lymphoma with unique phenotype and karyotype. Am J Hematol. 2000;64:64–6.

171. South ST, Frazer JK, Brothman AR, Chen Z. Unexpected cytogenetic finding in acute lymphoblastic leukemia: a case of del(5q) with a cryptic t(12;21). Cancer Genet Cytogenet. 2006;168:177–8.

172. Berger R, Le Coniat M, Derre J. 5q- anomaly in acute lymphoblastic leukemia. Cancer Genet Cytogenet. 1992;61:201–3.

173. Wynn TT, Heerema NA, Hammond S, Ranalli M, Kahwash SB. Acute lymphoblastic leukemia with hypereosinophilia: report of a case with 5q deletion and review of the literature. Pediatr Dev Pathol. 2003;6: 558–63.

174. Taborelli M, Tibiletti MG, Martin V, Pozzi B, Bertoni F, Capella C. Chromosome band 6q deletion pattern in malignant lymphomas. Cancer Genet Cytogenet. 2006;165:106–13.

175. Tilly H, Rossi A, Stamatoullas A et al. Prognostic value of chromosomal abnormalities in follicular lymphoma. Blood. 1994;84:1043–9.

176. Merup M, Moreno TC, Heyman M et al. 6q deletions in acute lymphoblastic leukemia and non-Hodgkin's lymphomas. Blood. 1998;91:3397–400.

177. Schouten HC, Sanger WG, Weisenburger DD, Armitage JO. Abnormalities involving chromosome 6 in newly diagnosed patients with non-Hodgkin's lymphoma. Nebraska Lymphoma Study Group. Cancer Genet Cytogenet. 1990;47:73–82.

178. Vitolo U, Gaidano G, Botto B et al. Rearrangements of bcl-6, bcl-2, c-myc and 6q deletion in B-diffuse large-cell lymphoma: clinical relevance in 71 patients. Ann Oncol. 1998;9:55–61.

179. Johansson B, Mertens F, Mitelman F. Cytogenetic deletion maps of hematologic neoplasms: circumstantial evidence for tumor suppressor loci. Genes Chromosomes Cancer. 1993;8:205–18.

180. Aamot HV, Torlakovic EE, Eide MB, Holte H, Heim S. Non-Hodgkin lymphoma with t(14;18): clonal evolution patterns and cytogenetic–pathologic–clinical correlations. J Cancer Res Clin Oncol 2007;133:455–70.

181. Viardot A, Moller P, Hogel J et al. Clinicopathologic correlations of genomic gains and losses in follicular lymphoma. J Clin Oncol. 2002;20:4523–30.

182. Schop RF, Kuehl WM, Van Wier SA et al. Waldenstrom macroglobulinemia neoplastic cells lack immunoglobulin heavy chain locus translocations but have frequent 6q deletions. Blood. 2002;100:2996–3001.

183. Schop RF, Fonseca R. Genetics and cytogenetics of Waldenstrom's macroglobulinemia. Semin Oncol. 2003;30:142–5.

184. Schop RF, Van Wier SA, Xu R et al. 6q deletion discriminates Waldenstrom macroglobulinemia from IgM monoclonal gammopathy of undetermined significance. Cancer Genet Cytogenet. 2006;169:150–3.

185. Amiel A, Mulchanov I, Elis A et al. Deletion of 6q27 in chronic lymphocytic leukemia and multiple myeloma detected by fluorescence in situ hybridization. Cancer Genet Cytogenet. 1999;112:53–6.

186. Cuneo A, Rigolin GM, Bigoni R et al. Chronic lymphocytic leukemia with 6q− shows distinct hematological features and intermediate prognosis. Leukemia. 2004;18:476–83.

187. Stilgenbauer S, Bullinger L, Benner A et al. Incidence and clinical significance of 6q deletions in B cell chronic lymphocytic leukemia. Leukemia. 1999;13:1331–4.

188. Sherratt T, Morelli C, Boyle JM, Harrison CJ. Analysis of chromosome 6 deletions in lymphoid malignancies provides evidence for a region of minimal deletion within a 2-megabase segment of 6q21. Chromosome Res. 1997;5:118–24.

189. Gutierrez NC, Garcia JL, Hernandez JM et al. Prognostic and biological significance of chromosomal imbalances assessed by comparative genomic hybridization in multiple myeloma. Blood 2004;104:2661–6.

190. Bosga-Bouwer AG, Kok K, Booman M et al. Array comparative genomic hybridization reveals a very high frequency of deletions of the long arm of chromosome 6 in testicular lymphoma. Genes Chromosomes Cancer. 2006;45:976–81.

191. Peters K, Zettl A, Starostik P et al. Genetic imbalances in primary gastric diffuse large B-cell lymphomas: comparison of comparative genomic hybridization, microsatellite, and cytogenetic analysis. Diagn Mol Pathol. 2000;9:58–65.

192. Fischer TC, Gellrich S, Muche JM et al. Genomic aberrations and survival in cutaneous T cell lymphomas. J Invest Dermatol. 2004;122:579–86.

193. Tien HF, Su IJ, Tang JL et al. Clonal chromosomal abnormalities as direct evidence for clonality in nasal T/natural killer cell lymphomas. Br J Haematol. 1997;97:621–5.

194. Cheng SH, Ng MH, Lau KM et al. 4q loss is potentially an important genetic event in MM tumorigenesis: identification of a tumor suppressor gene regulated by promoter methylation at 4q13.3, platelet factor 4. Blood 2006;109:2089–99.

195. Wong KF, Chan JK, Kwong YL. Identification of del(6)(q21q25) as a recurring chromosomal abnormality in putative NK cell lymphoma/leukaemia. Br J Haematol. 1997;98:922–6.

196. Heerema NA, Sather HN, Sensel MG et al. Clinical significance of deletions of chromosome arm 6q in childhood acute lymphoblastic leukemia: a report from the Children's Cancer Group. Leuk Lymphoma. 2000;36:467–78.

197. Mancini M, Vegna ML, Castoldi GL et al. Partial deletions of long arm of chromosome 6: biologic and clinical implications in adult acute lymphoblastic leukemia. Leukemia. 2002;16:2055–61.

198. Tosi S, Scherer SW, Giudici G, Czepulkowski B, Biondi A, Kearney L. Delineation of multiple deleted regions in 7q in myeloid disorders. Genes Chromosomes Cancer. 1999;25:384–92.

199. Brozek I, Babinska M, Kardas I et al. Cytogenetic analysis and clinical significance of chromosome 7 aberrations in acute leukaemia. J Appl Genet. 2003;44:401–12.

200. Baldus C, Fietz T, Rieder H, Schwartz S, Thiel E, Knauf W. MDR-1 expression and deletions of chromosomes 7 and 5(Q) separately indicate adverse prognosis in AML. Leuk Lymphoma. 2001;40:613–23.

201. Velloso ER, Michaux L, Ferrant A et al. Deletions of the long arm of chromosome 7 in myeloid disorders: loss of band 7q32 implies worst prognosis. Br J Haematol. 1996;92:574–81.

202. Chang KL, O'Donnell MR, Slovak ML et al. Primary myelodysplasia occurring in adults under 50 years old: a clinicopathologic study of 52 patients. Leukemia. 2002;16:623–31.

203. Baranger L, Baruchel A, Leverger G, Schaison G, Berger R. Monosomy-7 in childhood hemopoietic disorders. Leukemia. 1990;4:345–9.

204. McKenna RW. Myelodysplasia and myeloproliferative disorders in children. Am J Clin Pathol. 2004; 122(Suppl):S58–69.

205. Hasle H, Alonzo TA, Auvrignon A et al. Monosomy 7 and deletion 7q in children and adolescents with acute myeloid leukemia: an international retrospective study. Blood 2007;109:4641–7.

206. Evans JP, Czepulkowski B, Gibbons B, Swansbury GJ, Chessells JM. Childhood monosomy 7 revisited. Br J Haematol. 1988;69:41–5.

207. Trobaugh-Lotrario AD, Kletzel M, Quinones RR et al. Monosomy 7 associated with pediatric acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS): successful management by allogeneic hematopoietic stem cell transplant (HSCT). Bone Marrow Transplant. 2005;35:143–9.

208. Passmore SJ, Chessells JM, Kempski H, Hann IM, Brownbill PA, Stiller CA. Paediatric myelodysplastic syndromes and juvenile myelomonocytic leukaemia in the UK: a population-based study of incidence and survival. Br J Haematol. 2003;121:758–67.

209. Heerema NA, Nachman JB, Sather HN et al. Deletion of 7p or monosomy 7 in pediatric acute lymphoblastic leukemia is an adverse prognostic factor: a report from the Children's Cancer Group. Leukemia. 2004;18:939–47.

210. Tripputi P, Cassani B, Alfano R et al. Chromosome 7 monosomy and deletions in myeloproliferative diseases. Leuk Res. 2001;25:735–9.

211. Daghistani D, Toledano SR, Curless R. Monosomy 7 syndrome. Clinical heterogeneity in children and adolescents. Cancer Genet Cytogenet. 1990;44:263–9.

212. Terre C, Eclache V, Rousselot P et al. Report of 34 patients with clonal chromosomal abnormalities in Philadelphia-negative cells during imatinib treatment of Philadelphia-positive chronic myeloid leukemia. Leukemia. 2004;18:1340–6.

213. Lin Y, Bruyere H, Horsman DE et al. Philadelphia-negative clonal hematopoiesis following imatinib therapy in patients with chronic myeloid leukemia: a report of nine cases and analysis of predictive factors. Cancer Genet Cytogenet. 2006;170:16–23.

214. Algara P, Mateo MS, Sanchez-Beato M et al. Analysis of the IgV(H) somatic mutations in splenic marginal zone lymphoma defines a group of unmutated cases with frequent 7q deletion and adverse clinical course. Blood. 2002;99:1299–1304.

215. Corcoran MM, Mould SJ, Orchard JA et al. Dysregulation of cyclin dependent kinase 6 expression in splenic marginal zone lymphoma through chromosome 7q translocations. Oncogene. 1999;18:6271–7.

216. Viaggi S, Abbondandolo A, Carbone M et al. Uncommon cytogenetic findings in a case of splenic marginal zone lymphoma with aggressive clinical course. Cancer Genet Cytogenet. 2004;148:133–6.

217. Sole F, Salido M, Espinet B et al. Splenic marginal zone B-cell lymphomas: two cytogenetic subtypes, one with gain of 3q and the other with loss of 7q. Haematologica. 2001;86:71–7.

218. Dascalescu CM, Peoc'h M, Callanan M et al. Deletion 7q in B-cell low-grade lymphoid malignancies: a cytogenetic/fluorescence in situ hybridization and immunopathologic study. Cancer Genet Cytogenet. 1999;109:21–8.

219. Offit K, Louie DC, Parsa NZ, Noy A, Chaganti RS. Del (7)(q32) is associated with a subset of small lymphocytic lymphoma with plasmacytoid features. Blood. 1995;86:2365–70.

220. Hernandez JM, Garcia JL, Gutierrez NC et al. Novel genomic imbalances in B-cell splenic marginal zone lymphomas revealed by comparative genomic hybridization and cytogenetics. Am J Pathol. 2001;158:1843–50.

221. Garcia JL, Hernandez JM, Gutierrez NC et al. Abnormalities on 1q and 7q are associated with poor outcome in sporadic Burkitt's lymphoma. A cytogenetic and comparative genomic hybridization study. Leukemia. 2003;17:2016–24.

222. Hernandez JM, Mecucci C, Michaux L et al. del(7q) in chronic B-cell lymphoid malignancies. Cancer Genet Cytogenet. 1997;93:147–51.

223. Sanada M, Uike N, Ohyashiki K et al. Unbalanced translocation der(1;7)(q10;p10) defines a unique clinicopathological subgroup of myeloid neoplasms. Leukemia. 2007;21:992–7.

224. Moorman AV, Harrison CJ, Buck GA et al. Karyotype is an independent prognostic factor in adult acute lymphoblastic leukemia (ALL): analysis of cytogenetic data from patients treated on the Medical Research Council (MRC) UKALLXII/Eastern Cooperative Oncology Group (ECOG) 2993 trial. Blood. 2007;109:3189–97.

225. Strefford JC, Worley H, Barber K et al. Genome complexity in acute lymphoblastic leukemia is revealed by array-based comparative genomic hybridization. Oncogene 2007;26:4306–18.

226. Au WY, Gascoyne RD, Viswanatha DS, Connors JM, Klasa RJ, Horsman DE. Cytogenetic analysis in mantle cell lymphoma: a review of 214 cases. Leuk Lymphoma. 2002;43:783–91.

227. Flordal E, Berglund M, Rosenquist R et al. Clonal development of a blastoid mantle cell lymphoma studied with comparative genomic hybridization. Cancer Genet Cytogenet. 2002;139:38–43.

228. Vizcarra E, Martinez-Climent JA, Benet I et al. Identification of two subgroups of mantle cell leukemia with distinct clinical and biological features. Hematol J. 2001;2:234–41.

229. Rinaldi A, Kwee I, Poretti G et al. Comparative genome-wide profiling of post-transplant lymphoproliferative disorders and diffuse large B-cell lymphomas. Br J Haematol. 2006;134:27–36.

230. Chaganti SR, Gaidano G, Louie DC, Dalla-Favera R, Chaganti RS. Diffuse large cell lymphomas exhibit frequent deletions in 9p21–22 and 9q31–34 regions. Genes Chromosomes Cancer. 1995;12:32–6.

231. Karnan S, Tagawa H, Suzuki R et al. Analysis of chromosomal imbalances in de novo CD5-positive diffuse large-B-cell lymphoma detected by comparative genomic hybridization. Genes Chromosomes Cancer. 2004;39:77–81.

232. Pollak C, Hagemeijer A. Abnormalities of the short arm of chromosome 9 with partial loss of material in hematological disorders. Leukemia. 1987;1:541–8.

233. Zettl A, Rudiger T, Konrad MA et al. Genomic profiling of peripheral T-cell lymphoma, unspecified, and anaplastic large T-cell lymphoma delineates novel recurrent chromosomal alterations. Am J Pathol. 2004;164:1837–48.

234. Peniket A, Wainscoat J, Side L et al. Del (9q) AML: clinical and cytological characteristics and prognostic implications. Br J Haematol. 2005;129:210–20.

235. Appelbaum FR, Kopecky KJ, Tallman MS et al. The clinical spectrum of adult acute myeloid leukaemia associated with core binding factor translocations. Br J Haematol. 2006;135:165–73.

236. Frohling S, Schlenk RF, Krauter J et al. Acute myeloid leukemia with deletion 9q within a noncomplex karyotype is associated with CEBPA loss-of-function mutations. Genes Chromosomes Cancer. 2005; 42:427–32.

237. Leroy H, Roumier C, Huyghe P, Biggio V, Fenaux P, Preudhomme C. CEBPA point mutations in hematological malignancies. Leukemia. 2005;19:329–34.

238. Monni O, Knuutila S. 11q deletions in hematological malignancies. Leuk Lymphoma. 2001;40:259–66.

239. Zhu Y, Monni O, Franssila K et al. Deletions at 11q23 in different lymphoma subtypes. Haematologica. 2000; 85:908–12.

240. Dohner H, Stilgenbauer S, James MR et al. 11q deletions identify a new subset of B-cell chronic lymphocytic leukemia characterized by extensive nodal involvement and inferior prognosis. Blood. 1997;89:2516–22.

241. Byrd JC, Gribben JG, Peterson BL et al. Select high-risk genetic features predict earlier progression following chemoimmunotherapy with fludarabine and rituximab in chronic lymphocytic leukemia: justification for risk-adapted therapy. J Clin Oncol. 2006;24:437–43.

242. Dickinson JD, Smith LM, Sanger WG et al. Unique gene expression and clinical characteristics are associated with the 11q23 deletion in chronic lymphocytic leukaemia. Br J Haematol. 2005;128:460–71.

243. Aamot HV, Tjonnfjord GE, Delabie J, Heim S. Molecular cytogenetic analysis of leukemic mantle cell lymphoma with a cryptic t(11;14). Cancer Genet Cytogenet. 2006; 165:172–5.

244. Cuneo A, Bigoni R, Roberti MG et al. Molecular cytogenetic characterization of marginal zone B-cell lymphoma: correlation with clinicopathologic findings in 14 cases. Haematologica. 2001;86:64–70.

245. Bigoni R, Cuneo A, Milani R et al. Secondary chromosome changes in mantle cell lymphoma: cytogenetic and fluorescence in situ hybridization studies. Leuk Lymphoma. 2001;40:581–90.

246. Look AT. Molecular pathogenesis of MDS. Hematology Am Soc Hematol Educ Program 2005:156–60.

247. Sole F, Luno E, Sanzo C et al. Identification of novel cytogenetic markers with prognostic significance in a series of 968 patients with primary myelodysplastic syndromes. Haematologica. 2005;90:1168–78.

248. Gonzalez MB, Hernandez JM, Garcia JL et al. The value of fluorescence in situ hybridization for the detection of 11q in multiple myeloma. Haematologica. 2004; 89:1213–18.

249. Campbell LJ, Rayeroux KC, Arkell K, Catalano JV, Cole-Sinclair MF. Appearance of del(11q) in two patients with acute promyelocytic leukaemia treated with all-trans retinoic acid and combination chemotherapy. Br J Haematol. 2002;118:243–5.

250. Sato Y, Suto Y, Pietenpol J et al. TEL and KIP1 define the smallest region of deletions on 12p13 in hematopoietic malignancies. Blood. 1995;86:1525–33.

251. O'Connor HE, Butler TA, Clark R et al. Abnormalities of the ETV6 gene occur in the majority of patients with aberrations of the short arm of chromosome 12: a combined PCR and Southern blotting analysis. Leukemia. 1998;12:1099–106.

252. Heerema NA, Sather HN, Sensel MG et al. Prognostic significance of cytogenetic abnormalities of chromosome arm 12p in childhood acute lymphoblastic leukemia: a report from the Children's Cancer Group. Cancer. 2000;88:1945–54.

253. Wlodarska I, Marynen P, La Starza R, Mecucci C, Van den Berghe H. The ETV6, CDKN1B and D12S178 loci are involved in a segment commonly deleted in various 12p aberrations in different hematological malignancies. Cytogenet Cell Genet. 1996;72:229–35.

254. Hoglund M, Johansson B, Pedersen–Bjergaard J, Marynen P, Mitelman F. Molecular characterization of 12p abnormalities in hematologic malignancies: deletion of KIP1, rearrangement of TEL, and amplification of CCND2. Blood. 1996;87:324–30.

255. Cave H, Cacheux V, Raynaud S et al. ETV6 is the target of chromosome 12p deletions in t(12;21) childhood acute lymphocytic leukemia. Leukemia. 1997; 11:1459–64.

256. Kebriaei P, Anastasi J, Larson RA. Acute lymphoblastic leukaemia: diagnosis and classification. Best Pract Res Clin Haematol. 2002;15:597–21.

257. Attarbaschi A, Mann G, Konig M et al. Incidence and relevance of secondary chromosome abnormalities in childhood TEL/AML1+ acute lymphoblastic leukemia: an interphase FISH analysis. Leukemia. 2004;18:1611–16.

258. Kobayashi H, Rowley JD. Identification of cytogenetically undetected 12p13 translocations and associated deletions with fluorescence in situ hybridization. Genes Chromosomes Cancer. 1995;12:66–9.

259. Filatov LV, Saito M, Behm FG, Rivera GK, Raimondi SC. A subtle deletion of 12p by routine cytogenetics is found to be a translocation to 21q by fluorescence in situ hybridization: t(12;21)(p13;q22). Cancer Genet Cytogenet. 1996;89:136–40.

260. Penas EM, Cools J, Algenstaedt P et al. A novel cryptic translocation t(12;17)(p13;p12–p13) in a secondary acute myeloid leukemia results in a fusion of the ETV6 gene and the antisense strand of the PER1 gene. Genes Chromosomes Cancer. 2003;37:79–83.

261. Sole F, Espinet B, Sanz GF et al. Incidence, characterization and prognostic significance of chromosomal abnormalities in 640 patients with primary myelodysplastic syndromes. Grupo Cooperativo Espanol de Citogenetica Hematologica. Br J Haematol. 2000;108:346–56.

262. Cuneo A, Bigoni R, Rigolin GM et al. 13q14 deletion in non-Hodgkin's lymphoma: correlation with clinicopathologic features. Haematologica. 1999; 84:589–93.

263. Dohner H, Stilgenbauer S, Fischer K, Bentz M, Lichter P. Cytogenetic and molecular cytogenetic analysis of B cell chronic lymphocytic leukemia: specific chromosome aberrations identify prognostic subgroups of patients and point to loci of candidate genes. Leukemia. 1997;11(Suppl 2):S19–24.

264. Dohner H, Stilgenbauer S, Benner A et al. Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med. 2000;343:1910–16.

265. Chena C, Arrossagaray G, Scolnik M, Palacios MF, Slavutsky I. Interphase cytogenetic analysis in Argentinean B-cell chronic lymphocytic leukemia patients: association of trisomy 12 and del(13q14). Cancer Genet Cytogenet. 2003;146:154–60.

266. Sawyer JR, Waldron JA, Jagannath S, Barlogie B. Cytogenetic findings in 200 patients with multiple myeloma. Cancer Genet Cytogenet. 1995;82:41–9.

267. Schmidt-Wolf IG, Glasmacher A, Hahn-Ast C et al. Chromosomal aberrations in 130 patients with multiple myeloma studied by interphase FISH: diagnostic and prognostic relevance. Cancer Genet Cytogenet. 2006;167:20–5.

268. Zojer N, Konigsberg R, Ackermann J et al. Deletion of 13q14 remains an independent adverse prognostic variable in multiple myeloma despite its frequent detection by interphase fluorescence in situ hybridization. Blood. 2000;95:1925–30.

269. Carlebach M, Amiel A, Gaber E et al. Multiple myeloma: monoallelic deletions of the tumor suppressor genes TP53 and RB1 in long-term follow-up. Cancer Genet Cytogenet. 2000;117:57–60.

270. Gutierrez NC, Castellanos MV, Martin ML et al. Prognostic and biological implications of genetic abnormalities in multiple myeloma undergoing autologous stem cell transplantation: t(4;14) is the most relevant adverse prognostic factor, whereas RB deletion as a unique abnormality is not associated with adverse prognosis. Leukemia. 2007;21:143–50.

271. Konigsberg R, Ackermann J, Kaufmann H et al. Deletions of chromosome 13q in monoclonal gammopathy of undetermined significance. Leukemia. 2000;14:1975–9.

272. Fassas AB, Spencer T, Sawyer J et al. Both hypodiploidy and deletion of chromosome 13 independently confer poor prognosis in multiple myeloma. Br J Haematol. 2002;118:1041–7.

273. Tricot G, Barlogie B, Jagannath S et al. Poor prognosis in multiple myeloma is associated only with partial or complete deletions of chromosome 13 or abnormalities involving 11q and not with other karyotype abnormalities. Blood. 1995;86:4250–6.

274. Desikan R, Barlogie B, Sawyer J et al. Results of high-dose therapy for 1000 patients with multiple myeloma: durable complete remissions and superior survival in the absence of chromosome 13 abnormalities. Blood. 2000;95:4008–10.

275. Fassas AB, Tricot G. Chromosome 13 deletion/hypodiploidy and prognosis in multiple myeloma patients. Leuk Lymphoma. 2004;45:1083–91.

276. Kaufmann H, Kromer E, Nosslinger T et al. Both chromosome 13 abnormalities by metaphase cytogenetics and deletion of 13q by interphase FISH only are prognostically relevant in multiple myeloma. Eur J Haematol. 2003;71:179–83.

277. Mertens F, Johansson B, Heim S, Kristoffersson U, Mitelman F. Karyotypic patterns in chronic myeloproliferative disorders: report on 74 cases and review of the literature. Leukemia. 1991;5:214–20.

278. Najfeld V, Montella L, Scalise A, Fruchtman S. Exploring Polycythaemia vera with fluorescence in situ hybridization: additional cryptic 9p is the most frequent abnormality detected. Br J Haematol. 2002;119:558–66.

279. Bench AJ, Cross NC, Huntly BJ, Nacheva EP, Green AR. Myeloproliferative disorders. Best Pract Res Clin Haematol. 2001;14:531–51.

280. Djordjevic V, Dencic-Fekete M, Jovanovic J et al. Cytogenetics of agnogenic myeloid metaplasia: a study of 61 patients. Cancer Genet Cytogenet. 2007;173:57–62.

281. Reilly JT. Idiopathic myelofibrosis: pathogenesis, natural history and management. Blood Rev. 1997;11:233–42.

282. Reilly JT. Cytogenetic and molecular genetic abnormalities in agnogenic myeloid metaplasia. Semin Oncol. 2005;32:359–64.

283. Reilly JT, Snowden JA, Spearing RL et al. Cytogenetic abnormalities and their prognostic significance in idiopathic myelofibrosis: a study of 106 cases. Br J Haematol. 1997;98:96–102.

284. Werner M, Nolte M, Kaloutsi V, Buhr T, Kausche F, Georgii A. Karyotype findings and molecular analysis of the bcr gene rearrangement supplementing the histologic classification of chronic myeloproliferative disorders. Lab Invest. 1995;72:405–10.

285. Castro PD, Liang JC, Nagarajan L. Deletions of chromosome 5q13.3 and 17p loci cooperate in myeloid neoplasms. Blood. 2000;95:2138–43.

286. Trost D, Hildebrandt B, Beier M, Muller N, Germing U, Royer-Pokora B. Molecular cytogenetic profiling of complex karyotypes in primary myelodysplastic syndromes and acute myeloid leukemia. Cancer Genet Cytogenet. 2006;165:51–63.

287. Watson N, Dunlop L, Robson L, Sharma P, Smith A. 17p− syndrome arising from a novel dicentric translocation in a patient with acute myeloid leukemia. Cancer Genet Cytogenet. 2000;118:159–62.

288. Van Limbergen H, Poppe B, Michaux L et al. Identification of cytogenetic subclasses and recurring chromosomal aberrations in AML and MDS with complex karyotypes using M-FISH. Genes Chromosomes Cancer. 2002;33:60–72.

289. Alvarez S, Cigudosa JC. Gains, losses and complex karyotypes in myeloid disorders: a light at the end of the tunnel. Hematol Oncol. 2005;23:18–25.

290. Karrman K, Sallerfors B, Lenhoff S, Fioretos T, Johansson B. Cytogenetic evolution patterns in CML post-SCT. Bone Marrow Transplant. 2007;39:165–71.

291. Nakai H, Misawa S. Chromosome 17 abnormalities and inactivation of the p53 gene in chronic myeloid leukemia and their prognostic significance. Leuk Lymphoma. 1995;19:213–21.

292. Babicka L, Zemanova Z, Pavlistova L et al. Complex chromosomal rearrangements in patients with chronic myeloid leukemia. Cancer Genet Cytogenet. 2006;168:22–9.

293. Hoglund M, Sehn L, Connors JM et al. Identification of cytogenetic subgroups and karyotypic pathways of clonal evolution in follicular lymphomas. Genes Chromosomes Cancer. 2004;39:195–204.

294. Berglund M, Enblad G, Thunberg U et al. Genomic imbalances during transformation from follicular lymphoma to diffuse large B-cell lymphoma. Mod Pathol. 2007;20:63–75.

295. Gimenez S, Costa C, Espinet B et al. Comparative genomic hybridization analysis of cutaneous large B-cell lymphomas. Exp Dermatol. 2005;14:883–90.

296. Bea S, Colomo L, Lopez-Guillermo A et al. Clinicopathologic significance and prognostic value of chromosomal imbalances in diffuse large B-cell lymphomas. J Clin Oncol. 2004;22:3498–506.

297. Mao X, Lillington D, Scarisbrick JJ et al. Molecular cytogenetic analysis of cutaneous T-cell lymphomas: identification of common genetic alterations in Sezary syndrome and mycosis fungoides. Br J Dermatol. 2002;147:464–75.

298. Chen CY, Yao M, Tang JL et al. Chromosomal abnormalities of 200 Chinese patients with non-Hodgkin's lymphoma

in Taiwan: with special reference to T-cell lymphoma. Ann Oncol. 2004;15:1091–6.

299. Siu LL, Chan JK, Kwong YL. Natural killer cell malignancies: clinicopathologic and molecular features. Histol Histopathol. 2002;17:539–54.

300. Stokke T, DeAngelis P, Smedshammer L et al. Loss of chromosome 11q21–23.1 and 17p and gain of chromosome 6p are independent prognostic indicators in B-cell non-Hodgkin's lymphoma. Br J Cancer. 2001;85:1900–13.

301. Geisler CH, Philip P, Christensen BE et al. In B-cell chronic lymphocytic leukaemia chromosome 17 abnormalities and not trisomy 12 are the single most important cytogenetic abnormalities for the prognosis: a cytogenetic and immunophenotypic study of 480 unselected newly diagnosed patients. Leuk Res. 1997;21:1011–23.

302. Byrd JC, Smith L, Hackbarth ML et al. Interphase cytogenetic abnormalities in chronic lymphocytic leukemia may predict response to rituximab. Cancer Res. 2003;63:36–38.

303. Drach J, Ackermann J, Fritz E et al. Presence of a p53 gene deletion in patients with multiple myeloma predicts for short survival after conventional-dose chemotherapy. Blood. 1998;92:802–9.

304. Avet-Loiseau H, Attal M, Moreau P et al. Genetic abnormalities and survival in multiple myeloma: the experience of the Intergroupe Francophone du Myelome. Blood 2007;109:3489–95.

305. Chang H, Qi C, Yi QL, Reece D, Stewart AK. p53 gene deletion detected by fluorescence in situ hybridization is an adverse prognostic factor for patients with multiple myeloma following autologous stem cell transplantation. Blood. 2005;105:358–60.

306. Gertz MA, Lacy MQ, Dispenzieri A et al. Clinical implications of t(11;14)(q13;q32), t(4;14)(p16.3;q32), and −17p13 in myeloma patients treated with high-dose therapy. Blood. 2005;106:2837–40.

307. Chang H, Sloan S, Li D, Keith Stewart A. Multiple myeloma involving central nervous system: high frequency of chromosome 17p13.1 (p53) deletions. Br J Haematol. 2004;127:280–4.

308. Liu YC, Ito Y, Hsiao HH et al. Risk factor analysis in myelodysplastic syndrome patients with del(20q): prognosis revisited. Cancer Genet Cytogenet. 2006;171:9–16.

309. Mhawech P, Saleem A. Myelodysplastic syndrome: review of the cytogenetic and molecular data. Crit Rev Oncol Hematol. 2001;40:229–38.

310. Campbell LJ, Garson OM. The prognostic significance of deletion of the long arm of chromosome 20 in myeloid disorders. Leukemia. 1994;8:67–71.

311. Asimakopoulos FA, Green AR. Deletions of chromosome 20q and the pathogenesis of myeloproliferative disorders. Br J Haematol. 1996;95:219–26.

312. Dewald GW, Wright PI. Chromosome abnormalities in the myeloproliferative disorders. Semin Oncol. 1995;22:341–54.

313. Mufti GJ. Chromosomal deletions in the myelodysplastic syndrome. Leuk Res. 1992;16:35–41.

314. Heaney ML, Golde DW. Myelodysplasia. N Engl J Med. 1999;340:1649–60.

315. Kurtin PJ, Dewald GW, Shields DJ, Hanson CA. Hematologic disorders associated with deletions of chromosome 20q: a clinicopathologic study of 107 patients. Am J Clin Pathol. 1996;106:680–8.

316. Liu YC, Miyazawa K, Sashida G, Kodama A, Ohyashiki K. Deletion (20q) as the sole abnormality in Waldenstrom macroglobulinemia suggests distinct pathogenesis of 20q11 anomaly. Cancer Genet Cytogenet. 2006;169:69–72.

317. Hollings PE, Benjes SM, Rosman I, Fitzgerald PH. Deletion 20q in association with Philadelphia chromosome positive acute lymphoblastic leukemia. A report of two cases. Cancer Genet Cytogenet. 1995;79:32–5.

318. Blann MM, Velagaleti GV, Morgan DL, Martinez RE, Conlin PA, Tonk VS. Interstitial deletion of 20q in a patient with Waldenstrom macroglobulinemia following chemotherapy. Cancer Genet Cytogenet. 2002;132:145–8.

319. Adeyinka A, Dewald GW. Cytogenetics of chronic myeloproliferative disorders and related myelodysplastic syndromes. Hematol Oncol Clin North Am. 2003;17:1129–49.

320. Frank MB, Norwood TH, Willerford DM. Chimeric del20q in a case of chronic neutrophilic leukemia. Am J Hematol. 2000;64:229–31.

321. Matano S, Nakamura S, Kobayashi K, Yoshida T, Matsuda T, Sugimoto T. Deletion of the long arm of chromosome 20 in a patient with chronic neutrophilic leukemia: cytogenetic findings in chronic neutrophilic leukemia. Am J Hematol. 1997;54:72–5.

322. Reid AG, Swanton S, Grace C, Campbell LJ, Green AR, Nacheva EP. Double Philadelphia masquerading as chromosome 20q deletion – a new recurrent abnormality in chronic myeloid leukaemia blast crisis. Br J Haematol. 2003;123:442–8.

323. Byrd JC, Mrozek K, Dodge RK et al. Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: results from Cancer and Leukemia Group B (CALGB 8461). Blood. 2002;100:4325–36.

324. Cauwelier B, Cave H, Gervais C et al. Clinical, cytogenetic and molecular characteristics of 14 T-ALL patients carrying the TCRbeta-HOXA rearrangement: a study of the Groupe Francophone de Cytogenetique Hematologique. Leukemia. 2007;21:121–8.

325. Cauwelier B, Dastugue N, Cools J et al. Molecular cytogenetic study of 126 unselected T-ALL cases reveals high incidence of TCRbeta locus rearrangements and putative new T-cell oncogenes. Leukemia. 2006;20:1238–44.

326. Speleman F, Cauwelier B, Dastugue N et al. A new recurrent inversion, inv(7)(p15q34), leads to transcriptional activation of HOXA10 and HOXA11 in a subset of T-cell acute lymphoblastic leukemias. Leukemia. 2005;19:358–66.

327. Soulier J, Clappier E, Cayuela JM et al. HOXA genes are included in genetic and biologic networks defining human acute T-cell leukemia (T-ALL). Blood. 2005;106:274–86.

328. Pui CH, Behm FG, Singh B et al. Heterogeneity of presenting features and their relation to treatment outcome in 120 children with T-cell acute lymphoblastic leukemia. Blood. 1990;75:174–9.

329. Yamamoto M, Kakihana K, Kurosu T, Murakami N, Miura O. Clonal evolution with inv(11)(p15q22) and NUP98/DDX10 fusion gene in imatinib-resistant chronic myelogenous leukemia. Cancer Genet Cytogenet. 2005;157:104–8.

330. Arai Y, Hosoda F, Kobayashi H et al. The inv(11)(p15q22) chromosome translocation of de novo and therapy-related myeloid malignancies results in fusion of the nucleoporin gene, NUP98, with the putative RNA helicase gene, DDX10. Blood. 1997;89:3936–44.

331. Kobayashi H, Arai Y, Hosoda F et al. Inversion of chromosome 11 inv(11)(p15q22), as a recurring chromosomal aberration associated with de novo and secondary myeloid malignancies: identification of a P1 clone spanning the 11q22 breakpoint. Genes Chromosomes Cancer. 1997;19:150–5.

332. Johansson B, Mertens F, Mitelman F. Primary vs. secondary neoplasia-associated chromosomal abnormalities – balanced rearrangements vs. genomic imbalances? Genes Chromosomes Cancer. 1996;16:155–63.

333. Welborn J, Jenks H, Taplett J, Walling P. Inversion of chromosome 12 and lineage promiscuity in hematologic malignancies. Cancer Genet Cytogenet. 2004; 148:91–103.

334. Setoyama M, Tojo A, Nagamura F et al. A unique translocation of the TEL gene in a case of acute myelogenous leukemia with inv(12)(p13q15). Blood. 1998;92: 1454–5.

335. Larson RA, Wernli M, Le Beau MM et al. Short remission durations in therapy-related leukemia despite cytogenetic complete responses to high-dose cytarabine. Blood. 1988;72:1333–9.

336. Pui CH, Relling MV, Rivera GK et al. Epipodophyllotoxin-related acute myeloid leukemia: a study of 35 cases. Leukemia. 1995;9:1990–6.

337. Sessarego M, Defferrari R, Dejana AM et al. Cytogenetic analysis in essential thrombocythemia at diagnosis and at transformation. A 12–year study. Cancer Genet Cytogenet. 1989;43:57–65.

338. Raimondi SC, Shurtleff SA, Downing JR et al. 12p abnormalities and the TEL gene (ETV6) in childhood acute lymphoblastic leukemia. Blood. 1997;90:4559–66.

339. Maljaei SH, Brito-Babapulle V, Hiorns LR, Catovsky D. Abnormalities of chromosomes 8, 11, 14, and X in T-prolymphocytic leukemia studied by fluorescence in situ hybridization. Cancer Genet Cytogenet. 1998;103:110–16.

340. Liu P, Tarle SA, Hajra A et al. Fusion between transcription factor CBF beta/PEBP2 beta and a myosin heavy chain in acute myeloid leukemia. Science. 1993; 261:1041–4.

341. Loffler H, Gassmann W, Haferlach T. AML M1 and M2 with eosinophilia and AML M4Eo: diagnostic and clinical aspects. Leuk Lymphoma. 1995;18(Suppl 1):61–3.

342. Marlton P, Keating M, Kantarjian H et al. Cytogenetic and clinical correlates in AML patients with abnormalities of chromosome 16. Leukemia. 1995;9:965–71.

343. Wu Y, Slovak ML, Snyder DS, Arber DA. Coexistence of inversion 16 and the Philadelphia chromosome in acute and chronic myeloid leukemias: report of six cases and review of literature. Am J Clin Pathol. 2006;125: 260–6.

344. Rao PN, Flejter WL, Rahi S, Li H, Pettenati MJ. Identification of the inverted chromosome 16 using chromosome painting. Br J Haematol. 1999;104:618–20.

345. Belhadj K, Reyes F, Farcet JP et al. Hepatosplenic gammadelta T-cell lymphoma is a rare clinicopathologic entity with poor outcome: report on a series of 21 patients. Blood. 2003;102:4261–9.

346. Alonsozana EL, Stamberg J, Kumar D et al. Isochromosome 7q: the primary cytogenetic abnormality in hepatosplenic gammadelta T cell lymphoma. Leukemia. 1997;11:1367–72.

347. Francois A, Lesesve JF, Stamatoullas A et al. Hepatosplenic gamma/delta T-cell lymphoma: a report of two cases in immunocompromised patients, associated with isochromosome 7q. Am J Surg Pathol. 1997;21:781–90.

348. Wlodarska I, Martin-Garcia N, Achten R et al. Fluorescence in situ hybridization study of chromosome 7 aberrations in hepatosplenic T-cell lymphoma: isochromosome 7q as a common abnormality accumulating in forms with features of cytologic progression. Genes Chromosomes Cancer. 2002;33:243–51.

349. Fioretos T, Strombeck B, Sandberg T et al. Isochromosome 17q in blast crisis of chronic myeloid leukemia and in other hematologic malignancies is the result of clustered breakpoints in 17p11 and is not associated with coding TP53 mutations. Blood. 1999;94:225–32.

350. Sato Y, Izumi T, Kanamori H et al. t(1;3)(p36;p21) is a recurring therapy-related translocation. Genes Chromosomes Cancer. 2002;34:186–92.

351. Steurer M, Stauder R, Grunewald K et al. Hepatosplenic gammadelta-T-cell lymphoma with leukemic course after renal transplantation. Hum Pathol. 2002;33:253–8.

352. Michaux L, Wlodarska I, Rack K et al. Translocation t(1;6)(p35.3;p25.2): a new recurrent aberration in 'unmutated' B-CLL. Leukemia. 2005;19:77–82.

353. Willis TG, Jadayel DM, Du MQ et al. Bcl10 is involved in t(1;14)(p22;q32) of MALT B cell lymphoma and mutated in multiple tumor types. Cell. 1999;96:35–45.

354. Zhang Q, Siebert R, Yan M et al. Inactivating mutations and overexpression of BCL10, a caspase recruitment domain-containing gene, in MALT lymphoma with t(1;14)(p22;q32). Nat Genet. 1999;22:63–8.

355. Ye H, Dogan A, Karran L et al. BCL10 expression in normal and neoplastic lymphoid tissue. Nuclear localization in MALT lymphoma. Am J Pathol. 2000;157:1147–54.

356. Ohshima K, Muta H, Kawasaki C et al. Bcl10 expression, rearrangement and mutation in MALT lymphoma: correlation with expression of nuclear factor-kappaB. Int J Oncol. 2001;19:283–9.

357. Maes B, Demunter A, Peeters B, De Wolf-Peeters C. BCL10 mutation does not represent an important pathogenic mechanism in gastric MALT-type lymphoma, and the presence of the API2-MLT fusion is associated with aberrant nuclear BCL10 expression. Blood. 2002;99:1398–404.

358. Liu H, Ye H, Dogan A et al. T(11;18)(q21;q21) is associated with advanced mucosa-associated lymphoid tissue lymphoma that expresses nuclear BCL10. Blood. 2001;98:1182–7.

359. Wotherspoon AC, Soosay GN, Diss TC, Isaacson PG. Low-grade primary B-cell lymphoma of the lung. An immunohistochemical, molecular, and cytogenetic study of a single case. Am J Clin Pathol. 1990;94:655–60.

360. Shearer BM, Flynn HC, Knudson RA, Ketterling RP. Interphase FISH to detect PBX1/E2A fusion resulting from the der(19)t(1;19)(q23;p13.3) or t(1;19)(q23;p13.3) in paediatric patients with acute lymphoblastic leukaemia. Br J Haematol. 2005;129:45–52.

361. Crist WM, Carroll AJ, Shuster JJ et al. Poor prognosis of children with pre-B acute lymphoblastic leukemia is associated with the t(1;19)(q23;p13): a Pediatric Oncology Group study. Blood. 1990;76:117–22.

362. Piccaluga PP, Malagola M, Rondoni M et al. Poor outcome of adult acute lymphoblastic leukemia patients carrying the (1;19)(q23;p13) translocation. Leuk Lymphoma. 2006;47:469–72.

363. Bernstein J, Dastugue N, Haas OA et al. Nineteen cases of the t(1;22)(p13;q13) acute megakaryblastic leukaemia of infants/children and a review of 39 cases: report from a t(1;22) study group. Leukemia. 2000;14:216–18.

364. Trubia M, Albano F, Cavazzini F et al. Characterization of a recurrent translocation t(2;3)(p15-22;q26) occurring in acute myeloid leukaemia. Leukemia. 2006;20:48–54.

365. Cataldo KA, Jalal SM, Law ME et al. Detection of t(2;5) in anaplastic large cell lymphoma: comparison of immunohistochemical studies, FISH, and RT-PCR in paraffin-embedded tissue. Am J Surg Pathol. 1999;23:1386–92.

366. Duyster J, Bai RY, Morris SW. Translocations involving anaplastic lymphoma kinase (ALK). Oncogene. 2001;20:5623–37.

367. Lamant L, Meggetto F, al Saati T et al. High incidence of the t(2;5)(p23;q35) translocation in anaplastic large cell lymphoma and its lack of detection in Hodgkin's disease. Comparison of cytogenetic analysis, reverse transcriptase-polymerase chain reaction, and P-80 immunostaining. Blood. 1996;87:284–91.

368. Falini B. Anaplastic large cell lymphoma: pathological, molecular and clinical features. Br J Haematol. 2001;114:741–60.

369. Ott G, Katzenberger T, Siebert R et al. Chromosomal abnormalities in nodal and extranodal CD30+ anaplastic large cell lymphomas: infrequent detection of the t(2;5) in extranodal lymphomas. Genes Chromosomes Cancer. 1998;22:114–21.

370. Delsol G, Lamant L, Mariame B et al. A new subtype of large B-cell lymphoma expressing the ALK kinase and lacking the 2; 5 translocation. Blood. 1997;89:1483–90.

371. Gascoyne RD, Lamant L, Martin-Subero JI et al. ALK-positive diffuse large B-cell lymphoma is associated with clathrin-ALK rearrangements: report of 6 cases. Blood. 2003;102:2568–73.

372. De Paepe P, Baens M, van Krieken H et al. ALK activation by the CLTC-ALK fusion is a recurrent event in large B-cell lymphoma. Blood. 2003;102:2638–41.

373. Stachurski D, Miron PM, Al-Homsi S et al. Anaplastic lymphoma kinase-positive diffuse large B-cell lymphoma with a complex karyotype and cryptic 3' ALK gene insertion to chromosome 4 q22-24. Hum Pathol. 2007;38: 940–5.

374. Secker-Walker LM, Mehta A, Bain B. Abnormalities of 3q21 and 3q26 in myeloid malignancy: a United Kingdom Cancer Cytogenetic Group study. Br J Haematol. 1995;91:490–501.

375. Jotterand Bellomo M, Parlier V, Muhlematter D, Grob JP, Beris P. Three new cases of chromosome 3 rearrangement in bands q21 and q26 with abnormal thrombopoiesis bring further evidence to the existence of a 3q21q26 syndrome. Cancer Genet Cytogenet. 1992;59:138–60.

376. Cabello AI, Collado R, Ruiz MA et al. A retrospective analysis of myelodysplastic syndromes with thrombocytosis: reclassification of the cases by WHO proposals. Leuk Res. 2005;29:365–70.

377. Kwong YL. Translocation (3;5)(q21;q34) in erythroleukemia: a molecular and in situ hybridization study. Cancer Genet Cytogenet. 1998;103:15–19.

378. Shi G, Weh HJ, Martensen S, Seeger D, Hossfeld DK. 3p21 is a recurrent treatment-related breakpoint in myelodysplastic syndrome and acute myeloid leukemia. Cytogenet Cell Genet. 1996;74:295–9.

379. Charrin C, Belhabri A, Treille-Ritouet D et al. Structural rearrangements of chromosome 3 in 57 patients with acute myeloid leukemia: clinical, hematological and cytogenetic features. Hematol J. 2002;3:21–31.

380. Andersen MK, Christiansen DH, Pedersen-Bjergaard J. Centromeric breakage and highly rearranged chromosome derivatives associated with mutations of TP53 are common in therapy-related MDS and AML after therapy with alkylating agents: an M-FISH study. Genes Chromosomes Cancer. 2005;42:358–71.

381. Mertens F, Johansson B, Billstrom R, Engquist L, Mitelman F. A case of myelodysplastic syndrome with high platelet counts and a t(3;8)(q26;q24). Cancer Genet Cytogenet. 1987;27:1–4.

382. Lin P, Medeiros LJ, Yin CC, Abruzzo LV. Translocation (3;8)(q26;q24): a recurrent chromosomal abnormality in myelodysplastic syndrome and acute myeloid leukemia. Cancer Genet Cytogenet. 2006;166:82–5.

383. Wang HY, Bossler AD, Schaffer A et al. A novel t(3;8)(q27;q24.1) simultaneously involving both the BCL6 and MYC genes in a diffuse large B-cell lymphoma. Cancer Genet Cytogenet. 2007;172:45–53.

384. Bernicot I, Douet-Guilbert N, Le Bris MJ et al. Characterization of IGH rearrangements in non-Hodgkin's B-cell lymphomas by fluorescence in situ hybridization. Anticancer Res. 2005;25:3179–82.

385. Oscier DG, Gardiner AC. Lymphoid neoplasms. Best Pract Res Clin Haematol. 2001;14:609–30.

386. Nanjangud G, Rao PH, Hegde A et al. Spectral karyotyping identifies new rearrangements, translocations, and clinical associations in diffuse large B-cell lymphoma. Blood. 2002;99:2554–61.

387. Au WY, Horsman DE, Connors JM, Klasa RJ, Gascoyne RD. Cytogenetic findings in reactive lymphoid hyperplasia: significance of non-clonal t(3;14) and t(3;22). Am J Hematol. 2002;70:133–8.

388. Nucifora G, Rowley JD. AML1 and the 8;21 and 3;21 translocations in acute and chronic myeloid leukemia. Blood. 1995;86:1–14.

389. Zent C, Rowley JD, Nucifora G. Rearrangements of the AML1/CBFA2 gene in myeloid leukemia with the 3;21 translocation: in vitro and in vivo studies. Leukemia. 1997;11(Suppl 3):273–8.

390. Nucifora G, Laricchia-Robbio L, Senyuk V. EVI1 and hematopoietic disorders: history and perspectives. Gene. 2006;368:1–11.

391. Drabkin HA, Erickson P. Down syndrome and leukemia, an update. Prog Clin Biol Res. 1995;393:169–76.

392. Bacher U, Schnittger S, Kern W et al. Acute myeloid leukemia (AML) with t(8;21)(q22;q22) relapsing as AML with t(3;21)(q26;q22). Cancer Genet Cytogenet. 2006;168:172–4.

393. Hayette S, Tigaud I, Maguer-Satta V et al. Recurrent involvement of the MLL gene in adult T-lineage acute lymphoblastic leukemia. Blood. 2002;99:4647–9.

394. Hilden JM, Kersey JH. The MLL (11q23) and AF-4 (4q21) genes disrupted in t(4;11) acute leukemia: molecular and clinical studies. Leuk Lymphoma. 1994;14:189–95.

395. Secker-Walker LM, Prentice HG, Durrant J, Richards S, Hall E, Harrison G. Cytogenetics adds independent prognostic information in adults with acute lymphoblastic leukaemia on MRC trial UKALL XA. MRC Adult Leukaemia Working Party. Br J Haematol. 1997;96:601–10.

396. Heerema NA, Sather HN, Ge J et al. Cytogenetic studies of infant acute lymphoblastic leukemia: poor prognosis of infants with t(4;11) – a report of the Children's Cancer Group. Leukemia. 1999;13:679–86.

397. Cimino G, Elia L, Mancini M et al. Clinico-biologic features and treatment outcome of adult pro-B-ALL patients enrolled in the GIMEMA 0496 study: absence of the ALL1/AF4 and of the BCR/ABL fusion genes correlates with a significantly better clinical outcome. Blood. 2003;102:2014–20.

398. Chesi M, Nardini E, Lim RS, Smith KD, Kuehl WM, Bergsagel PL. The t(4;14) translocation in myeloma dysregulates both FGFR3 and a novel gene, MMSET, resulting in IgH/MMSET hybrid transcripts. Blood. 1998;92:3025–34.

399. Chesi M, Nardini E, Brents LA et al. Frequent translocation t(4;14)(p16.3;q32.3) in multiple myeloma is associated with increased expression and activating mutations of fibroblast growth factor receptor 3. Nat Genet. 1997;16:260–64.

400. Chang H, Sloan S, Li D et al. The t(4;14) is associated with poor prognosis in myeloma patients undergoing autologous stem cell transplant. Br J Haematol. 2004;125:64–8.

401. Keats JJ, Reiman T, Belch AR, Pilarski LM. Ten years and counting: so what do we know about t(4;14)(p16;q32) multiple myeloma. Leuk Lymphoma. 2006;47:2289–300.

402. Keats JJ, Maxwell CA, Taylor BJ et al. Overexpression of transcripts originating from the MMSET locus characterizes all t(4;14)(p16;q32)-positive multiple myeloma patients. Blood. 2005;105:4060–9.

403. Avet-Loiseau H, Facon T, Grosbois B et al. Oncogenesis of multiple myeloma:14q32 and 13q chromosomal abnormalities are not randomly distributed, but correlate with natural history, immunological features, and clinical presentation. Blood. 2002;99:2185–91.

404. Avet-Loiseau H, Facon T, Daviet A et al. 14q32 translocations and monosomy 13 observed in monoclonal gammopathy of undetermined significance delineate a multistep process for the oncogenesis of multiple myeloma. Intergroupe Francophone du Myelome. Cancer Res. 1999;59:4546–50.

405. Keats JJ, Reiman T, Maxwell CA et al. In multiple myeloma, t(4;14)(p16;q32) is an adverse prognostic factor irrespective of FGFR3 expression. Blood. 2003; 101:1520–29.

406. Moreau P, Facon T, Leleu X et al. Recurrent 14q32 translocations determine the prognosis of multiple myeloma, especially in patients receiving intensive chemotherapy. Blood. 2002;100:1579–83.

407. Fonseca R, Blood E, Rue M et al. Clinical and biologic implications of recurrent genomic aberrations in myeloma. Blood. 2003;101:4569–75.

408. Gough SM, Benjes SM, McDonald M, Heaton D, Ganly P, Morris CM. Translocation (5;10)(q22;q24) in a case of acute lymphoblastic leukemia. Cancer Genet Cytogenet. 2006;165:36–40.

409. Steer EJ, Cross NC. Myeloproliferative disorders with translocations of chromosome 5q31–35: role of the platelet-derived growth factor receptor Beta. Acta Haematol. 2002;107:113–22.

410. David M, Cross NC, Burgstaller S et al. Durable responses to imatinib in patients with PDGFRB fusion gene-positive and BCR-ABL-negative chronic myeloproliferative disorders. Blood. 2007;109:61–4.

411. Sakai R, Fujimaki K, Yamazaki E et al. Acute myelomonocytic leukemia with dysplastic bone marrow eosinophils showing t(5; 17)(q13;q11) and a secondary chromosomal aberration, inv(16)(p13q02). Int J Hematol. 2006;84:417–20.

412. Gozzetti A, Tozzuoli D, Crupi R et al. A novel t(6;7)(p24;q21) in a chronic myelocytic leukemia in complete cytogenetic remission after therapy with imatinib mesylate. Cancer Genet Cytogenet. 2004;148:152–4.

413. von Lindern M, Fornerod M, van Baal S et al. The translocation (6;9), associated with a specific subtype of acute myeloid leukemia, results in the fusion of two genes, dek and can, and the expression of a chimeric, leukemia-specific dek-can mRNA. Mol Cell Biol. 1992;12:1687–97.

414. Soekarman D, von Lindern M, Daenen S et al. The translocation (6;9) (p23;q34) shows consistent rearrangement of two genes and defines a myeloproliferative disorder with specific clinical features. Blood. 1992;79:2990–7.

415. Wong KF, Chan JK, Sin VC. T-cell prolymphocytic leukemia with a novel translocation (6;11)(q21;q23). Cancer Genet Cytogenet. 1999;111:149–51.

416. Yamamoto K, Nakamura Y, Saito K, Furusawa S. Expression of the NUP98/HOXA9 fusion transcript in the blast crisis of Philadelphia chromosome-positive chronic myelogenous leukaemia with t(7;11)(p15;p15). Br J Haematol. 2000;109:423–6.

417. Ahuja HG, Popplewell L, Tcheurekdjian L, Slovak ML. NUP98 gene rearrangements and the clonal evolution of chronic myelogenous leukemia. Genes Chromosomes Cancer. 2001;30:410–5.

418. Slater RM, von Drunen E, Kroes WG et al. t(7;12)(q36;p13) and t(7;12)(q32;p13) – ranslocations involving ETV6 in children 18 months of age or younger with myeloid disorders. Leukemia. 2001;15:915–20.

419. Tosi S, Harbott J, Teigler-Schlegel A et al. t(7;12)(q36;p13), a new recurrent translocation involving

ETV6 in infant leukemia. Genes Chromosomes Cancer. 2000;29:325–32.

420. D'Achille P, Seymour JF, Campbell LJ. Translocation (14;18)(q32;q21) in acute lymphoblastic leukemia: a study of 12 cases and review of the literature. Cancer Genet Cytogenet. 2006;171:52–6.

421. Nacheva E, Dyer MJ, Fischer P et al. C-MYC translocations in de novo B-cell lineage acute leukemias with t(14;18)(cell lines Karpas 231 and 353). Blood. 1993;82:231–40.

422. Dunphy CH, van Deventer HW, Carder KJ, Rao KW, Dent GA. Mature B-cell acute lymphoblastic leukemia with associated translocations (14;18)(q32;q21) and (8;9)(q24;p13). A Burkitt variant? Arch Pathol Lab Med. 2003;127:610–13.

423. Lillington DM, Monard S, Johnson PW et al. The t(14;18) in a patient with de novo acute lymphoblastic leukemia is associated with t(8;9). Leukemia. 1994;8:560–3.

424. Reiter A, Walz C, Watmore A et al. The t(8;9)(p22;p24) is a recurrent abnormality in chronic and acute leukemia that fuses PCM1 to JAK2. Cancer Res. 2005;65:2662–7.

425. Murati A, Gelsi-Boyer V, Adelaide J et al. PCM1-JAK2 fusion in myeloproliferative disorders and acute erythroid leukemia with t(8;9) translocation. Leukemia. 2005; 19:1692–6.

426. Macpherson N, Lesack D, Klasa R et al. Small noncleaved, non-Burkitt's (Burkit-like) lymphoma: cytogenetics predict outcome and reflect clinical presentation. J Clin Oncol. 1999;17:1558–67.

427. McClure RF, Remstein ED, Macon WR et al. Adult B-cell lymphomas with Burkitt-like morphology are phenotypically and genotypically heterogeneous with aggressive clinical behavior. Am J Surg Pathol. 2005;29:1652–60.

428. Cairo MS, Sposto R, Perkins SL et al. Burkitt's and Burkitt-like lymphoma in children and adolescents: a review of the Children's Cancer Group experience. Br J Haematol. 2003;120:660–70.

429. Lones MA, Sanger WG, Le Beau MM et al. Chromosome abnormalities may correlate with prognosis in Burkitt/Burkitt-like lymphomas of children and adolescents: a report from Children's Cancer Group Study CCG-E08. J Pediatr Hematol Oncol. 2004;26:169–78.

430. Hallek M, Bergsagel PL, Anderson KC. Multiple myeloma: increasing evidence for a multistep transformation process. Blood. 1998;91:3–21.

431. Chaganti RS, Nanjangud G, Schmidt H, Teruya-Feldstein J. Recurring chromosomal abnormalities in non-Hodgkin's lymphoma: biologic and clinical significance. Semin Hematol. 2000;37:396–411.

432. Vega F, Medeiros LJ. Chromosomal translocations involved in non-Hodgkin lymphomas. Arch Pathol Lab Med. 2003;127:1148–60.

433. Hao S, Sanger W, Onciu M, Lai R, Schlette EJ, Medeiros LJ. Mantle cell lymphoma with 8q24 chromosomal abnormalities: a report of 5 cases with blastoid features. Mod Pathol. 2002;15:1266–72.

434. Au WY, Horsman DE, Gascoyne RD, Viswanatha DS, Klasa RJ, Connors JM. The spectrum of lymphoma with 8q24 aberrations: a clinical, pathological and cytogenetic study of 87 consecutive cases. Leuk Lymphoma. 2004;45:519–28.

435. Velloso ER, Mecucci C, Michaux L et al. Translocation t(8;16)(p11;p13) in acute non-lymphocytic leukemia: report on two new cases and review of the literature. Leuk Lymphoma. 1996;21:137–42.

436. Camos M, Esteve J, Jares P et al. Gene expression profiling of acute myeloid leukemia with translocation t(8;16)(p11;p13) and MYST3-CREBBP rearrangement reveals a distinctive signature with a specific pattern of HOX gene expression. Cancer Res. 2006;66:6947–54.

437. Panagopoulos I, Teixeira MR, Micci F et al. Acute myeloid leukemia with inv(8)(p11q13). Leuk Lymphoma. 2000;39:651–6.

438. Harris NL, Jaffe ES, Diebold J et al. World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues: report of the Clinical Advisory Committee meeting – Airlie House, Virginia, November 1997. J Clin Oncol. 1999;17:3835–49.

439. Khoury H, Dalal BI, Nevill TJ et al. Acute myelogenous leukemia with t(8;21) – identification of a specific immunophenotype. Leuk Lymphoma. 2003;44:1713–18.

440. Schnittger S, Kohl TM, Haferlach T et al. KIT-D816 mutations in AML1-ETO-positive AML are associated with impaired event-free and overall survival. Blood. 2006;107:1791–9.

441. Rowley JD. Identification of a translocation with quinacrine fluorescence in a patient with acute leukemia. Ann Genet. 1973;16:109–12.

442. Erickson PF, Robinson M, Owens G, Drabkin HA. The ETO portion of acute myeloid leukemia t(8;21) fusion transcript encodes a highly evolutionarily conserved, putative transcription factor. Cancer Res. 1994;54:1782–6.

443. Andrieu V, Radford-Weiss I, Troussard X et al. Molecular detection of t(8;21)/AML1-ETO in AML M1/M2: correlation with cytogenetics, morphology and immunophenotype. Br J Haematol. 1996;92:855–65.

444. Byrd JC, Weiss RB. Recurrent granulocytic sarcoma. An unusual variation of acute myelogenous leukemia associated with 8;21 chromosomal translocation and blast expression of the neural cell adhesion molecule. Cancer. 1994;73:2107–12.

445. Bloomfield CD, Lawrence D, Byrd JC et al. Frequency of prolonged remission duration after high-dose cytarabine intensification in acute myeloid leukemia varies by cytogenetic subtype. Cancer Res. 1998;58:4173–9.

446. Leroy H, Botton SD, Grardel-Duflos N et al. Prognostic value of real-time quantitative PCR (RQ-PCR) in AML with t(8;21). Leukemia 2005;19:367–72.

447. Kuchenbauer F, Schnittger S, Look T et al. Identification of additional cytogenetic and molecular genetic abnormalities in acute myeloid leukaemia with t(8;21)/AML1-ETO. Br J Haematol. 2006;134:616–19.

448. Yuan Y, Zhou L, Miyamoto T et al. AML1-ETO expression is directly involved in the development of acute myeloid leukemia in the presence of additional mutations. Proc Natl Acad Sci USA. 2001;98:10398–403.

449. Schoch C, Haase D, Haferlach T et al. Fifty-one patients with acute myeloid leukemia and translocation t(8;21)(q22;q22): an additional deletion in 9q is an adverse prognostic factor. Leukemia. 1996;10:1288–95.

450. Rubnitz JE, Raimondi SC, Tong X et al. Favorable impact of the t(9;11) in childhood acute myeloid leukemia. J Clin Oncol. 2002;20:2302–9.

451. Offit K, Parsa NZ, Filippa D, Jhanwar SC, Chaganti RS. t(9;14)(p13;q32) denotes a subset of low-grade non-Hodgkin's lymphoma with plasmacytoid differentiation. Blood. 1992;80:2594–9.

452. Cook JR, Aguilera NI, Reshmi-Skarja S et al. Lack of PAX5 rearrangements in lymphoplasmacytic lymphomas: reassessing the reported association with t(9;14). Hum Pathol. 2004;35:447–54.

453. Radich JP, Gooley T, Bryant E et al. The significance of bcr-abl molecular detection in chronic myeloid leukemia patients 'late,' 18 months or more after transplantation. Blood. 2001;98:1701–7.

454. Huntly BJ, Bench AJ, Delabesse E et al. Derivative chromosome 9 deletions in chronic myeloid leukemia: poor prognosis is not associated with loss of ABL-BCR expression, elevated BCR-ABL levels, or karyotypic instability. Blood. 2002;99:4547–53.

455. Lundan T, Volin L, Ruutu T, Knuutila S, Porkka K. Allogeneic stem cell transplantation reverses the poor prognosis of CML patients with deletions in derivative chromosome 9. Leukemia. 2005;19:138–40.

456. Gleissner B, Gokbuget N, Bartram CR et al. Leading prognostic relevance of the BCR-ABL translocation in adult acute B-lineage lymphoblastic leukemia: a prospective study of the German Multicenter Trial Group and confirmed polymerase chain reaction analysis. Blood. 2002;99:1536–43.

457. Suryanarayan K, Hunger SP, Kohler S et al. Consistent involvement of the bcr gene by 9;22 breakpoints in pediatric acute leukemias. Blood. 1991;77:324–30.

458. Brisco MJ, Sykes PJ, Dolman G et al. Effect of the Philadelphia chromosome on minimal residual disease in acute lymphoblastic leukemia. Leukemia. 1997; 11:1497–500.

459. Tempescul A, Guillerm G, Douet-Guilbert N, Morel F, Le Bris MJ, De Braekeleer M. Translocation (10;17)(p15;q21) is a recurrent anomaly in acute myeloblastic leukemia. Cancer Genet Cytogenet. 2007;172:74–6.

460. Kaloutsi V, Hadjileontis C, Tsatalas C, Sambani C, Kostopoulos I, Papadimitriou C. Occurrence of a variant Philadelphia translocation, t(10;22), in de novo acute megakaryoblastic leukemia. Cancer Genet Cytogenet. 2004;152:52–5.

461. Kodet R, Mrhalova M, Krskova L et al. Mantle cell lymphoma: improved diagnostics using a combined approach of immunohistochemistry and identification of t(11;14)(q13;q32) by polymerase chain reaction and fluorescence in situ hybridization. Virchows Arch. 2003;442:538–47.

462. Belaud-Rotureau MA, Parrens M, Dubus P, Garroste JC, de Mascarel A, Merlio JP. A comparative analysis of FISH, RT-PCR, PCR, and immunohistochemistry for the diagnosis of mantle cell lymphomas. Mod Pathol. 2002;15:517–25.

463. Frater JL, Tsiftsakis EK, Hsi ED, Pettay J, Tubbs RR. Use of novel t(11;14) and t(14;18) dual-fusion fluorescence in situ hybridization probes in the differential diagnosis of lymphomas of small lymphocytes. Diagn Mol Pathol. 2001;10:214–22.

464. Troussard X, Avet-Loiseau H, Macro M et al. Cyclin D1 expression in patients with multiple myeloma. Hematol J. 2000;1:181–5.

465. Pruneri G, Fabris S, Baldini L et al. Immunohistochemical analysis of cyclin D1 shows deregulated expression in multiple myeloma with the t(11;14). Am J Pathol. 2000; 156:1505–13.

466. Panani AD, Ferti AD, Papaxoinis C, Raptis SA, Roussos C. Cytogenetic data as a prognostic factor in multiple myeloma patients: involvement of 1p12 region an

adverse prognostic factor. Anticancer Res. 2004;24:4141–6.

467. Bosch F, Campo E, Jares P et al. Increased expression of the PRAD-1/CCND1 gene in hairy cell leukaemia. Br J Haematol. 1995;91:1025–30.

468. Melnick A, Licht JD. Deconstructing a disease: RARalpha, its fusion partners, and their roles in the pathogenesis of acute promyelocytic leukemia. Blood. 1999;93:3167–215.

469. Licht JD, Chomienne C, Goy A et al. Clinical and molecular characterization of a rare syndrome of acute promyelocytic leukemia associated with translocation (11;17). Blood. 1995;85:1083–94.

470. Streubel B, Lamprecht A, Dierlamm J et al. T(14;18)(q32;q21) involving IGH and MALT1 is a frequent chromosomal aberration in MALT lymphoma. Blood. 2003;101:2335–9.

471. Isaacson PG, Du MQ. Gastrointestinal lymphoma: where morphology meets molecular biology. J Pathol. 2005; 205:255–74.

472. Auer IA, Gascoyne RD, Connors JM et al. t(11;18)(q21;q21) is the most common translocation in MALT lymphomas. Ann Oncol. 1997;8:979–85.

473. Barth TF, Bentz M, Leithauser F et al. Molecular-cytogenetic comparison of mucosa-associated marginal zone B-cell lymphoma and large B-cell lymphoma arising in the gastro-intestinal tract. Genes Chromosomes Cancer. 2001;31:316–25.

474. Barth TF, Bentz M, Leithauser F et al. Pathogenic complexity of gastric B-cell lymphoma. Blood. 2002; 100:1095–1096; author reply 1096-7.

475. Maes B, De Wolf-Peeters C. Marginal zone cell lymphoma – an update on recent advances. Histopathology. 2002;40:117–26.

476. Wotherspoon AC, Dogan A, Du MQ. Mucosa-associated lymphoid tissue lymphoma. Curr Opin Hematol. 2002; 9:50–5.

477. Liu H, Ye H, Ruskone-Fourmestraux A et al. T(11;18) is a marker for all stage gastric MALT lymphomas that will not respond to *H. pylori* eradication. Gastroenterology. 2002;122:1286–94.

478. Chuang SS, Lee C, Hamoudi RA et al. High frequency of t(11;18) in gastric mucosa-associated lymphoid tissue lymphomas in Taiwan, including one patient with high-grade transformation. Br J Haematol. 2003;120:97–100.

479. Streubel B, Ye H, Du MQ, Isaacson PG, Chott A, Raderer M. Translocation t(11;18)(q21;q21) is not predictive of response to chemotherapy with 2CdA in patients with gastric MALT lymphoma. Oncology. 2004;66:476–80.

480. Raynaud SD, Dastugue N, Zoccola D, Shurtleff SA, Mathew S, Raimondi SC. Cytogenetic abnormalities associated with the t(12;21): a collaborative study of 169 children with t(12;21)-positive acute lymphoblastic leukemia. Leukemia. 1999;13:1325–30.

481. Reid AG, Seppa L, von der Weid N, Niggli FK, Betts DR. A t(12;17)(p13;q12) identifies a distinct TEL rearrangement-negative subtype of precursor-B acute lymphoblastic leukemia. Cancer Genet Cytogenet. 2006;165:64–9.

482. Eguchi-Ishimae M, Eguchi M, Ishii E et al. Breakage and fusion of the TEL (ETV6) gene in immature B lymphocytes induced by apoptogenic signals. Blood. 2001;97:737–43.

483. Turhan N, Yurur-Kutlay N, Topcuoglu P et al. Translocation (13;17)(q14;q25) as a novel chromosomal abnormality in acute myeloid leukemia-M4. Leuk Res. 2006;30:903–5.

484. Kienast J, Berdel WE. c-maf in multiple myeloma: an oncogene enhancing tumor – stroma interactions. Cancer Cell. 2004;5:109–10.

485. Hurt EM, Wiestner A, Rosenwald A et al. Overexpression of c-maf is a frequent oncogenic event in multiple myeloma that promotes proliferation and pathological interactions with bone marrow stroma. Cancer Cell. 2004;5:191–9.

486. Segel MJ, Paltiel O, Zimran A et al. Geographic variance in the frequency of the t(14;18) translocation in follicular lymphoma: an Israeli series compared to the world. Blood Cells Mol Dis. 1998;24:62–72.

487. Godon A, Moreau A, Talmant P et al. Is t(14;18)(q32;q21) a constant finding in follicular lymphoma? An interphase FISH study on 63 patients. Leukemia. 2003;17:255–9.

488. Jacobson JO, Wilkes BM, Kwaiatkowski DJ, Medeiros LJ, Aisenberg AC, Harris NL. bcl-2 rearrangements in de novo diffuse large cell lymphoma. Association with distinctive clinical features. Cancer. 1993;72:231–6.

489. Knutsen T. Cytogenetic mechanisms in the pathogenesis and progression of follicular lymphoma. Cancer Surv. 1997;30:163–92.

490. Yunis JJ, Frizzera G, Oken MM, McKenna J, Theologides A, Arnesen M. Multiple recurrent genomic defects in follicular lymphoma. A possible model for cancer. N Engl J Med. 1987;316:79–84.

491. Okano A, Nakano S, Namura K et al. Unique three-way translocation, t(3;14;18)(q27;q32;q21), in follicular lymphoma. Cancer Genet Cytogenet. 2005;159:174–6.

492. Kramer MH, Raghoebier S, Beverstock GC, de Jong D, Kluin PM, Kluin-Nelemans JC. De novo acute B-cell leukemia with translocation t(14;18): an entity with a poor prognosis. Leukemia. 1991;5:473–8.

493. Mufti GJ, Hamblin TJ, Oscier DG, Johnson S. Common ALL with pre-B-cell features showing (8;14) and (14;18) chromosome translocations. Blood. 1983;62:1142–6.

494. Gluck WL, Bigner SH, Borowitz MJ, Brenckman WD Jr. Acute lymphoblastic leukemia of Burkitt's type (L3 ALL) with 8;22 and 14;18 translocations and absent surface immunoglobulins. Am J Clin Pathol. 1986;85:636–40.

495. Thangavelu M, Olopade O, Beckman E et al. Clinical, morphologic, and cytogenetic characteristics of patients with lymphoid malignancies characterized by both t(14;18)(q32;q21) and t(8;14)(q24;q32) or t(8;22)(q24;q11). Genes Chromosomes Cancer. 1990; 2:147–58.

496. Marosi C, Bettelheim P, Chott A et al. Simultaneous occurrence of t(14;18) and t(8;22) common acute lymphoblastic leukemia. Ann Hematol. 1992;64:101–4.

497. Haralambieva E, Kleiverda K, Mason DY, Schuuring E, Kluin PM. Detection of three common translocation breakpoints in non-Hodgkin's lymphomas by fluorescence in situ hybridization on routine paraffin-embedded tissue sections. J Pathol. 2002;198:163–70.

498. Sanchez-Vega B, Vega F, Medeiros LJ, Lee MS, Luthra R. Quantification of bcl-2/JH fusion sequences and a control gene by multiplex real-time PCR coupled with automated amplicon sizing by capillary electrophoresis. J Mol Diagn. 2002;4:223–9.

499. Shaminie J, Peh SC, Tan MJ. Improvement in the detection rate of t(14;18) translocation on paraffin-embedded tissue: a combination approach using PCR and FISH. Pathology. 2003;35:414–21.

500. Barrans SL, Evans PA, O'Connor SJ, Owen RG, Morgan GJ, Jack AS. The detection of t(14;18) in archival lymph nodes: development of a fluorescence in situ hybridization (FISH)-based method and evaluation by comparison with polymerase chain reaction. J Mol Diagn. 2003;5:168–75.

501. Liu Y, Hernandez AM, Shibata D, Cortopassi GA. BCL2 translocation frequency rises with age in humans. Proc Natl Acad Sci USA. 1994;91:8910–4.

502. Schmitt C, Balogh B, Grundt A et al. The bcl-2/IgH rearrangement in a population of 204 healthy individuals: occurrence, age and gender distribution, breakpoints, and detection method validity. Leuk Res. 2006;30:745–50.

503. Summers KE, Goff LK, Wilson AG, Gupta RK, Lister TA, Fitzgibbon J. Frequency of the Bcl-2/IgH rearrangement in normal individuals: implications for the monitoring of disease in patients with follicular lymphoma. J Clin Oncol. 2001;19:420–4.

504. Zuckerman E, Zuckerman T, Sahar D et al. The effect of antiviral therapy on t(14;18) translocation and immunoglobulin gene rearrangement in patients with chronic hepatitis C virus infection. Blood. 2001;97:1555–9.

505. Zuckerman E, Zuckerman T, Sahar D et al. bcl-2 and immunoglobulin gene rearrangement in patients with hepatitis C virus infection. Br J Haematol. 2001;112:364–9.

506. Delage R, Jacques L, Massinga-Loembe M et al. Persistent polyclonal B-cell lymphocytosis: further evidence for a genetic disorder associated with B-cell abnormalities. Br J Haematol. 2001;114:666–70.

507. Mossafa H, Tapia S, Flandrin G, Troussard X. Chromosomal instability and ATR amplification gene in patients with persistent and polyclonal B-cell lymphocytosis (PPBL). Leuk Lymphoma. 2004;45:1401–6.

508. Callet-Bauchu E, Gazzo S, Poncet C et al. Distinct chromosome 3 abnormalities in persistent polyclonal B-cell lymphocytosis. Genes Chromosomes Cancer. 1999; 26:221–8.

509. Himmelmann A, Gautschi O, Nawrath M, Bolliger U, Fehr J, Stahel RA. Persistent polyclonal B-cell lymphocytosis is an expansion of functional IgD(+)CD27(+) memory B cells. Br J Haematol. 2001;114:400–5.

510. Michaux L, Dierlamm J, Wlodarska I, Bours V, Van den Berghe H, Hagemeijer A. t(14;19)/BCL3 rearrangements in lymphoproliferative disorders: a review of 23 cases. Cancer Genet Cytogenet. 1997;94:36–43.

511. Huh YO, Abruzzo LV, Rassidakis GZ et al. The t(14;19)(q32;q13)-positive small B-cell leukaemia: a clinicopathologic and cytogenetic study of seven cases. Br J Haematol. 2007;136:220–8.

512. Soma LA, Gollin SM, Remstein ED et al. Splenic small B-cell lymphoma with IGH/BCL3 translocation. Hum Pathol. 2006;37:218–30.

513. Wang ZG, Delva L, Gaboli M et al. Role of PML in cell growth and the retinoic acid pathway. Science. 1998; 279:1547–51.

514. Wang ZG, Ruggero D, Ronchetti S et al. PML is essential for multiple apoptotic pathways. Nat Genet. 1998; 20:266–72.

515. Reiter A, Lengfelder E, Grimwade D. Pathogenesis, diagnosis and monitoring of residual disease in acute promyelocytic leukaemia. Acta Haematol. 2004;112:55–67.

516. de Botton S, Chevret S, Sanz M et al. Additional chromosomal abnormalities in patients with acute promyelocytic leukaemia (APL) do not confer poor prognosis: results of APL 93 trial. Br J Haematol. 2000;111:801–6.

517. Owatari S, Uozumi K, Haraguchi K et al. A new cytogenetic abnormality, t(2;7)(q33;q36), in acute promyelocytic leukemia. Cancer Genet Cytogenet. 2007;173:71–4.

518. Wan TS, Ma SK, Au WY, Liu HS, Chan JC, Chan LC. Trisomy 21 and other chromosomal abnormalities in acute promyelocytic leukemia. Cancer Genet Cytogenet. 2003;140:170–3.

519. Gallagher RE, Willman CL, Slack JL et al. Association of PML-RAR alpha fusion mRNA type with pretreatment hematologic characteristics but not treatment outcome in acute promyelocytic leukemia: an intergroup molecular study. Blood. 1997;90:1656–63.

520. Rennert H, Golde T, Wilson RB, Spitalnik SL, Van Deerlin VM, Leonard DG. A novel, non-nested reverse-transcriptase polymerase chain reaction (RT-PCR) test for the detection of the t(15;17) translocation: a comparative study of RT-PCR cytogenetics, and fluorescence in situ hybridization. Mol Diagn. 1999;4:195–209.

521. Garcia-Casado Z, Cervera J, Valencia A et al. A t(17;20)(q21;q12) masking a variant t(15;17)(q22;q21) in a patient with acute promyelocytic leukemia. Cancer Genet Cytogenet. 2006;168:73–6.

522. Yamamoto K, Hamaguchi H, Nagata K, Kobayashi M, Takashima T, Taniwaki M. A new complex translocation (15;20;17)(q22;p13;q21) in acute promyelocytic leukemia. Cancer Genet Cytogenet. 1998;101:89–94.

523. Yunis JJ, Mayer MG, Arnesen MA, Aeppli DP, Oken MM, Frizzera G. bcl-2 and other genomic alterations in the prognosis of large-cell lymphoma. N Engl J Med. 1989;320:1047–54.

524. Dierlamm J, Wlodarska I, Michaux L et al. Genetic abnormalities in marginal zone B-cell lymphoma. Hematol Oncol. 2000;18:1–13.

525. Dierlamm J. Genetic abnormalities in marginal zone B-cell lymphoma. Haematologica. 2003;88:8–12.

526. Wotherspoon AC, Finn TM, Isaacson PG. Trisomy 3 in low-grade B-cell lymphomas of mucosa-associated lymphoid tissue. Blood. 1995;85:2000–4.

527. Lau LC, Kee SK, Tien SL, Koh Mickey BC. Trisomy 8 as sole cytogenetic abnormality in a case of chronic lymphocytic leukemia. Cancer Genet Cytogenet. 2007;172:66–9.

528. Sierra M, Hernandez JM, Garcia JL et al. Hematological, immunophenotypic, and cytogenetic characteristics of acute myeloblastic leukemia with trisomy 11. Cancer Genet Cytogenet. 2005;160:68–72.

529. Heinonen K, Mrozek K, Lawrence D et al. Clinical characteristics of patients with de novo acute myeloid leukaemia and isolated trisomy 11: a Cancer and Leukemia Group B study. Br J Haematol. 1998;101:513–20.

530. Kwong YL, Wong KF. Acute myeloid leukemia with trisomy 11: a molecular cytogenetic study. Cancer Genet Cytogenet. 1997;99:19–23.

531. Bilhou-Nabera C, Lesesve JF, Marit G et al. Trisomy 11 in acute myeloid leukemia: ten cases. Leukemia. 1994; 8:2240–1.

532. Foon KA, Rai KR, Gale RP. Chronic lymphocytic leukemia: new insights into biology and therapy. Ann Intern Med. 1990;113:525–39.

533. Tsimberidou AM, Keating MJ. Richter syndrome: biology, incidence, and therapeutic strategies. Cancer. 2005;103:216–28.

534. Batanian JR, Slovak ML, Mohamed A, Dobin S, Luthardt FW, Keitges EA. Trisomy 15 is frequently observed as a minor clone in patients with anemia/MDS/NHL and as a major clone in patients with AML. Cancer Genet Cytogenet. 2000;121:186–9.

535. Carbone P, Santoro A, Giglio MC, Mirto S, Granata G, Barbata G. Cytogenetic findings in secondary acute non-lymphocytic leukemia. Cancer Genet Cytogenet. 1992;58:18–23.

536. Krugmann J, Tzankov A, Dirnhofer S et al. Unfavourable prognosis of patients with trisomy 18q21 detected by fluorescence in situ hybridisation in t(11;18) negative, surgically resected, gastrointestinal B cell lymphomas. J Clin Pathol. 2004;57:360–4.

537. Grois N, Nowotny H, Tyl E, Krieger O, Kier P, Haas OA. Is trisomy 22 in acute myeloid leukemia a primary abnormality or only a secondary change associated with inversion 16? Cancer Genet Cytogenet. 1989;43:119–29.

538. Boissel N, Renneville A, Biggio V et al. Prevalence, clinical profile, and prognosis of NPM mutations in AML with normal karyotype. Blood. 2005;106:3618–20.

539. Boissel N, Leroy H, Brethon B et al. Incidence and prognostic impact of c-Kit, FLT3, and Ras gene mutations in core binding factor acute myeloid leukemia (CBF-AML). Leukemia. 2006;20:965–70.

540. Lo-Coco F, Ammatuna E. The biology of acute promyelocytic leukemia and its impact on diagnosis and treatment. Hematology Am Soc Hematol Educ Program. 2006: 156–61.

541. Bouley J, Deriano L, Delic J, Merle-Beral H. New molecular markers in resistant B-CLL. Leuk Lymphoma. 2006;47:791–801.

542. Hochhaus A. Cytogenetic and molecular mechanisms of resistance to imatinib. Semin Hematol. 2003;40:69–79.

543. Hochhaus A, Erben P, Ernst T, Mueller MC. Resistance to targeted therapy in chronic myelogenous leukemia. Semin Hematol. 2007;44:15–24.

544. Hochhaus A, Kreil S, Corbin AS et al. Molecular and chromosomal mechanisms of resistance to imatinib (STI571) therapy. Leukemia. 2002;16:2190–6.

545. Hochhaus A, La Rosee P. Imatinib therapy in chronic myelogenous leukemia: strategies to avoid and overcome resistance. Leukemia. 2004;18:1321–31.

546. Fojo T. Multiple paths to a drug resistance phenotype: mutations, translocations, deletions and amplification of coding genes or promoter regions, epigenetic changes and microRNAs. Drug Resist Updat 2007;10:59–67.

547. Branford S, Hughes T. Detection of BCR-ABL mutations and resistance to imatinib mesylate. Methods Mol Med. 2006;125:93–106.

548. Branford S, Rudzki Z, Walsh S et al. High frequency of point mutations clustered within the adenosine triphosphate-binding region of BCR/ABL in patients with chronic myeloid leukemia or Ph-positive acute lymphoblastic leukemia who develop imatinib (STI571) resistance. Blood. 2002;99:3472–5.

549. Branford S, Rudzki Z, Walsh S et al. Detection of BCR-ABL mutations in patients with CML treated with imatinib is virtually always accompanied by clinical resistance, and mutations in the ATP phosphate-binding loop (P-loop) are associated with a poor prognosis. Blood. 2003;102:276–83.

550. Corbin AS, La Rosee P, Stoffregen EP, Druker BJ, Deininger MW. Several Bcr-Abl kinase domain mutants associated with imatinib mesylate resistance remain sensitive to imatinib. Blood. 2003;101:4611–4.

551. Donato NJ, Wu JY, Stapley J et al. Imatinib mesylate resistance through BCR-ABL independence in chronic myelogenous leukemia. Cancer Res. 2004;64:672–7.

552. Gambacorti-Passerini CB, Gunby RH, Piazza R, Galietta A, Rostagno R, Scapozza L. Molecular mechanisms of resistance to imatinib in Philadelphia-chromosome-positive leukaemias. Lancet Oncol. 2003;4:75–85.

553. Melo JV, Chuah C. Resistance to imatinib mesylate in chronic myeloid leukaemia. Cancer Lett 2006; 249:121–32.

554. Shah NP, Nicoll JM, Nagar B et al. Multiple BCR-ABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. Cancer Cell. 2002;2:117–25.

555. Shah NP, Tran C, Lee FY, Chen P, Norris D, Sawyers CL. Overriding imatinib resistance with a novel ABL kinase inhibitor. Science. 2004;305:399–401.

556. Soverini S, Martinelli G, Rosti G et al. ABL mutations in late chronic phase chronic myeloid leukemia patients with up-front cytogenetic resistance to imatinib are associated with a greater likelihood of progression to blast crisis and shorter survival: a study by the GIMEMA Working Party on Chronic Myeloid Leukemia. J Clin Oncol. 2005;23:4100–9.

557. Bernasconi P, Calatroni S, Giardini I et al. ABL1 amplification in T-cell acute lymphoblastic leukemia. Cancer Genet Cytogenet. 2005;162:146–50.

558. Graux C, Cools J, Melotte C et al. Fusion of NUP214 to ABL1 on amplified episomes in T-cell acute lymphoblastic leukemia. Nat Genet. 2004;36:1084–9.

559. De Keersmaecker K, Graux C, Odero MD et al. Fusion of EML1 to ABL1 in T-cell acute lymphoblastic leukemia with cryptic t(9;14)(q34;q32). Blood. 2005;105:4849–52.

560. Morris SW, Kirstein MN, Valentine MB et al. Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. Science. 1994;263:1281–4.

561. Raetz EA, Perkins SL, Carlson MA, Schooler KP, Carroll WL, Virshup DM. The nucleophosmin-anaplastic lymphoma kinase fusion protein induces c-Myc expression in pediatric anaplastic large cell lymphomas. Am J Pathol. 2002;161:875–83.

562. Lamant L, de Reynies A, Duplantier MM et al. Gene-expression profiling of systemic anaplastic large-cell lymphoma reveals differences based on ALK status and two distinct morphologic ALK+subtypes. Blood. 2007;109: 2156–64.

563. Kalinova M, Krskova L, Brizova H, Kabickova E, Kepak T, Kodet R. Quantitative PCR detection of NPM/ALK fusion gene and CD30 gene expression in patients with anaplastic large cell lymphoma – residual disease monitoring and a correlation with the disease status. Leuk Res. 2007.(epub ahead of print)

564. Ma Z, Cools J, Marynen P et al. Inv(2)(p23q35) in anaplastic large-cell lymphoma induces constitutive anaplastic lymphoma kinase (ALK) tyrosine kinase activation by fusion to ATIC, an enzyme involved in purine nucleotide biosynthesis. Blood. 2000;95:2144–9.

565. Lamant L, Dastugue N, Pulford K, Delsol G, Mariame B. A new fusion gene TPM3-ALK in anaplastic large cell lymphoma created by a (1;2)(q25;p23) translocation. Blood. 1999;93:3088–95.

566. Hernandez L, Pinyol M, Hernandez S et al. TRK-fused gene (TFG) is a new partner of ALK in anaplastic large cell lymphoma producing two structurally different TFG-ALK translocations. Blood. 1999;94:3265–8.

567. Tort F, Pinyol M, Pulford K et al. Molecular characterization of a new ALK translocation involving moesin (MSN-ALK) in anaplastic large cell lymphoma. Lab Invest. 2001;81:419–26.

568. Colomo L, Loong F, Rives S et al. Diffuse large B-cell lymphomas with plasmablastic differentiation represent a heterogeneous group of disease entities. Am J Surg Pathol. 2004;28:736–47.

569. Rudzki Z, Rucinska M, Jurczak W et al. ALK-positive diffuse large B-cell lymphoma: two more cases and a brief literature review. Pol J Pathol. 2005;56:37–45.

570. Stoffel A, Le Beau MM. The API2/MALT1 fusion product may lead to germinal center B cell lymphomas by suppression of apoptosis. Hum Hered. 2001;51:1–7.

571. Hosokawa Y, Suzuki H, Suzuki Y, Takahashi R, Seto M. Antiapoptotic function of apoptosis inhibitor 2-MALT1 fusion protein involved in t(11;18)(q21;q21) mucosa-associated lymphoid tissue lymphoma. Cancer Res. 2004;64:3452–7.

572. Stankovic T, Hubank M, Cronin D et al. Microarray analysis reveals that TP53- and ATM-mutant B-CLLs share a defect in activating proapoptotic responses after DNA damage but are distinguished by major differences in activating prosurvival responses. Blood. 2004;103:291–300.

573. Pettitt AR, Sherrington PD, Stewart G, Cawley JC, Taylor AM, Stankovic T. p53 dysfunction in B-cell chronic lymphocytic leukemia: inactivation of ATM as an alternative to TP53 mutation. Blood. 2001; 98:814–22.

574. Stankovic T, Stewart GS, Fegan C et al. Ataxia telangiectasia mutated-deficient B-cell chronic lymphocytic leukemia occurs in pregerminal center cells and results in defective damage response and unrepaired chromosome damage. Blood. 2002;99:300–9.

575. Starostik P, Manshouri T, O'Brien S et al. Deficiency of the ATM protein expression defines an aggressive subgroup of B-cell chronic lymphocytic leukemia. Cancer Res. 1998;58:4552–7.

576. Bullrich F, Rasio D, Kitada S et al. ATM mutations in B-cell chronic lymphocytic leukemia. Cancer Res. 1999;59:24–7.

577. Lossos IS, Thorstenson YR, Wayne TL, Oefner PJ, Levy R, Chu G. Mutation of the ATM gene is not involved in the pathogenesis of either follicle center lymphoma or its transformation to higher-grade lymphoma. Leuk Lymphoma. 2002;43:1079–85.

578. Soverini S, Cavo M, Cellini C et al. Cyclin D1 overexpression is a favorable prognostic variable for newly diagnosed multiple myeloma patients treated with high-dose chemotherapy and single or double autologous transplantation. Blood. 2003;102:1588–94.

579. Markovic O, Marisavljevic D, Cemerikic V, Suvajdzic N, Milic N, Colovic M. Immunohistochemical analysis of cyclin D1 and p53 in multiple myeloma: relationship to proliferative activity and prognostic significance. Med Oncol. 2004;21:73–80.

580. Rasmussen T, Knudsen LM, Johnsen HE. Frequency and prognostic relevance of cyclin D1 dysregulation in multiple myeloma. Eur J Haematol. 2001;67:296–301.

581. Hoechtlen-Vollmar W, Menzel G, Bartl R, Lamerz R, Wick M, Seidel D. Amplification of cyclin D1 gene in multiple myeloma: clinical and prognostic relevance. Br J Haematol. 2000;109:30–8.

582. Specht K, Haralambieva E, Bink K et al. Different mechanisms of cyclin D1 overexpression in multiple myeloma revealed by fluorescence in situ hybridization and quantitative analysis of mRNA levels. Blood. 2004;104:1120–6.

583. Vasef MA, Medeiros LJ, Yospur LS, Sun NC, McCourty A, Brynes RK. Cyclin D1 protein in multiple myeloma and plasmacytoma: an immunohistochemical study using fixed, paraffin-embedded tissue sections. Mod Pathol. 1997;10:927–32.

584. Reed JC. Bcl-2 family proteins: regulators of apoptosis and chemoresistance in hematologic malignancies. Semin Hematol. 1997;34:9–19.

585. Tsujimoto Y, Cossman J, Jaffe E, Croce CM. Involvement of the bcl-2 gene in human follicular lymphoma. Science. 1985;228:1440–3.

586. Tang SC, Visser L, Hepperle B, Hanson J, Poppema S. Clinical significance of bcl-2-MBR gene rearrangement and protein expression in diffuse large-cell non-Hodgkin's lymphoma: an analysis of 83 cases. J Clin Oncol. 1994;12:149–54.

587. Hermine O, Haioun C, Lepage E et al. Prognostic significance of bcl-2 protein expression in aggressive non-Hodgkin's lymphoma. Groupe d'Etude des Lymphomes de l'Adulte (GELA). Blood. 1996;87:265–72.

588. Gascoyne RD, Adomat SA, Krajewski S et al. Prognostic significance of Bcl-2 protein expression and Bcl-2 gene rearrangement in diffuse aggressive non-Hodgkin's lymphoma. Blood. 1997;90:244–51.

589. Kramer MH, Hermans J, Parker J et al. Clinical significance of bcl2 and p53 protein expression in diffuse large B-cell lymphoma: a population-based study. J Clin Oncol. 1996;14:2131–8.

590. Rantanen S, Monni O, Joensuu H, Franssila K, Knuutila S. Causes and consequences of BCL2 overexpression in diffuse large B-cell lymphoma. Leuk Lymphoma. 2001;42:1089–98.

591. Rao PH, Houldsworth J, Dyomina K et al. Chromosomal and gene amplification in diffuse large B-cell lymphoma. Blood. 1998;92:234–40.

592. Monni O, Joensuu H, Franssila K, Klefstrom J, Alitalo K, Knuutila S. BCL2 overexpression associated with

chromosomal amplification in diffuse large B-cell lymphoma. Blood. 1997;90:1168–74.

593. Mollejo M, Menarguez J, Cristobal E et al. Monocytoid B cells. A comparative clinical pathological study of their distribution in different types of low-grade lymphomas. Am J Surg Pathol. 1994;18:1131–39.

594. Villuendas R, Piris MA, Orradre JL, Mollejo M, Rodriguez R, Morente M. Different bcl-2 protein expression in high-grade B-cell lymphomas derived from lymph node or mucosa-associated lymphoid tissue. Am J Pathol. 1991;139:989–93.

595. Skinnider BF, Horsman DE, Dupuis B, Gascoyne RD. Bcl-6 and Bcl-2 protein expression in diffuse large B-cell lymphoma and follicular lymphoma: correlation with 3q27 and 18q21 chromosomal abnormalities. Hum Pathol. 1999;30:803–8.

596. Kersten MJ, Jong Dd D, Raemaekers JM, Kluin PM, Hagenbeek A. Beyond the International Prognostic Index: new prognostic factors in follicular lymphoma and diffuse large-cell lymphoma. A meeting report of the Second International Lunenburg Lymphoma Workshop. Hematol J. 2004;5:202–8.

597. Gascoyne RD. Pathologic prognostic factors in diffuse aggressive non-Hodgkin's lymphoma. Hematol Oncol Clin North Am. 1997;11:847–62.

598. Hill ME, MacLennan KA, Cunningham DC et al. Prognostic significance of BCL-2 expression and bcl-2 major breakpoint region rearrangement in diffuse large cell non-Hodgkin's lymphoma: a British National Lymphoma Investigation Study. Blood. 1996;88:1046–51.

599. Villuendas R, Sanchez-Beato M, Martinez JC et al. Loss of p16/INK4A protein expression in non-Hodgkin's lymphomas is a frequent finding associated with tumor progression. Am J Pathol. 1998;153:887–97.

600. Barrans SL, Carter I, Owen RG et al. Germinal center phenotype and bcl-2 expression combined with the International Prognostic Index improves patient risk stratification in diffuse large B-cell lymphoma. Blood. 2002;99:1136–43.

601. Mounier N, Briere J, Gisselbrecht C et al. Rituximab plus CHOP (R-CHOP) overcomes bcl-2-associated resistance to chemotherapy in elderly patients with diffuse large B-cell lymphoma (DLBCL). Blood. 2003;101:4279–84.

602. Maartense E, Kramer MH, le Cessie S et al. Lack of prognostic significance of BCL2 and p53 protein overexpression in elderly patients with diffuse large B-cell non-Hodgkin's lymphoma: results from a population-based non-Hodgkin's lymphoma registry. Leuk Lymphoma. 2004;45:101–7.

603. Ghia P, Boussiotis VA, Schultze JL et al. Unbalanced expression of bcl-2 family proteins in follicular lymphoma: contribution of CD40 signaling in promoting survival. Blood. 1998;91:244–51.

604. Rassidakis GZ, Jones D, Lai R et al. BCL-2 family proteins in peripheral T-cell lymphomas: correlation with tumour apoptosis and proliferation. J Pathol. 2003;200:240–8.

605. Villalva C, Bougrine F, Delsol G, Brousset P. Bcl-2 expression in anaplastic large cell lymphoma. Am J Pathol. 2001;158:1889–90.

606. ten Berge RL, Meijer CJ, Dukers DF et al. Expression levels of apoptosis-related proteins predict clinical outcome in anaplastic large cell lymphoma. Blood. 2002;99:4540–6.

607. Rassidakis GZ, Medeiros LJ, Vassilakopoulos TP et al. BCL-2 expression in Hodgkin and Reed–Sternberg cells of classical Hodgkin disease predicts a poorer prognosis in patients treated with ABVD or equivalent regimens. Blood. 2002;100:3935–41.

608. Vassallo J, Metze K, Traina F, de Souza CA, Lorand-Metze I. The prognostic relevance of apoptosis-related proteins in classical Hodgkin's lymphomas. Leuk Lymphoma. 2003; 44:483–8.

609. Campos L, Rouault JP, Sabido O et al. High expression of bcl-2 protein in acute myeloid leukemia cells is associated with poor response to chemotherapy. Blood. 1993; 81:3091–6.

610. Coustan-Smith E, Kitanaka A, Pui CH et al. Clinical relevance of BCL-2 overexpression in childhood acute lymphoblastic leukemia. Blood. 1996;87:1140–6.

611. Jardin F, Ruminy P, Bastard C, Tilly H. The BCL6 proto-oncogene: a leading role during germinal center development and lymphomagenesis. Pathol Biol (Paris). 2007;55(1):73–83.

612. Kirn D, Mauch P, Shaffer K et al. Large-cell and immunoblastic lymphoma of the mediastinum: prognostic features and treatment outcome in 57 patients. J Clin Oncol. 1993;11:1336–43.

613. Dent AL, Vasanwala FH, Toney LM. Regulation of gene expression by the proto-oncogene BCL-6. Crit Rev Oncol Hematol. 2002;41:1–9.

614. Baron BW, Nucifora G, McCabe N et al. Identification of the gene associated with the recurring chromosomal translocations t(3;14)(q27;q32) and t(3;22)(q27;q11) in B-cell lymphomas. Proc Natl Acad Sci USA. 1993;90:5262–6.

615. Ye BH, Lista F, Lo Coco F et al. Alterations of a zinc finger-encoding gene, BCL-6, in diffuse large-cell lymphoma. Science. 1993;262:747–50.

616. Dalla-Favera R, Ye BH, Cattoretti G et al. BCL-6 in diffuse large-cell lymphomas. Important Adv Oncol. 1996:139–48.

617. Lo Coco F, Ye BH, Lista F et al. Rearrangements of the BCL6 gene in diffuse large cell non-Hodgkin's lymphoma. Blood. 1994;83:1757–9.

618. Otsuki T, Yano T, Clark HM et al. Analysis of LAZ3 (BCL-6) status in B-cell non-Hodgkin's lymphomas: results of rearrangement and gene expression studies and a mutational analysis of coding region sequences. Blood. 1995;85:2877–84.

619. Chaganti SR, Chen W, Parsa N et al. Involvement of BCL6 in chromosomal aberrations affecting band 3q27 in B-cell non-Hodgkin lymphoma. Genes Chromosomes Cancer. 1998;23:323–7.

620. Ohshima A, Miura I, Hashimoto K et al. Rearrangements of the BCL6 gene and chromosome aberrations affecting 3q27 in 54 patients with non-Hodgkin's lymphoma. Leuk Lymphoma. 1997;27:329–34.

621. Phan RT, Dalla-Favera R. The BCL6 proto-oncogene suppresses p53 expression in germinal-centre B cells. Nature. 2004;432:635–9.

622. Margalit O, Amram H, Amariglio N et al. BCL6 is regulated by p53 through a response element frequently disrupted in B-cell non-Hodgkin lymphoma. Blood. 2006;107:1599–607.

623. Onizuka T, Moriyama M, Yamochi T et al. BCL-6 gene product, a 92- to 98-kD nuclear phosphoprotein, is highly expressed in germinal center B cells and their neoplastic counterparts. Blood. 1995;86:28–37.

624. Flenghi L, Bigerna B, Fizzotti M et al. Monoclonal antibodies PG-B6a and PG-B6p recognize, respectively, a highly conserved and a formol-resistant epitope on the human BCL-6 protein amino-terminal region. Am J Pathol. 1996;148:1543–55.

625. Falini B, Bigerna B, Pasqualucci L et al. Distinctive expression pattern of the BCL-6 protein in nodular lymphocyte predominance Hodgkin's disease. Blood. 1996;87:465–71.

626. Dogan A, Bagdi E, Munson P, Isaacson PG. CD10 and BCL-6 expression in paraffin sections of normal lymphoid tissue and B-cell lymphomas. Am J Surg Pathol. 2000;24:846–52.

627. Artiga MJ, Saez AI, Romero C et al. A short mutational hot spot in the first intron of BCL-6 is associated with increased BCL-6 expression and with longer overall survival in large B-cell lymphomas. Am J Pathol. 2002;160:1371–80.

628. Offit K, Lo Coco F, Louie DC et al. Rearrangement of the bcl-6 gene as a prognostic marker in diffuse large-cell lymphoma. N Engl J Med. 1994;331:74–80.

629. Lossos IS, Jones CD, Warnke R et al. Expression of a single gene, BCL-6, strongly predicts survival in patients with diffuse large B-cell lymphoma. Blood. 2001;98:945–51.

630. Akasaka T, Ueda C, Kurata M et al. Nonimmunoglobulin (non-Ig)/BCL6 gene fusion in diffuse large B-cell lymphoma results in worse prognosis than Ig/BCL6. Blood. 2000;96:2907–9.

631. Alizadeh AA, Eisen MB, Davis RE et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature. 2000;403:503–11.

632. Rosenwald A, Wright G, Chan WC et al. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. N Engl J Med. 2002;346:1937–47.

633. Melnick A. Reprogramming specific gene expression pathways in B-cell lymphomas. Cell Cycle. 2005;4:239–41.

634. Polo JM, Dell'Oso T, Ranuncolo SM et al. Specific peptide interference reveals BCL6 transcriptional and oncogenic mechanisms in B-cell lymphoma cells. Nat Med. 2004;10:1329–35.

635. Akasaka T, Lossos IS, Levy R. BCL6 gene translocation in follicular lymphoma: a harbinger of eventual transformation to diffuse aggressive lymphoma. Blood. 2003;102:1443–8.

636. Sarsotti E, Marugan I, Benet I et al. Bcl-6 mutation status provides clinically valuable information in early-stage B-cell chronic lymphocytic leukemia. Leukemia. 2004;18:743–6.

637. Sahota SS, Davis Z, Hamblin TJ, Stevenson FK. Somatic mutation of bcl-6 genes can occur in the absence of V(H) mutations in chronic lymphocytic leukemia. Blood. 2000;95:3534–40.

638. Ye H, Gong L, Liu H et al. MALT lymphoma with t(14;18)(q32;q21)/IGH-MALT1 is characterized by strong cytoplasmic MALT1 and BCL10 expression. J Pathol. 2005;205:293–301.

639. Chuang SS, Liu H, Martin-Subero JI, Huang WT, Siebert R, Ye H. Pulmonary MALT lymphoma with strong nuclear BCL10 expression and novel translocation t(1;2)(p22;p12)/IGK-BCL10. J Clin Pathol. 2007;60:727–8.

640. Bullinger L, Rucker FG, Kurz S et al. Gene expression profiling identifies distinct subclasses of core binding factor acute myeloid leukemia. Blood. 2007;110:1291–300.

641. Illmer T, Schaich M, Ehninger G, Thiede C. Tyrosine kinase mutations of JAK2 are rare events in AML but influence prognosis of patients with CBF-leukemias. Haematologica. 2007;92:137–8.

642. Stentoft J, Hokland P, Ostergaard M, Hasle H, Nyvold CG. Minimal residual core binding factor AMLs by real time quantitative PCR – initial response to chemotherapy predicts event free survival and close monitoring of peripheral blood unravels the kinetics of relapse. Leuk Res. 2006;30:389–95.

643. Bienz M, Ludwig M, Leibundgut EO et al. Risk assessment in patients with acute myeloid leukemia and a normal karyotype. Clin Cancer Res. 2005;11:1416–24.

644. Chapiro E, Russell L, Radford-Weiss I et al. Overexpression of CEBPA resulting from the translocation t(14;19)(q32;q13) of human precursor B acute lymphoblastic leukemia. Blood. 2006;108:3560–3.

645. Akasaka T, Balasas T, Russell LJ et al. Five members of the CEBP transcription factor family are targeted by recurrent IGH translocations in B-cell precursor acute lymphoblastic leukemia (BCP-ALL). Blood. 2007;109:3451–61.

646. Chevallier N, Corcoran CM, Lennon C et al. ETO protein of t(8;21) AML is a corepressor for Bcl-6 B-cell lymphoma oncoprotein. Blood 2004;103:1454–63.

647. Weisser M, Haferlach C, Hiddemann W, Schnittger S. The quality of molecular response to chemotherapy is predictive for the outcome of AML1-ETO-positive AML and is independent of pretreatment risk factors. Leukemia 2007;21:1177–82.

648. Tobal K, Liu Yin JA. Diagnosis and monitoring of AML1-MTG8 (ETO)-positive acute myeloid leukemia by qualitative and real-time quantitative RT-PCR. Methods Mol Med. 2006;125:149–61.

649. Vieira L, Marques B, Cavaleiro C et al. Molecular cytogenetic characterization of rearrangements involving 12p in leukemia. Cancer Genet Cytogenet. 2005;157:134–9.

650. Odero MD, Carlson K, Calasanz MJ, Lahortiga I, Chinwalla V, Rowley JD. Identification of new translocations involving ETV6 in hematologic malignancies by fluorescence in situ hybridization and spectral karyotyping. Genes Chromosomes Cancer. 2001;31:134–42.

651. Yagasaki F, Wakao D, Yokoyama Y et al. Fusion of ETV6 to fibroblast growth factor receptor 3 in peripheral T-cell lymphoma with a t(4;12)(p16;p13) chromosomal translocation. Cancer Res. 2001;61:8371–4.

652. Odero MD, Vizmanos JL, Roman JP et al. A novel gene, MDS2, is fused to ETV6/TEL in a t(1;12)(p36.1;p13) in a patient with myelodysplastic syndrome. Genes Chromosomes Cancer. 2002;35:11–19.

653. Qiao Y, Ogawa S, Hangaishi A et al. Identification of a novel fusion gene, TTL, fused to ETV6 in acute lymphoblastic leukemia with t(12;13)(p13;q14), and its implication in leukemogenesis. Leukemia. 2003;17:1112–20.

654. Peeters P, Raynaud SD, Cools J et al. Fusion of TEL, the ETS-variant gene 6 (ETV6), to the receptor-associated kinase JAK2 as a result of t(9;12) in a lymphoid and t(9;15;12) in a myeloid leukemia. Blood. 1997;90:2535–40.

655. Andreasson P, Johansson B, Arheden K, Billstrom R, Mitelman F, Hoglund M. Deletions of CDKN1B and ETV6 in acute myeloid leukemia and myelodysplastic syndromes without cytogenetic evidence of 12p abnormalities. Genes Chromosomes Cancer. 1997;19:77–83.

656. Asakura K, Uchida H, Miyachi H et al. TEL/AML1 overcomes drug resistance through transcriptional repression of multidrug resistance-1 gene expression. Mol Cancer Res. 2004;2:339–47.

657. Ramakers-van Woerden NL, Pieters R, Loonen AH et al. TEL/AML1 gene fusion is related to in vitro drug sensitivity for L-asparaginase in childhood acute lymphoblastic leukemia. Blood. 2000;96:1094–9.

658. von Bergh AR, van Drunen E, van Wering ER et al. High incidence of t(7;12)(q36;p13) in infant AML but not in infant ALL, with a dismal outcome and ectopic expression of HLXB9. Genes Chromosomes Cancer. 2006;45:731–9.

659. Birg F, Rosnet O, Carbuccia N, Birnbaum D. The expression of FMS, KIT and FLT3 in hematopoietic malignancies. Leuk Lymphoma. 1994;13:223–7.

660. Birg F, Courcoul M, Rosnet O et al. Expression of the FMS/KIT-like gene FLT3 in human acute leukemias of the myeloid and lymphoid lineages. Blood. 1992;80:2584–93.

661. Carow CE, Levenstein M, Kaufmann SH et al. Expression of the hematopoietic growth factor receptor FLT3 (STK-1/Flk2) in human leukemias. Blood. 1996;87:1089–96.

662. Stacchini A, Fubini L, Severino A, Sanavio F, Aglietta M, Piacibello W. Expression of type III receptor tyrosine kinases FLT3 and KIT and responses to their ligands by acute myeloid leukemia blasts. Leukemia. 1996;10:1584–91.

663. Brown P, Levis M, Shurtleff S, Campana D, Downing J, Small D. FLT3 inhibition selectively kills childhood acute lymphoblastic leukemia cells with high levels of FLT3 expression. Blood. 2005;105:812–20.

664. Kussick SJ, Stirewalt DL, Yi HS et al. A distinctive nuclear morphology in acute myeloid leukemia is strongly associated with loss of HLA-DR expression and FLT3 internal tandem duplication. Leukemia. 2004;18:1591–8.

665. Ozeki K, Kiyoi H, Hirose Y et al. Biologic and clinical significance of the FLT3 transcript level in acute myeloid leukemia. Blood. 2004;103:1901–8.

666. Whitman SP, Archer KJ, Feng L et al. Absence of the wild-type allele predicts poor prognosis in adult de novo acute myeloid leukemia with normal cytogenetics and the internal tandem duplication of FLT3: a cancer and leukemia group B study. Cancer Res. 2001;61:7233–9.

667. Stirewalt DL, Kopecky KJ, Meshinchi S et al. Size of FLT3 internal tandem duplication has prognostic significance in patients with acute myeloid leukemia. Blood. 2006;107:3724–6.

668. Callens C, Chevret S, Cayuela JM et al. Prognostic implication of FLT3 and Ras gene mutations in patients with acute promyelocytic leukemia (APL): a retrospective study from the European APL Group. Leukemia. 2005;19:1153–60.

669. Gale RE, Hills R, Pizzey AR et al. Relationship between FLT3 mutation status, biologic characteristics, and response to targeted therapy in acute promyelocytic leukemia. Blood. 2005;106:3768–76.

670. Au WY, Fung A, Chim CS et al. FLT-3 aberrations in acute promyelocytic leukaemia: clinicopathological associations and prognostic impact. Br J Haematol. 2004;125:463–9.

671. Arrigoni P, Beretta C, Silvestri D et al. FLT3 internal tandem duplication in childhood acute myeloid leukaemia: association with hyperleucocytosis in acute promyelocytic leukaemia. Br J Haematol. 2003;120:89–92.

672. Meshinchi S, Woods WG, Stirewalt DL et al. Prevalence and prognostic significance of FLT3 internal tandem duplication in pediatric acute myeloid leukemia. Blood. 2001;97:89–94.

673. Shih LY, Huang CF, Wang PN et al. Acquisition of FLT3 or N-ras mutations is frequently associated with progression of myelodysplastic syndrome to acute myeloid leukemia. Leukemia. 2004;18:466–75.

674. Shih LY, Lin TL, Wang PN et al. Internal tandem duplication of fms-like tyrosine kinase 3 is associated with poor outcome in patients with myelodysplastic syndrome. Cancer. 2004;101:989–98.

675. Georgiou G, Karali V, Zouvelou C et al. Serial determination of FLT3 mutations in myelodysplastic syndrome patients at diagnosis, follow up or acute myeloid leukaemia transformation: incidence and their prognostic significance. Br J Haematol. 2006;134:302–6.

676. Katoh M, Katoh M. Human FOX gene family. Int J Oncol. 2004;25:1495–500.

677. Streubel B, Vinatzer U, Lamprecht A, Raderer M, Chott A. T(3;14)(p14.1;q32) involving IGH and FOXP1 is a novel recurrent chromosomal aberration in MALT lymphoma. Leukemia. 2005;19:652–8.

678. Wlodarska I, Veyt E, De Paepe P et al. FOXP1, a gene highly expressed in a subset of diffuse large B-cell lymphoma, is recurrently targeted by genomic aberrations. Leukemia. 2005;19:1299–305.

679. Nakamura S, Ye H, Bacon CM et al. Clinical impact of genetic aberrations in gastric MALT lymphoma: a comprehensive analysis using interphase fluorescence in situ hybridisation. Gut. 2007;56:1358–63.

680. Barrans SL, Fenton JA, Banham A, Owen RG, Jack AS. Strong expression of FOXP1 identifies a distinct subset of diffuse large B-cell lymphoma (DLBCL) patients with poor outcome. Blood. 2004;104:2933–5.

681. Banham AH, Connors JM, Brown PJ et al. Expression of the FOXP1 transcription factor is strongly associated with inferior survival in patients with diffuse large B-cell lymphoma. Clin Cancer Res. 2005;11:1065–72.

682. Senff NJ, Hoefnagel JJ, Jansen PM et al. Reclassification of 300 primary cutaneous B-cell lymphomas according to the new WHO-EORTC classification for cutaneous lymphomas: comparison with previous classifications and identification of prognostic markers. J Clin Oncol. 2007;25:1581–7.

683. Fonseca R, Blood EA, Oken MM et al. Myeloma and the t(11;14)(q13;q32); evidence for a biologically defined unique subset of patients. Blood. 2002;99:3735–41.

684. Stevenson F, Sahota S, Zhu D et al. Insight into the origin and clonal history of B-cell tumors as revealed by analysis of immunoglobulin variable region genes. Immunol Rev. 1998;162:247–59.

685. Oscier DG, Gardiner AC, Mould SJ et al. Multivariate analysis of prognostic factors in CLL: clinical stage, IGVH gene mutational status, and loss or mutation of the p53 gene are independent prognostic factors. Blood. 2002; 100:1177–84.

686. Hamblin TJ, Orchard JA, Gardiner A, Oscier DG, Davis Z, Stevenson FK. Immunoglobulin V genes and CD38 expression in CLL. Blood. 2000;95:2455–7.

687. Catherwood MA, Matthews C, Niblock R, Dobbin E, Morris TC, Alexander HD. ZAP-70 mRNA quantification in B-cell chronic lymphocytic leukaemia. Eur J Haematol. 2006;76:294–8.

688. Crespo M, Bosch F, Villamor N et al. ZAP-70 expression as a surrogate for immunoglobulin-variable-region mutations in chronic lymphocytic leukemia. N Engl J Med. 2003;348:1764–75.

689. Durig J, Nuckel H, Cremer M et al. ZAP-70 expression is a prognostic factor in chronic lymphocytic leukemia. Leukemia. 2003;17:2426–34.

690. Huttmann A, Klein-Hitpass L, Thomale J et al. Gene expression signatures separate B-cell chronic lymphocytic leukaemia prognostic subgroups defined by ZAP-70 and CD38 expression status. Leukemia. 2006;20:1774–82.

691. Orchard JA, Ibbotson RE, Davis Z et al. ZAP-70 expression and prognosis in chronic lymphocytic leukaemia. Lancet. 2004;363:105–11.

692. Rassenti LZ, Huynh L, Toy TL et al. ZAP-70 compared with immunoglobulin heavy-chain gene mutation status as a predictor of disease progression in chronic lymphocytic leukemia. N Engl J Med. 2004;351:893–901.

693. Wiestner A, Rosenwald A, Barry TS et al. ZAP-70 expression identifies a chronic lymphocytic leukemia subtype with unmutated immunoglobulin genes, inferior clinical outcome, and distinct gene expression profile. Blood. 2003;101:4944–51.

694. Damle RN, Wasil T, Fais F et al. Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood. 1999;94:1840–7.

695. D'Arena G, Musto P, Cascavilla N et al. CD38 expression correlates with adverse biological features and predicts poor clinical outcome in B-cell chronic lymphocytic leukemia. Leuk Lymphoma. 2001;42:109–14.

696. Del Poeta G, Maurillo L, Venditti A et al. Clinical significance of CD38 expression in chronic lymphocytic leukemia. Blood. 2001;98:2633–9.

697. Ghia P, Guida G, Stella S et al. The pattern of CD38 expression defines a distinct subset of chronic lymphocytic leukemia (CLL) patients at risk of disease progression. Blood. 2003;101:1262–9.

698. Hamblin TJ, Orchard JA, Ibbotson RE et al. CD38 expression and immunoglobulin variable region mutations are independent prognostic variables in chronic lymphocytic leukemia, but CD38 expression may vary during the course of the disease. Blood. 2002;99:1023–9.

699. Ibrahim S, Keating M, Do KA et al. CD38 expression as an important prognostic factor in B-cell chronic lymphocytic leukemia. Blood. 2001;98:181–16.

700. Krober A, Seiler T, Benner A et al. V(H) mutation status, CD38 expression level, genomic aberrations, and survival in chronic lymphocytic leukemia. Blood. 2002;100:1410–16.

701. Thornton PD, Fernandez C, Giustolisi GM et al. CD38 expression as a prognostic indicator in chronic lymphocytic leukaemia. Hematol J. 2004;5:145–51.

702. Austen B, Powell JE, Alvi A et al. Mutations in the ATM gene lead to impaired overall and treatment-free survival that is independent of IGVH mutation status in patients with B-CLL. Blood. 2005;106:3175–82.

703. Buhl AM, Jurlander J, Jorgensen FS et al. Identification of a gene on chromosome 12q22 uniquely overexpressed in chronic lymphocytic leukemia. Blood. 2006;107:2904–11.

704. Kienle D, Krober A, Katzenberger T et al. VH mutation status and VDJ rearrangement structure in mantle cell lymphoma: correlation with genomic aberrations, clinical characteristics, and outcome. Blood. 2003;102:3003–9.

705. Lacronique V, Boureux A, Valle VD et al. A TEL-JAK2 fusion protein with constitutive kinase activity in human leukemia. Science. 1997;278:1309–12.

706. James C, Ugo V, Le Couedic JP et al. A unique clonal JAK2 mutation leading to constitutive signalling causes Polycythaemia vera. Nature. 2005;434:1144–8.

707. Levine RL, Wadleigh M, Cools J et al. Activating mutation in the tyrosine kinase JAK2 in Polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. Cancer Cell. 2005;7:387–97.

708. Kralovics R, Passamonti F, Buser AS et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med. 2005;352:1779–90.

709. Zhao R, Xing S, Li Z et al. Identification of an acquired JAK2 mutation in Polycythemia vera. J Biol Chem. 2005;280:22788–92.

710. Baxter EJ, Scott, L. M. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. Lancet. 2005;365:1054–61.

711. Bousquet M, Le Guellec S, Quelen C, Rigal-Huguet F, Delsol G, Brousset P. Frequent detection of the JAK2 V617F mutation in bone marrow core biopsy specimens from chronic myeloproliferative disorders using the TaqMan polymerase chain reaction single nucleotide polymorphism genotyping assay. A retrospective study with pathologic correlations. Hum Pathol. 2006;37:1458–64.

712. Tefferi A, Lasho TL, Schwager SM et al. The clinical phenotype of wild-type, heterozygous, and homozygous JAK2V617F in Polycythemia vera. Cancer. 2006; 106:631–5.

713. Aboudola S, Murugesan G, Szpurka H et al. Bone marrow phospho-STAT5 expression in non-CML chronic myeloproliferative disorders correlates with JAK2 V617F mutation and provides evidence of in vivo JAK2 activation. Am J Surg Pathol. 2007;31:233–9.

714. Goemans BF, Zwaan CM, Miller M et al. Mutations in KIT and RAS are frequent events in pediatric core-binding factor acute myeloid leukemia. Leukemia. 2005;19:1536–42.

715. Gari M, Goodeve A, Wilson G et al. c-kit proto-oncogene exon 8 in-frame deletion plus insertion mutations in acute myeloid leukaemia. Br J Haematol. 1999;105:894–900.

716. Fontalba A, Real PJ, Fernandez-Luna JL, Agirre X, Prosper F, Richard C. Identification of c-Kit gene mutations in patients with Polycythemia vera. Leuk Res. 2006; 30:1325–6.

717. Pardanani A, Reeder TL, Kimlinger TK et al. Flt-3 and c-kit mutation studies in a spectrum of chronic myeloid disorders including systemic mast cell disease. Leuk Res. 2003;27:739–42.

718. Lorenzo F, Nishii K, Monma F, Kuwagata S, Usui E, Shiku H. Mutational analysis of the KIT gene in myelodysplastic syndrome (MDS) and MDS-derived leukemia. Leuk Res. 2006;30:1235–9.

719. Cho-Vega JH, Vega F, Rassidakis G, Medeiros LJ. Primary cutaneous marginal zone B-cell lymphoma. Am J Clin Pathol. 2006;125(Suppl):S38-49.

720. Cook JR, Sherer M, Craig FE, Shekhter-Levin S, Swerdlow SH. T(14;18)(q32;q21) involving MALT1 and IGH genes in an extranodal diffuse large B-cell lymphoma. Hum Pathol. 2003;34:1212–15.

721. Takada S, Yoshino T, Taniwaki M et al. Involvement of the chromosomal translocation t(11;18) in some mucosa-associated lymphoid tissue lymphomas and diffuse large B-cell lymphomas of the ocular adnexa: evidence from multiplex reverse transcriptase-polymerase chain reaction and fluorescence in situ hybridization on using formalin-fixed, paraffin-embedded specimens. Mod Pathol. 2003; 16:445–52.

722. Johnstone RW, Cretney E, Smyth MJ. P-glycoprotein protects leukemia cells against caspase-dependent, but not caspase-independent, cell death. Blood. 1999;93:1075–85.

723. Juranka PF, Zastawny RL, Ling V. P-glycoprotein: multidrug-resistance and a superfamily of membrane-associated transport proteins. Faseb J. 1989;3:2583–92.

724. Leith CP, Kopecky KJ, Chen IM et al. Frequency and clinical significance of the expression of the multidrug resistance proteins MDR1/P-glycoprotein, MRP1, and LRP in acute myeloid leukemia: a Southwest Oncology Group Study. Blood. 1999;94:1086–99.

725. Wood P, Burgess R, MacGregor A, Yin JA. P-glycoprotein expression on acute myeloid leukaemia blast cells at diagnosis predicts response to chemotherapy and survival. Br J Haematol. 1994;87:509–14.

726. Musto P, Melillo L, Lombardi G, Matera R, di Giorgio G, Carotenuto M. High risk of early resistant relapse for leukaemic patients with presence of multidrug resistance associated P-glycoprotein positive cells in complete remission. Br J Haematol. 1991;77:50–3.

727. Guerci A, Merlin JL, Missoum N et al. Predictive value for treatment outcome in acute myeloid leukemia of cellular daunorubicin accumulation and P-glycoprotein expression simultaneously determined by flow cytometry. Blood. 1995;85:2147–53.

728. Leith CP, Kopecky KJ, Godwin J et al. Acute myeloid leukemia in the elderly: assessment of multidrug resistance (MDR1) and cytogenetics distinguishes biologic subgroups with remarkably distinct responses to standard chemotherapy. A Southwest Oncology Group study. Blood. 1997;89:3323–9.

729. Zochbauer S, Gsur A, Brunner R, Kyrle PA, Lechner K, Pirker R. P-glycoprotein expression as unfavorable prognostic factor in acute myeloid leukemia. Leukemia. 1994;8:974–7.

730. Steinbach D, Furchtbar S, Sell W et al. Contrary to adult patients, expression of the multidrug resistance gene (MDR1) fails to define a poor prognostic group in childhood AML. Leukemia. 2003;17:470–1.

731. Schaich M, Soucek S, Thiede C, Ehninger G, Illmer T. MDR1 and MRP1 gene expression are independent predictors for treatment outcome in adult acute myeloid leukaemia. Br J Haematol. 2005;128:324–32.

732. Schaich M, Harbich-Brutscher E, Pascheberg U, et al. Association of specific cytogenetic aberrations with mdr1 gene expression in adult myeloid leukemia and its implication in treatment outcome. Haematologica. 2002;87:455–64.

733. List AF, Kopecky KJ, Willman CL et al. Benefit of cyclosporine modulation of drug resistance in patients with poor-risk acute myeloid leukemia: a Southwest Oncology Group study. Blood. 2001;98:3212–20.

734. List AF, Kopecky KJ, Willman CL et al. Cyclosporine inhibition of P-glycoprotein in chronic myeloid leukemia blast phase. Blood. 2002;100:1910–2.

735. van den Heuvel-Eibrink MM, Sonneveld P, Pieters R. The prognostic significance of membrane transport-associated multidrug resistance (MDR) proteins in leukemia. Int J Clin Pharmacol Ther. 2000;38:94–110.

736. el Rouby S, Thomas A, Costin D et al. p53 gene mutation in B-cell chronic lymphocytic leukemia is associated with drug resistance and is independent of MDR1/MDR3 gene expression. Blood. 1993;82:3452–9.

737. Tanaka PY, Calore EE. P-glycoprotein expression in non-Hodgkin's lymphomas of human immunodeficiency virus infected patients. Pathol Res Pract. 2007;203:1–7.

738. Yasunami T, Wang YH, Tsuji K, Takanashi M, Yamada Y, Motoji T. Multidrug resistance protein expression of adult T-cell leukemia/lymphoma. Leuk Res. 2007; 31:465–70.

739. Yamaguchi M, Kita K, Miwa H et al. Frequent expression of P-glycoprotein/MDR1 by nasal T-cell lymphoma cells. Cancer. 1995;76:2351–6.

740. Grier DG, Thompson A, Kwasniewska A, McGonigle GJ, Halliday HL, Lappin TR. The pathophysiology of HOX genes and their role in cancer. J Pathol. 2005;205:154–71.

741. Schoch C, Schnittger S, Klaus M, Kern W, Hiddemann W, Haferlach T. AML with 11q23/MLL abnormalities as defined by the WHO classification: incidence, partner chromosomes, FAB subtype, age distribution, and prognostic impact in an unselected series of 1897 cytogenetically analyzed AML cases. Blood. 2003;102:2395–402.

742. Harrison CJ. The detection and significance of chromosomal abnormalities in childhood acute lymphoblastic leukaemia. Blood Rev. 2001;15:49–59.

743. Pui CH, Sandlund JT, Pei D et al. Improved outcome for children with acute lymphoblastic leukemia: results of Total Therapy Study XIIIB at St Jude Children's Research Hospital. Blood. 2004;104:2690–6.

744. Ferrando AA, Neuberg DS, Staunton J et al. Gene expression signatures define novel oncogenic pathways in T cell acute lymphoblastic leukemia. Cancer Cell. 2002;1:75–87.

745. Basecke J, Whelan JT, Griesinger F, Bertrand FE. The MLL partial tandem duplication in acute myeloid leukaemia. Br J Haematol. 2006;135:438–49.

746. Whitman SP, Ruppert AS, Marcucci G et al. Long-term disease-free survivors with cytogenetically normal acute myeloid leukemia and MLL partial tandem duplication: a Cancer and Leukemia Group B study. Blood 2007;107:5164–7.

747. Pardanani AD, Levine RL, Lasho T et al. MPL515 mutations in myeloproliferative and other myeloid disorders: a study of 1182 patients. Blood. 2006;108:3472–6.

748. Pikman Y, Lee BH, Mercher T et al. MPLW515L is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia. PLoS Med. 2006;3:e270.

749. Bennett M, Stroncek DF. Recent advances in the bcr-abl negative chronic myeloproliferative diseases. J Transl Med. 2006;4:41.

750. Lasho TL, Pardanani A, McClure RF et al. Concurrent MPL515 and JAK2V617F mutations in myelofibrosis: chronology of clonal emergence and changes in mutant allele burden over time. Br J Haematol. 2006;135:683–7.

751. Moliterno AR, Williams DM, Rogers O, Spivak JL. Molecular mimicry in the chronic myeloproliferative disorders: reciprocity between quantitative JAK2 V617F and Mpl expression. Blood. 2006;108:3913–15.

752. Falini B, Fizzotti M, Pucciarini A et al. A monoclonal antibody (MUM1p) detects expression of the MUM1/IRF4 protein in a subset of germinal center B cells, plasma cells, and activated T cells. Blood. 2000;95:2084–92.

753. Tsuboi K, Iida S, Inagaki H et al. MUM1/IRF4 expression as a frequent event in mature lymphoid malignancies. Leukemia. 2000;14:449–56.

754. Natkunam Y, Warnke RA, Montgomery K, Falini B, van De Rijn M. Analysis of MUM1/IRF4 protein expression using tissue microarrays and immunohistochemistry. Mod Pathol. 2001;14:686–94.

755. Hallermann C, Niermann C, Fischer RJ, Schulze HJ. New prognostic relevant factors in primary cutaneous diffuse large B-cell lymphomas. J Am Acad Dermatol. 2007;56:588–97.

756. Sundram U, Kim Y, Mraz-Gernhard S, Hoppe R, Natkunam Y, Kohler S. Expression of the bcl-6 and MUM1/IRF4 proteins correlate with overall and disease-specific survival in patients with primary cutaneous large B-cell lymphoma: a tissue microarray study. J Cutan Pathol. 2005;32:227–34.

757. Hans CP, Weisenburger DD, Greiner TC et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood. 2004;103:275–82.

758. Boxer LM, Dang CV. Translocations involving c-myc and c-myc function. Oncogene. 2001;20:5595–610.

759. Taub R, Kirsch I, Morton C et al. Translocation of the c-myc gene into the immunoglobulin heavy chain locus in human Burkitt lymphoma and murine plasmacytoma cells. Proc Natl Acad Sci USA. 1982;79:7837–41.

760. Kramer MH, Hermans J, Wijburg E et al. Clinical relevance of BCL2, BCL6, and MYC rearrangements in diffuse large B-cell lymphoma. Blood. 1998;92:3152–62.

761. Ladanyi M, Offit K, Chaganti RS. Variant t(8;14) translocations in non-Burkitt's non-Hodgkin's lymphomas. Blood. 1992;79:1377–9.

762. Ladanyi M, Offit K, Jhanwar SC, Filippa DA, Chaganti RS. MYC rearrangement and translocations involving band 8q24 in diffuse large cell lymphomas. Blood. 1991;77:1057–63.

763. Au WY, Horsman DE, Viswanatha DS, Connors JM, Klasa RJ, Gascoyne RD. 8q24 translocations in blastic transformation of mantle cell lymphoma. Haematologica. 2000;85:1225–7.

764. Drach J, Kaufmann H, Urbauer E, Schreiber S, Ackermann J, Huber H. The biology of multiple myeloma. J Cancer Res Clin Oncol. 2000;126:441–7.

765. Shou Y, Martelli ML, Gabrea A et al. Diverse karyotypic abnormalities of the c-myc locus associated with c-myc dysregulation and tumor progression in multiple myeloma. Proc Natl Acad Sci USA. 2000;97:228–33.

766. Fabris S, Storlazzi CT, Baldini L et al. Heterogeneous pattern of chromosomal breakpoints involving the MYC locus in multiple myeloma. Genes Chromosomes Cancer. 2003;37:261–9.

767. Chan NP, Ma ES, Wan TS, Chan LC. The spectrum of acute lymphoblastic leukemia with mature B-cell phenotype. Leuk Res. 2003;27:231–4.

768. Brito-Babapulle V, Crawford A, Khokhar T et al. Translocations t(14;18) and t(8;14) with rearranged bcl-2 and c-myc in a case presenting as B-ALL (L3). Leukemia. 1991;5:83–7.

769. Monaco S, Tsao L, Murty VV et al. Pediatric ALK+ anaplastic large cell lymphoma with t(3;8)(q26.2;q24) translocation and c-myc rearrangement terminating in a leukemic phase. Am J Hematol. 2007;82:59–64.

770. Chang CC, Liu YC, Cleveland RP, Perkins SL. Expression of c-Myc and p53 correlates with clinical outcome in diffuse large B-cell lymphomas. Am J Clin Pathol. 2000;113:512–18.

771. Pagnano KB, Vassallo J, Lorand-Metze I, Costa FF, Saad ST. p53, Mdm2, and c-Myc overexpression is associated with a poor prognosis in aggressive non-Hodgkin's lymphomas. Am J Hematol. 2001;67:84–92.

772. Nagy B, Lundan T, Larramendy ML et al. Abnormal expression of apoptosis-related genes in haematological malignancies: overexpression of MYC is poor prognostic sign in mantle cell lymphoma. Br J Haematol. 2003;120:434–41.

773. Kanungo A, Medeiros LJ, Abruzzo LV, Lin P. Lymphoid neoplasms associated with concurrent t(14;18) and 8q24/c-MYC translocation generally have a poor prognosis. Mod Pathol. 2006;19:25–33.

774. Inghirami G, Macri L, Cesarman E, Chadburn A, Zhong J, Knowles DM. Molecular characterization of CD30+ anaplastic large-cell lymphoma: high frequency of c-myc proto-oncogene activation. Blood. 1994;83:3581–90.

775. Joos S, Falk MH, Lichter P et al. Variable breakpoints in Burkitt lymphoma cells with chromosomal t(8;14) translocation separate c-myc and the IgH locus up to several hundred kb. Hum Mol Genet. 1992;1:625–32.

776. Einerson RR, Law ME, Blair HE et al. Novel FISH probes designed to detect IGK-MYC and IGL-MYC rearrangements in B-cell lineage malignancy identify a new breakpoint cluster region designated BVR2. Leukemia. 2006;20:1790–9.

777 Grisendi S, Bernardi R, Rossi M et al. Role of nucleophosmin in embryonic development and tumorigenesis. Nature. 2005;437:147–53.

778. Mrozek K, Dohner H, Bloomfield CD. Influence of new molecular prognostic markers in patients with karyotypically normal acute myeloid leukemia: recent advances. Curr Opin Hematol. 2007;14:106–14.

779. Naoe T, Suzuki T, Kiyoi H, Urano T. Nucleophosmin: a versatile molecule associated with hematological malignancies. Cancer Sci. 2006;97:963–9.

780. Zhang Y, Zhang M, Yang L, Xiao Z. NPM1 mutations in myelodysplastic syndromes and acute myeloid leukemia with normal karyotype. Leuk Res. 2007;31:109–11.

781. Schnittger S, Schoch C, Kern W et al. Nucleophosmin gene mutations are predictors of favorable prognosis in acute myelogenous leukemia with a normal karyotype. Blood. 2005;106:3733–9.

782. Falini B, Mecucci C, Tiacci E et al. Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. N Engl J Med. 2005;352:254–66.

783. Pasqualucci L, Liso A, Martelli MP et al. Mutated nucleophosmin detects clonal multilineage involvement in acute myeloid leukemia: Impact on WHO classification. Blood. 2006;108:4146–55.

784. Caudill JS, Sternberg AJ, Li CY, Tefferi A, Lasho TL, Steensma DP. C-terminal nucleophosmin mutations are uncommon in chronic myeloid disorders. Br J Haematol. 2006;133:638–41.

785. Chen W, Rassidakis GZ, Li J et al. High frequency of NPM1 gene mutations in acute myeloid leukemia with prominent nuclear invaginations ('cuplike'nuclei). Blood. 2006;108:1783–4.

786. Alcalay M, Tiacci E, Bergomas R et al. Acute myeloid leukemia bearing cytoplasmic nucleophosmin (NPMc+ AML) shows a distinct gene expression profile characterized by up-regulation of genes involved in stem-cell maintenance. Blood. 2005;106:899–902.

787. Dohner K, Schlenk RF, Habdank M et al. Mutant nucleophosmin (NPM1) predicts favorable prognosis in younger adults with acute myeloid leukemia and normal cytogenetics: interaction with other gene mutations. Blood. 2005;106:3740–6.

788. Verhaak RG, Goudswaard CS, van Putten W et al. Mutations in nucleophosmin (NPM1) in acute myeloid leukemia (AML): association with other gene abnormalities and previously established gene expression signatures and their favorable prognostic significance. Blood. 2005;106:3747–54.

789. Thiede C, Koch S, Creutzig E et al. Prevalence and prognostic impact of NPM1 mutations in 1485 adult patients with acute myeloid leukemia (AML). Blood. 2006;107:4011–20.

790. Gorello P, Cazzaniga G, Alberti F et al. Quantitative assessment of minimal residual disease in acute myeloid leukemia carrying nucleophosmin (NPM1) gene mutations. Leukemia. 2006;20:1103–8.

791. Falini B, Martelli MP, Bolli N et al. Immunohistochemistry predicts nucleophosmin (NPM) mutations in acute myeloid leukemia. Blood. 2006;108:1999–2005.

792. Brown P, McIntyre E, Rau R et al. The incidence and clinical significance of nucleophosmin mutations in childhood AML. Blood. 2007;110:979–85.

793. Maier H, Hagman J. Roles of EBF and Pax-5 in B lineage commitment and development. Semin Immunol. 2002;14:415–22.

794. Busslinger M, Urbanek P. The role of BSAP (Pax-5) in B-cell development. Curr Opin Genet Dev. 1995;5:595–601.

795. Iida S, Rao PH, Nallasivam P et al. The t(9;14)(p13;q32) chromosomal translocation associated with lymphoplasmacytoid lymphoma involves the PAX-5 gene. Blood. 1996;88:4110–17.

796. Amakawa R, Ohno H, Fukuhara S. t(9;14)(p13;q32) involving the PAX-5 gene: a unique subtype of 14q32 translocation in B cell non-Hodgkin's lymphoma. Int J Hematol. 1999;69:65–9.

797. Morrison AM, Jager U, Chott A, Schebesta M, Haas OA, Busslinger M. Deregulated PAX-5 transcription from a translocated IgH promoter in marginal zone lymphoma. Blood. 1998;92:3865–78.

798. Pardanani A, Brockman SR, Paternoster SF et al. FIP1L1-PDGFRA fusion: prevalence and clinicopathologic correlates in 89 consecutive patients with moderate to severe eosinophilia. Blood. 2004;104:3038–45.

799. Pardanani A, Ketterling RP, Brockman SR et al. CHIC2 deletion, a surrogate for FIP1L1-PDGFRA fusion, occurs in systemic mastocytosis associated with eosinophilia and predicts response to imatinib mesylate therapy. Blood. 2003;102:3093–6.

800. Klion AD, Robyn J, Akin C et al. Molecular remission and reversal of myelofibrosis in response to imatinib mesylate treatment in patients with the myeloproliferative variant of hypereosinophilic syndrome. Blood. 2004;103:473–8.

801. Gotlib J. Molecular classification and pathogenesis of eosinophilic disorders:2005 update. Acta Haematol. 2005;114:7–25.

802. Gotlib J, Cools J, Malone JM 3rd, Schrier SL, Gilliland DG, Coutre SE. The FIP1L1-PDGFRalpha fusion tyrosine kinase in hypereosinophilic syndrome and chronic eosinophilic leukemia: implications for diagnosis, classification, and management. Blood. 2004;103:2879–91.

803. Score J, Curtis C, Waghorn K et al. Identification of a novel imatinib responsive KIF5B-PDGFRA fusion gene following screening for PDGFRA overexpression in patients with hypereosinophilia. Leukemia. 2006;20:827–32.

804. Baxter EJ, Hochhaus A, Bolufer P et al. The t(4;22)(q12;q11) in atypical chronic myeloid leukaemia fuses BCR to PDGFRA. Hum Mol Genet. 2002;11:1391–7.

805. Walz C, Curtis C, Schnittger S et al. Transient response to imatinib in a chronic eosinophilic leukemia associated with ins(9;4)(q33;q12q25) and a CDK5RAP2-PDGFRA fusion gene. Genes Chromosomes Cancer. 2006;45:950–6.

806. Sucic M, Zadro R, Burazer B et al. Acute promyelocytic leukemia M3: cytomorphologic, immunophenotypic, cytogenetic, and molecular variants. J Hematother Stem Cell Res. 2002;11:941–50.

807. Brockman SR, Paternoster SF, Ketterling RP, Dewald GW. New highly sensitive fluorescence in situ hybridization method to detect PML/RARA fusion in acute promyelocytic leukemia. Cancer Genet Cytogenet. 2003;145:144–51.

808. Chen SJ, Zelent A, Tong JH et al. Rearrangements of the retinoic acid receptor alpha and promyelocytic leukemia zinc finger genes resulting from t(11;17)(q23;q21) in a patient with acute promyelocytic leukemia. J Clin Invest. 1993;91:2260–7.

809. Chen Z, Brand NJ, Chen A et al. Fusion between a novel Kruppel-like zinc finger gene and the retinoic acid receptor-alpha locus due to a variant t(11;17) translocation associated with acute promyelocytic leukaemia. Embo J. 1993;12:1161–7.

810. Redner RL, Rush EA, Faas S, Rudert WA, Corey SJ. The t(5;17) variant of acute promyelocytic leukemia expresses a nucleophosmin–retinoic acid receptor fusion. Blood. 1996;87:882–6.

811. Wells RA, Catzavelos C, Kamel-Reid S. Fusion of retinoic acid receptor alpha to NuMA, the nuclear mitotic apparatus protein, by a variant translocation in acute promyelocytic leukaemia. Nat Genet. 1997;17:109–13.

812. Arnould C, Philippe C, Bourdon V, Gr goire MJ, Berger R, Jonveaux P. The signal transducer and activator of transcription STAT5b gene is a new partner of retinoic acid receptor alpha in acute promyelocytic–like leukaemia. Hum Mol Genet. 1999;8:1741–9.

813. Rossi V, Levati L, Biondi A. Diagnosis and monitoring of PML-RARA-positive acute promyelocytic leukemia by

qualitative RT-PCR. Methods Mol Med. 2006; 125:115–26.

814. Gurrieri C, Nafa K, Merghoub T et al. Mutations of the PML tumor suppressor gene in acute promyelocytic leukemia. Blood. 2004;103:2358–62.

815. Caruccio L, Bettinotti M, Director-Myska AE, Arthur DC, Stroncek D. The gene overexpressed in polycythemia rubra vera, PRV-1, and the gene encoding a neutrophil alloantigen, NB1, are alleles of a single gene, CD177, in chromosome band 19q13.31. Transfusion. 2006;46:441–7.

816. Griesshammer M, Klippel S, Strunck E et al. PRV-1 mRNA expression discriminates two types of essential thrombocythemia. Ann Hematol. 2004;83:364–70.

817. Radich JP, Kopecky KJ, Willman CL et al. N-ras mutations in adult de novo acute myelogenous leukemia: prevalence and clinical significance. Blood. 1990;76:801–7.

818. Neubauer A, Dodge RK, George SL et al. Prognostic importance of mutations in the ras proto-oncogenes in de novo acute myeloid leukemia. Blood. 1994;83:1603–11.

819. Coghlan DW, Morley AA, Matthews JP, Bishop JF. The incidence and prognostic significance of mutations in codon 13 of the N-ras gene in acute myeloid leukemia. Leukemia. 1994;8:1682–7.

820. Rasmussen T, Kuehl M, Lodahl M, Johnsen HE, Dahl IM. Possible roles for activating RAS mutations in the MGUS to MM transition and in the intramedullary to extramedullary transition in some plasma cell tumors. Blood. 2005;105:317–23.

821. Intini D, Agnelli L, Ciceri G et al. Relevance of Ras gene mutations in the context of the molecular heterogeneity of multiple myeloma. Hematol Oncol. 2007;25:6–10.

822. Matsuda K, Shimada A, Yoshida N et al. Spontaneous improvement of hematologic abnormalities in patients having juvenile myelomonocytic leukemia with specific RAS mutations. Blood. 2007;109:5477–80.

823. Shaun N, Thomas, B. Cell cycle regulation. In: Degos LL, D.C., Lowenberg B, eds. Textbook of Malignant Hematology, 2nd edn. London and New York: Taylor & Francis, 2005.

824. Godbout R, Dryja TP, Squire J, Gallie BL, Phillips RA. Somatic inactivation of genes on chromosome 13 is a common event in retinoblastoma. Nature. 1983; 304:451–3.

825. Zhu YM, Haynes AP, Keith FJ, Russell NH. Abnormalities of retinoblastoma gene expression in hematological malignancies. Leuk Lymphoma. 1995;18:61–7.

826. Jamal R, Gale RE, Shaun N, Thomas B, Wheatley K, Linch DC. The retinoblastoma gene (rb1) in acute myeloid leukaemia: analysis of gene rearrangements, protein expression and comparison of disease outcome. Br J Haematol. 1996;94:342–51.

827. Sanchez-Beato M, Martinez-Montero JC, Doussis-Anagnostopoulou TA et al. Anomalous retinoblastoma protein expression in Sternberg–Reed cells in Hodgkin's disease: a comparative study with p53 and Ki67 expression. Br J Cancer. 1996;74:1056–62.

828. Krug U, Ganser A, Koeffler HP. Tumor suppressor genes in normal and malignant hematopoiesis. Oncogene. 2002; 21:3475–95.

829. Tsai T, Davalath S, Rankin C et al. Tumor suppressor gene alteration in adult acute lymphoblastic leukemia (ALL). Analysis of retinoblastoma (Rb) and p53 gene expression in lymphoblasts of patients with de novo, relapsed, or refractory ALL treated in Southwest Oncology Group studies. Leukemia. 1996;10:1901–10.

830. Morente MM, Piris MA, Abraira V et al. Adverse clinical outcome in Hodgkin's disease is associated with loss of retinoblastoma protein expression, high Ki67 proliferation index, and absence of Epstein–Barr virus-latent membrane protein 1 expression. Blood. 1997;90:2429–36.

831. Rassidakis GZ, Lai R, Herling M, Cromwell C, Schmitt-Graeff A, Medeiros LJ. Retinoblastoma protein is frequently absent or phosphorylated in anaplastic large-cell lymphoma. Am J Pathol. 2004;164:2259–67.

832. Sauerbrey A, Stammler G, Zintl F, Volm M. Expression of the retinoblastoma tumor suppressor gene (RB-1) in acute leukemia. Leuk Lymphoma. 1998;28:275–83.

833. Dao DD, Sawyer JR, Epstein J, Hoover RG, Barlogie B, Tricot G. Deletion of the retinoblastoma gene in multiple myeloma. Leukemia. 1994;8:1280–4.

834. Wu KL, Beverloo B, Lokhorst HM et al. Abnormalities of chromosome 1p/q are highly associated with chromosome 13/13q deletions and are an adverse prognostic factor for the outcome of high-dose chemotherapy in patients with multiple myeloma. Br J Haematol. 2007;136:615–23.

835. Miyoshi H, Shimizu K, Kozu T, Maseki N, Kaneko Y, Ohki M. t(8;21) breakpoints on chromosome 21 in acute myeloid leukemia are clustered within a limited region of a single gene, AML1. Proc Natl Acad Sci USA. 1991; 88:10431–4.

836. Zhang Y, Emmanuel N, Kamboj G et al. PRDX4, a member of the peroxiredoxin family, is fused to AML1 (RUNX1) in an acute myeloid leukemia patient with a t(X;21)(p22;q22). Genes Chromosomes Cancer. 2004; 40:365–70.

837. Ramsey H, Christopherson K, Hromas R. Forced expression of AML1-AMP19, a fusion transcript generated from

a radiation-associated t(19;21) leukemia, blocks myeloid differentiation. Leuk Res. 2004;28:863–8.

838. Mitani K, Ogawa S, Tanaka T et al. Generation of the AML1-EVI-1 fusion gene in the t(3;21)(q26;q22) causes blastic crisis in chronic myelocytic leukemia. Embo J. 1994;13:504–10.

839. Golub TR, Barker GF, Bohlander SK et al. Fusion of the TEL gene on 12p13 to the AML1 gene on 21q22 in acute lymphoblastic leukemia. Proc Natl Acad Sci USA. 1995;92:4917–21.

840. Gattenloehner S, Chuvpilo S, Langebrake C et al. Novel RUNX1 isoforms determine the fate of acute myeloid leukemia cells by controlling CD56 expression. Blood. 2007;110:2027–33.

841. Jeandidier E, Dastugue N, Mugneret F et al. Abnormalities of the long arm of chromosome 21 in 107 patients with hematopoietic disorders: a collaborative retrospective study of the Groupe Francais de Cytogenetique Hematologique. Cancer Genet Cytogenet. 2006; 166:1–11.

842. Steensma DP, Gibbons RJ, Mesa RA, Tefferi A, Higgs DR. Somatic point mutations in RUNX1/CBFA2/AML1 are common in high-risk myelodysplastic syndrome, but not in myelofibrosis with myeloid metaplasia. Eur J Haematol. 2005;74:47–53.

843. Taketani T, Taki T, Takita J et al. AML1/RUNX1 mutations are infrequent, but related to AML-M0, acquired trisomy 21, and leukemic transformation in pediatric hematologic malignancies. Genes Chromosomes Cancer. 2003;38:1–7.

844. Niimi H, Harada H, Harada Y et al. Hyperactivation of the RAS signaling pathway in myelodysplastic syndrome with AML1/RUNX1 point mutations. Leukemia. 2006;20:635–44.

845. Dicker F, Haferlach C, Kern W, Haferlach T, Schnittger S. Trisomy 13 is strongly associated with AML1/RUNX1 mutations and increased FLT3-expression in acute myeloid leukemia. Blood. 2007;110:1308–16.

846. Lane DP. Cancer. p53, guardian of the genome. Nature. 1992;358:15–6.

847. Lowe SW, Ruley HE, Jacks T, Housman DE. p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. Cell. 1993;74:957–67.

848. Vogelstein B, Kinzler KW. p53 function and dysfunction. Cell. 1992;70:523–6.

849. Imamura J, Miyoshi I, Koeffler HP. p53 in hematologic malignancies. Blood. 1994;84:2412–21.

850. Soussi T, Beroud C. Assessing TP53 status in human tumours to evaluate clinical outcome. Nat Rev Cancer. 2001;1:233–40.

851. Aguilar-Santelises M, Rottenberg ME, Lewin N, Mellstedt H, Jondal M. Bcl-2, Bax and p53 expression in B-CLL in relation to in vitro survival and clinical progression. Int J Cancer. 1996;69:114–19.

852. Dohner H, Fischer K, Bentz M et al. p53 gene deletion predicts for poor survival and non-response to therapy with purine analogs in chronic B-cell leukemias. Blood. 1995;85:1580–9.

853. Sturm I, Bosanquet AG, Hermann S, Guner D, Dorken B, Daniel PT. Mutation of p53 and consecutive selective drug resistance in B-CLL occurs as a consequence of prior DNA-damaging chemotherapy. Cell Death Differ. 2003;10:477–84.

854. Wattel E, Preudhomme C, Hecquet B et al. p53 mutations are associated with resistance to chemotherapy and short survival in hematologic malignancies. Blood. 1994;84:3148–57.

855. Lozanski G, Heerema NA, Flinn IW et al. Alemtuzumab is an effective therapy for chronic lymphocytic leukemia with p53 mutations and deletions. Blood. 2004;103:3278–81.

856. Carter A, Lin K, Sherrington PD, Pettitt AR. Detection of p53 dysfunction by flow cytometry in chronic lymphocytic leukaemia. Br J Haematol. 2004;127:425–8.

857. Baldini L, Guffanti A, Cro L et al. Poor prognosis in nonvillous splenic marginal zone cell lymphoma is associated with p53 mutations. Br J Haematol. 1997;99:375–8.

858. Greiner TC, Moynihan MJ, Chan WC et al. p53 mutations in mantle cell lymphoma are associated with variant cytology and predict a poor prognosis. Blood. 1996;87:4302–10.

859. Sanchez-Beato M, Saez AI, Navas IC et al. Overall survival in aggressive B-cell lymphomas is dependent on the accumulation of alterations in p53, p16, and p27. Am J Pathol. 2001;159:205–13.

860. Moller MB, Ino Y, Gerdes AM, Skjodt K, Louis DN, Pedersen NT. Aberrations of the p53 pathway components p53, MDM2 and CDKN2A appear independent in diffuse large B cell lymphoma. Leukemia. 1999;13:453–9.

861. Ichikawa A, Kinoshita T, Watanabe T et al. Mutations of the p53 gene as a prognostic factor in aggressive B-cell lymphoma. N Engl J Med. 1997;337:529–34.

862. Ichikawa A. Prognostic and predictive significance of p53 mutation in aggressive B-cell lymphoma. Int J Hematol. 2000;71:211–20.

863. Kerbauy F, Colleoni G, Saad S et al. Detection and possible prognostic relevance of p53 gene mutations in diffuse

large B-cell lymphoma. An analysis of 51 cases and review of the literature. Leuk Lymphoma. 2004;45:2071–8.

864. Pescarmona E, Pignoloni P, Puopolo M et al. p53 overexpression identifies a subset of nodal peripheral T-cell lymphomas with a distinctive biological profile and poor clinical outcome. J Pathol. 2001;195:361–6.

865. Menssen HD, Siehl JM, Thiel E. Wilms tumor gene (WT1) expression as a panleukemic marker. Int J Hematol. 2002;76:103–9.

866. Sugiyama H. Wilms' tumor gene WT1: its oncogenic function and clinical application. Int J Hematol. 2001;73:177–87.

867. Sugiyama H. Wilms tumor gene WT1 as a tumor marker for leukemic blast cells and its role in leukemogenesis. Methods Mol Med. 2002;68:223–37.

868. Yang L, Han Y, Saurez Saiz F, Minden MD. A tumor suppressor and oncogene: the WT1 story. Leukemia. 2007;21:868–76.

869. Svedberg H, Richter J, Gullberg U. Forced expression of the Wilms tumor 1 (WT1) gene inhibits proliferation of human hematopoietic CD34(+) progenitor cells. Leukemia. 2001;15:1914–22.

870. Ellisen LW, Carlesso N, Cheng T, Scadden DT, Haber DA. The Wilms tumor suppressor WT1 directs stage-specific quiescence and differentiation of human hematopoietic progenitor cells. Embo J. 2001;20:1897–909.

871. King-Underwood L, Renshaw J, Pritchard-Jones K. Mutations in the Wilms' tumor gene WT1 in leukemias. Blood. 1996;87:2171–9.

872 Bergmann L, Maurer U, Weidmann E. Wilms tumor gene expression in acute myeloid leukemias. Leuk Lymphoma. 1997;25:435–43.

873. Cilloni D, Gottardi E, De Micheli D et al. Quantitative assessment of WT1 expression by real time quantitative PCR may be a useful tool for monitoring minimal residual disease in acute leukemia patients. Leukemia. 2002;16:2115–21.

874. Cilloni D, Saglio G. WT1 as a universal marker for minimal residual disease detection and quantification in myeloid leukemias and in myelodysplastic syndrome. Acta Haematol. 2004;112:79–84.

875. Trka J, Kalinova M, Hrusak O et al. Real-time quantitative PCR detection of WT1 gene expression in children with AML: prognostic significance, correlation with disease status and residual disease detection by flow cytometry. Leukemia. 2002;16:1381–9.

876. Schmid D, Heinze G, Linnerth B et al. Prognostic significance of WT1 gene expression at diagnosis in adult de novo acute myeloid leukemia. Leukemia. 1997; 11:639–43.

877 Garg M, Moore H, Tobal K, Liu Yin JA. Prognostic significance of quantitative analysis of WT1 gene transcripts by competitive reverse transcription polymerase chain reaction in acute leukaemia. Br J Haematol. 2003;123:49–59.

878. Barragan E, Cervera J, Bolufer P et al. Prognostic implications of Wilms' tumor gene (WT1) expression in patients with de novo acute myeloid leukemia. Haematologica. 2004;89:926–33.

879. Gaiger A, Linnerth B, Mann G et al. Wilms' tumour gene (wt1) expression at diagnosis has no prognostic relevance in childhood acute lymphoblastic leukaemia treated by an intensive chemotherapy protocol. Eur J Haematol. 1999;63:86–93.

880. Miwa H, Beran M, Saunders GF. Expression of the Wilms' tumor gene (WT1) in human leukemias. Leukemia. 1992;6:405–9.

881. Menssen HD, Renkl HJ, Rodeck U et al. Presence of Wilms' tumor gene (wt1) transcripts and the WT1 nuclear protein in the majority of human acute leukemias. Leukemia. 1995;9:1060–7.

882. Galimberti S, Guerrini F, Carulli G et al. Significant coexpression of WT1 and MDR1 genes in acute myeloid leukemia patients at diagnosis. Eur J Haematol. 2004;72:45–51.

883. Ostergaard M, Olesen LH, Hasle H, Kjeldsen E, Hokland P. WT1 gene expression: an excellent tool for monitoring minimal residual disease in 70% of acute myeloid leukaemia patients – results from a single-centre study. Br J Haematol. 2004;125:590–600.

884. Kreuzer KA, Saborowski A, Lupberger J et al. Fluorescent 5'-exonuclease assay for the absolute quantification of Wilms' tumour gene (WT1) mRNA: implications for monitoring human leukaemias. Br J Haematol. 2001;114:313–18.

885. Bagg A, Kallakury BV. Molecular pathology of leukemia and lymphoma. Am J Clin Pathol. 1999;112:S76-92.

886. Arber DA. Molecular diagnostic approach to non-Hodgkin's lymphoma. J Mol Diagn. 2000;2:178–90.

887. Hummel M, Bentink S, Berger H et al. A biologic definition of Burkitt's lymphoma from transcriptional and genomic profiling. N Engl J Med. 2006;354:2419–30.

888. Dave SS, Fu K, Wright GW et al. Molecular diagnosis of Burkitt's lymphoma. N Engl J Med. 2006;354:2431–42.

889. Fenaux P, Preudhomme C, Lai JL, Morel P, Beuscart R, Bauters F. Cytogenetics and their prognostic value in de novo acute myeloid leukaemia: a report on 283 cases. Br J Haematol. 1989;73:61–7.

890. Pui CH, Crist WM, Look AT. Biology and clinical significance of cytogenetic abnormalities in childhood acute lymphoblastic leukemia. Blood. 1990;76:1449–63.

891. VinSheth FJ, Sheth JJ, Patel AI, Shah AD, Verhest A. Usefulness of cytogenetics in leukemias. Indian J Cancer. 2002;39:139–42.

892. Bain BJ. Cytogenetic and molecular genetic aspects of eosinophilic leukaemias. Br J Haematol. 2003;122:173–9.

893. Caligiuri MA, Strout MP, Gilliland DG. Molecular biology of acute myeloid leukemia. Semin Oncol. 1997;24:32–44.

894 Campo E. Genetic and molecular genetic studies in the diagnosis of B-cell lymphomas I: mantle cell lymphoma, follicular lymphoma, and Burkitt's lymphoma. Hum Pathol. 2003;34:330–5.

895. Capello D, Cerri M, Muti G et al. Molecular histogenesis of posttransplantation lymphoproliferative disorders. Blood. 2003;102:3775–85.

896. Dohner H, Stilgenbauer S, Dohner K, Bentz M, Lichter P. Chromosome aberrations in B-cell chronic lymphocytic leukemia: reassessment based on molecular cytogenetic analysis. J Mol Med. 1999;77:266–281.

897. Dybkaer K, Iqbal J, Zhou G, Chan WC. Molecular diagnosis and outcome prediction in diffuse large B-cell lymphoma and other subtypes of lymphoma. Clin Lymphoma. 2004;5:19–28.

898. Graux C, Cools J, Michaux L, Vandenberghe P, Hagemeijer A. Cytogenetics and molecular genetics of T-cell acute lymphoblastic leukemia: from thymocyte to lymphoblast. Leukemia. 2006;20:1496–510.

899 Grimwade D, Lo Coco F. Acute promyelocytic leukemia: a model for the role of molecular diagnosis and residual disease monitoring in directing treatment approach in acute myeloid leukemia. Leukemia. 2002;16:1959–73.

900. Medeiros LJ, Carr J. Overview of the role of molecular methods in the diagnosis of malignant lymphomas. Arch Pathol Lab Med. 1999;123:1189–207.

901. Meyer-Monard S, Parlier V, Passweg J et al. Combination of broad molecular screening and cytogenetic analysis for genetic risk assignment and diagnosis in patients with acute leukemia. Leukemia. 2006;20:247–53.

902. Westbrook CA. The role of molecular techniques in the clinical management of leukemia. Lessons from the Philadelphia chromosome. Cancer. 1992;70:1695–700.

903 Zhan F, Huang Y, Colla S et al. The molecular classification of multiple myeloma. Blood. 2006;108:2020–8.

904. Delabesse E, Burtin ML, Millien C et al. Rapid, multifluorescent TCRG Vgamma and Jgamma typing: application to T cell acute lymphoblastic leukemia and to the detection of minor clonal populations. Leukemia. 2000;14:1143–52.

905. Fodinger M, Winkler K, Mannhalter C, Chott A. Combined polymerase chain reaction approach for clonality detection in lymphoid neoplasms. Diagn Mol Pathol. 1999;8:80–91.

906. Meier VS, Rufle A, Gudat F. Simultaneous evaluation of T- and B-cell clonality, t(11;14) and t(14;18), in a single reaction by a four-color multiplex polymerase chain reaction assay and automated high-resolution fragment analysis: a method for the rapid molecular diagnosis of lymphoproliferative disorders applicable to fresh frozen and formalin-fixed, paraffin-embedded tissues, blood, and bone marrow aspirates. Am J Pathol. 2001;159:2031–43.

907. Evans PA, Pott C, Groenen PJ et al. Significantly improved PCR-based clonality testing in B-cell malignancies by use of multiple immunoglobulin gene targets. Report of the BIOMED-2 Concerted Action BHM4-CT98-3936. Leukemia. 2007;21:207–14.

908. Bruggemann M, White H, Gaulard P et al. Powerful strategy for polymerase chain reaction-based clonality assessment in T-cell malignancies Report of the BIOMED-2 Concerted Action BHM4 CT98-3936. Leukemia. 2007;21:215–21.

909. Basecke J, Griesinger F, Trumper L, Brittinger G. Leukemia- and lymphoma-associated genetic aberrations in healthy individuals. Ann Hematol. 2002;81:64–75.

910. Gruppioni E, Corti B, Altimari A et al. Application of a fluorescent PCR method for molecular diagnosis of post-transplant lymphoproliferative disorders on routine tissue sections. Diagn Mol Pathol. 2005;14:170–6.

911. Knowles DM, Cesarman E, Chadburn A et al. Correlative morphologic and molecular genetic analysis demonstrates three distinct categories of posttransplantation lymphoproliferative disorders. Blood. 1995;85:552–65.

912. Bagg A, Braziel RM, Arber DA, Bijwaard KE, Chu AY. Immunoglobulin heavy chain gene analysis in lymphomas: a multi-center study demonstrating the heterogeneity of performance of polymerase chain reaction assays. J Mol Diagn. 2002;4:81–9.

913. Derksen PW, Langerak AW, Kerkhof E et al. Comparison of different polymerase chain reaction-based approaches for clonality assessment of immunoglobulin heavy-chain gene rearrangements in B-cell neoplasia. Mod Pathol. 1999;12:794–805.

914. Diaz-Cano S. PCR-based alternative for diagnosis of immunoglobulin heavy chain gene rearrangement: principles, practice, and polemics. Diagn Mol Pathol. 1996;5:3–9.

915. Theriault C, Galoin S, Valmary S et al. PCR analysis of immunoglobulin heavy chain (IgH) and TcR-gamma chain gene rearrangements in the diagnosis of lymphoproliferative disorders: results of a study of 525 cases. Mod Pathol. 2000;13:1269–79.

916. van Dongen JJ, Langerak AW, Bruggemann M et al. Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: report of the BIOMED-2 Concerted Action BMH4-CT98-3936. Leukemia. 2003;17:2257–317.

917. Assaf C, Hummel M, Steinhoff M et al. Early TCR-beta and TCR-gamma PCR detection of T-cell clonality indicates minimal tumor disease in lymph nodes of cutaneous T-cell lymphoma: diagnostic and prognostic implications. Blood. 2005;105:503–10.

918. Diss TC, Pan L, Peng H, Wotherspoon AC, Isaacson PG. Sources of DNA for detecting B cell monoclonality using PCR. J Clin Pathol. 1994;47:493–6.

919. Miller JE, Wilson SS, Jaye DL, Kronenberg M. An automated semiquantitative B and T cell clonality assay. Mol Diagn. 1999;4:101–17.

920. Segal GH, Jorgensen T, Masih AS, Braylan RC. Optimal primer selection for clonality assessment by polymerase chain reaction analysis: I. Low grade B-cell lymphoproliferative disorders of nonfollicular center cell type. Hum Pathol. 1994;25:1269–75.

921. Segal GH, Jorgensen T, Scott M, Braylan RC. Optimal primer selection for clonality assessment by polymerase chain reaction analysis: II. Follicular lymphomas. Hum Pathol. 1994;25:1276–82.

922. Achille A, Scarpa A, Montresor M et al. Routine application of polymerase chain reaction in the diagnosis of monoclonality of B-cell lymphoid proliferations. Diagn Mol Pathol. 1995;4:14–24.

923. Galimberti S, Brizzi F, Mameli M, Petrini M. An advantageous method to evaluate IgH rearrangement and its role in minimal residual disease detection. Leuk Res. 1999;23:921–9.

924. Lozano MD, Tierens A, Greiner TC, Wickert RS, Weisenburger DD, Chan WC. Clonality analysis of B-lymphoid proliferations using the polymerase chain reaction. Cancer. 1996;77:1349–55.

925. Hoeve MA, Krol AD, Philippo K et al. Limitations of clonality analysis of B cell proliferations using CDR3 polymerase chain reaction. Mol Pathol. 2000;53:194–200.

926. Tonegawa S. Somatic generation of antibody diversity. Nature. 1983;302:575–81.

927. Kuppers R, Goossens T, Klein U. The role of somatic hypermutation in the generation of deletions and duplications in human Ig V region genes and chromosomal translocations. Curr Top Microbiol Immunol. 1999;246:193–8.

928. Gonzalez D, Garcia-Sanz R. Incomplete DJH rearrangements. Methods Mol Med. 2005;113:165–73.

929. Abdel-Reheim FA, Edwards E, Arber DA. Utility of a rapid polymerase chain reaction panel for the detection of molecular changes in B-cell lymphoma. Arch Pathol Lab Med. 1996;120:357–63.

930. Gong JZ, Zheng S, Chiarle R et al. Detection of immunoglobulin kappa light chain rearrangements by polymerase chain reaction. An improved method for detecting clonal B-cell lymphoproliferative disorders. Am J Pathol. 1999;155:355–63.

931. Signoretti S, Murphy M, Cangi MG, Puddu P, Kadin ME, Loda M. Detection of clonal T-cell receptor gamma gene rearrangements in paraffin-embedded tissue by polymerase chain reaction and nonradioactive single-strand conformational polymorphism analysis. Am J Pathol. 1999;154:67–75.

932. Dictor M, Warenholt J, Isinger A. Resolving T-cell receptor clonality in two and genotype in four multiplex polymerase chain reactions. Haematologica. 2005;90:1524–32.

933. Cairns SM, Taylor JM, Gould PR, Spagnolo DV. Comparative evaluation of PCR-based methods for the assessment of T cell clonality in the diagnosis of T cell lymphoma. Pathology. 2002;34:320–5.

934. Greiner TC. Advances in molecular hematopathology: T-cell receptor gamma and bcl-2 genes. Am J Pathol. 1999;154:7–9.

935. Luo V, Lessin SR, Wilson RB et al. Detection of clonal T-cell receptor gamma gene rearrangements using fluorescent-based PCR and automated high-resolution capillary electrophoresis. Mol Diagn. 2001;6:169–79.

936. Lee SC, Berg KD, Racke FK, Griffin CA, Eshleman JR. Pseudo-spikes are common in histologically benign lymphoid tissues. J Mol Diagn. 2000;2:145–52.

937. Holm N, Flaig MJ, Yazdi AS, Sander CA. The value of molecular analysis by PCR in the diagnosis of cutaneous lymphocytic infiltrates. J Cutan Pathol. 2002;29:447–52.

938. Ashton-Key M, Diss TC, Pan L, Du MQ, Isaacson PG. Molecular analysis of T-cell clonality in ulcerative jejunitis and enteropathy-associated T-cell lymphoma. Am J Pathol. 1997;151:493–8.

939. Delfau-Larue MH, Laroche L, Wechsler J et al. Diagnostic value of dominant T-cell clones in peripheral blood in 363 patients presenting consecutively with a

clinical suspicion of cutaneous lymphoma. Blood. 2000;96:2987–92.

940. Kolowos W, Herrmann M, Ponner BB et al. Detection of restricted junctional diversity of peripheral T cells in SLE patients by spectratyping. Lupus. 1997;6:701–7.

941 Hall FC, Thomson K, Procter J, McMichael AJ, Wordsworth BP. TCR beta spectratyping in RA: evidence of clonal expansions in peripheral blood lymphocytes. Ann Rheum Dis. 1998;57:319–22.

942. Wack A, Cossarizza A, Heltai S et al. Age-related modifications of the human alphabeta T cell repertoire due to different clonal expansions in the CD4+ and CD8+ subsets. Int Immunol. 1998;10:1281–8.

943. Schwab R, Szabo P, Manavalan JS et al. Expanded CD4+ and CD8+ T cell clones in elderly humans. J Immunol. 1997;158:4493–9.

944. Posnett DN, Sinha R, Kabak S, Russo C. Clonal populations of T cells in normal elderly humans: the T cell equivalent to 'benign monoclonal gammapathy'. J Exp Med. 1994;179:609–18.

945. Lantelme E, Granziero L, Angman L, Giachino C. Clonal predominance, but preservation of a polyclonal reservoir, in the normal alpha beta T-cell repertoire. Hum Immunol. 1997;53:49–56.

946. Chamberlain WD, Falta MT, Kotzin BL. Functional subsets within clonally expanded CD8(+) memory T cells in elderly humans. Clin Immunol. 2000;94:160–72.

947. Ponti R, Quaglino P, Novelli M et al. T-cell receptor gamma gene rearrangement by multiplex polymerase chain reaction/heteroduplex analysis in patients with cutaneous T-cell lymphoma (mycosis fungoides/Sezary syndrome) and benign inflammatory disease: correlation with clinical, histological and immunophenotypical findings. Br J Dermatol. 2005;153:565–73.

948 Sabnani I, Zucker MJ, Tsang P, Palekar S. Clonal T-large granular lymphocyte proliferation in solid organ transplant recipients. Transplant Proc. 2006;38:3437–40.

949. Bernasconi P, Alessandrino EP, Boni M et al. Karyotype in myelodysplastic syndromes: relations to morphology, clinical evolution, and survival. Am J Hematol. 1994;46:270–7.

950. Greenberg P, Cox C, LeBeau MM et al. International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood. 1997;89:2079–88.

951. Howe RB, Porwit-MacDonald A, Wanat R, Tehranchi R, Hellstrom-Lindberg E. The WHO classification of MDS does make a difference. Blood. 2004;103:3265–70.

952. Wattel E, Lai JL, Hebbar M et al. De novo myelodysplastic syndrome (MDS) with deletion of the long arm of chromosome 20: a subtype of MDS with distinct hematological and prognostic features? Leuk Res. 1993;17:921–6.

953. Aul C, Gattermann N, Heyll A, Germing U, Derigs G, Schneider W. Primary myelodysplastic syndromes: analysis of prognostic factors in 235 patients and proposals for an improved scoring system. Leukemia. 1992;6:52–9.

954. Bennett JM, Kouides PA, Forman SJ. The myelodysplastic syndromes: morphology, risk assessment, and clinical management (2002). Int J Hematol. 2002;76(Suppl 2):228–38.

955. Greenberg PL, Sanz GF, Sanz MA. Prognostic scoring systems for risk assessment in myelodysplastic syndromes. Forum (Genova). 1999;9:17–31.

956. Morel P, Hebbar M, Lai JL et al. Cytogenetic analysis has strong independent prognostic value in de novo myelodysplastic syndromes and can be incorporated in a new scoring system: a report on 408 cases. Leukemia. 1993;7:1315–23.

957. Sanz GF, Sanz MA, Greenberg PL. Prognostic factors and scoring systems in myelodysplastic syndromes. Haematologica. 1998;83:358–68.

968. Wu HK, Kitamura K, Xi YR, Wang YT, Liu SL, Zhou DB. Cytogenetic analysis of bone marrow from patients with primary myelodysplastic syndrome. J Int Med Res. 1992;20:254–66.

959. Matutes E, Oscier D, Garcia-Marco J et al. Trisomy 12 defines a group of CLL with atypical morphology: correlation between cytogenetic, clinical and laboratory features in 544 patients. Br J Haematol. 1996;92:382–8.

960. Neilson JR, Auer R, White D et al. Deletions at 11q identify a subset of patients with typical CLL who show consistent disease progression and reduced survival. Leukemia. 1997;11:1929–32.

961. Trbusek M, Malcikova J, Smardova J et al. Inactivation of p53 and deletion of ATM in B-CLL patients in relation to IgVH mutation status and previous treatment. Leukemia. 2006;20:1159–61.

962. Caligaris-Cappio F. Biology of chronic lymphocytic leukemia. Rev Clin Exp Hematol. 2000;4:5–21.

963. Hamblin TJ, Davis Z, Gardiner A, Oscier DG, Stevenson FK. Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. Blood. 1999;94:1848–54.

964. Han T, Henderson ES, Emrich LJ, Sandberg AA. Prognostic significance of karyotypic abnormalities in B cell chronic lymphocytic leukemia: an update. Semin Hematol. 1987;24:257–63.

965. Juliusson G, Oscier DG, Fitchett M et al. Prognostic subgroups in B-cell chronic lymphocytic leukemia defined by

specific chromosomal abnormalities. N Engl J Med. 1990;323:720–4.

966. Mandelli F, De Rossi G, Mancini P et al. Prognosis in chronic lymphocytic leukemia: a retrospective multicentric study from the GIMEMA group. J Clin Oncol. 1987;5:398–406.

967. Mayr C, Speicher MR, Kofler DM et al. Chromosomal translocations are associated with poor prognosis in chronic lymphocytic leukemia. Blood. 2006;107:742–51.

968. Ripolles L, Ortega M, Ortuno F et al. Genetic abnormalities and clinical outcome in chronic lymphocytic leukemia. Cancer Genet Cytogenet. 2006;171:57–64.

969. Stilgenbauer S, Bullinger L, Lichter P, Dohner H. Genetics of chronic lymphocytic leukemia: genomic aberrations and V(H) gene mutation status in pathogenesis and clinical course. Leukemia. 2002;16:993–1007.

970. van der Velden VH, Hochhaus A, Cazzaniga G, Szczepanski T, Gabert J, van Dongen JJ. Detection of minimal residual disease in hematologic malignancies by real-time quantitative PCR: principles, approaches, and laboratory aspects. Leukemia. 2003;17:1013–34.

971. Gabert J, Beillard E, van der Velden VH et al. Standardization and quality control studies of 'real-time' quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia – a Europe Against Cancer program. Leukemia. 2003;17:2318–57.

972. Venditti A, Maurillo L, Buccisano F et al. Multidimensional flow cytometry for detection of minimal residual disease in acute myeloid leukemia. Leuk Lymphoma. 2003;44:445–50.

973. San-Miguel JF, Vidriales MB, Orfao A. Immunological evaluation of minimal residual disease (MRD) in acute myeloid leukaemia (AML). Best Pract Res Clin Haematol. 2002;15:105–18.

974. Van Dongen JJM, Szczepanski, T. et al. Detection of minimal residual disease in lymphoid malignancies. In: Degos L, Linch, D. C., Lowenberg, B., eds. Textbook of Malignant Hematology, (2nd edn). London and New York: Taylor & Francis; 2005:267–307.

975. Esteve J, Villamor N,Colomer D et al. Different clinical value of minimal residual disease after autologous and allogeneic stem cell transplantation for chronic lymphocytic leukemia. Blood. 2002;99:1873–4.

976. Maloum K, Sutton L, Baudet S et al. Novel flow-cytometric analysis based on BCD5+ subpopulations for the evaluation of minimal residual disease in chronic lymphocytic leukaemia. Br J Haematol. 2002;119:970–5.

977. Rawstron AC, Kennedy B, Evans PA et al. Quantitation of minimal disease levels in chronic lymphocytic leukemia using a sensitive flow cytometric assay improves the prediction of outcome and can be used to optimize therapy. Blood. 2001;98:29–35.

978. Thiede C. Diagnostic chimerism analysis after allogeneic stem cell transplantation: new methods and markers. Am J Pharmacogenomics. 2004;4:177–87.

979. Thiede C, Bornhauser M, Ehninger G. Strategies and clinical implications of chimerism diagnostics after allogeneic hematopoietic stem cell transplantation. Acta Haematol. 2004;112:16–23.

980. van Dongen JJ, Breit TM, Adriaansen HJ, Beishuizen A, Hooijkaas H. Detection of minimal residual disease in acute leukemia by immunological marker analysis and polymerase chain reaction. Leukemia. 1992;6(Suppl 1):47–59.

981. van der Velden VH, Boeckx N, van Wering ER, van Dongen JJ. Detection of minimal residual disease in acute leukemia. J Biol Regul Homeost Agents. 2004; 18:146–54.

982. Meyer C, Schneider B, Reichel M et al. Diagnostic tool for the identification of MLL rearrangements including unknown partner genes. Proc Natl Acad Sci USA. 2005;102:449–54.

983. Khouri IF, Keating MJ, Saliba RM, Champlin RE. Long-term follow-up of patients with CLL treated with allogeneic hematopoietic transplantation. Cytotherapy. 2002;4:217–21.

984. Magnac C, Sutton L, Cazin B et al. Detection of minimal residual disease in B chronic lymphocytic leukemia (CLL). Hematol Cell Ther. 1999;41:13–18.

985. Campana D, Coustan-Smith E. Minimal residual disease studies by flow cytometry in acute leukemia. Acta Haematol. 2004;112:8–15.

986. Bruggemann M, Pott C, Ritgen M, Kneba M. Significance of minimal residual disease in lymphoid malignancies. Acta Haematol. 2004;112:111–19.

987. Mauro FR, Foa R, Giannarelli D et al. Clinical characteristics and outcome of young chronic lymphocytic leukemia patients: a single institution study of 204 cases. Blood. 1999;94:448–54.

988. Keating MJ, O'Brien S, Albitar M et al. Early results of a chemoimmunotherapy regimen of fludarabine, cyclophosphamide, and rituximab as initial therapy for chronic lymphocytic leukemia. J Clin Oncol. 2005;23:4079–88.

989. Wierda W, O'Brien S, Wen S et al. Chemoimmunotherapy with fludarabine, cyclophosphamide, and rituximab for

relapsed and refractory chronic lymphocytic leukemia. J Clin Oncol. 2005;23:4070–78.

990. Montserrat E. Treatment of chronic lymphocytic leukemia: achieving minimal residual disease-negative status as a goal. J Clin Oncol. 2005;23:2884–5.

991. Montserrat E, Moreno C, Esteve J, Urbano-Ispizua A, Gine E, Bosch F. How I treat refractory CLL. Blood. 2006;107:1276–83.

992. Moreton P, Kennedy B, Lucas G et al. Eradication of minimal residual disease in B-cell chronic lymphocytic leukemia after alemtuzumab therapy is associated with prolonged survival. J Clin Oncol. 2005;23:2971–9.

993. Robertson LE, Huh YO, Butler JJ et al. Response assessment in chronic lymphocytic leukemia after fludarabine plus prednisone: clinical, pathologic, immunophenotypic, and molecular analysis. Blood. 1992;80:29–36.

994. Esteve J, Villamor N, Colomer D et al. Stem cell transplantation for chronic lymphocytic leukemia: different outcome after autologous and allogeneic transplantation and correlation with minimal residual disease status. Leukemia. 2001;15:445–51.

995. Provan D, Bartlett-Pandite L, Zwicky C et al. Eradication of polymerase chain reaction-detectable chronic lymphocytic leukemia cells is associated with improved outcome after bone marrow transplantation. Blood. 1996;88:2228–35.

996. Milligan DW, Fernandes S, Dasgupta R et al. Results of the MRC pilot study show autografting for younger patients with chronic lymphocytic leukemia is safe and achieves a high percentage of molecular responses. Blood. 2005;105:397–404.

997. Pfitzner T, Engert A, Wittor H et al. A real-time PCR assay for the quantification of residual malignant cells in B cell chronic lymphatic leukemia. Leukemia. 2000;14:754–66.

998. Bottcher S, Ritgen M, Pott C et al. Comparative analysis of minimal residual disease detection using four-color flow cytometry, consensus IgH-PCR, and quantitative IgH PCR in CLL after allogeneic and autologous stem cell transplantation. Leukemia. 2004;18:1637–45.

999. Noy A, Verma R, Glenn M et al. Clonotypic polymerase chain reaction confirms minimal residual disease in CLL nodular PR: results from a sequential treatment CLL protocol. Blood. 2001;97:1929–36.

1000. Moreno C, Villamor N, Colomer D et al. Clinical significance of minimal residual disease, as assessed by different techniques, after stem cell transplantation for chronic lymphocytic leukemia. Blood. 2006;107:4563–9.

1001. Doney KC, Chauncey T, Appelbaum FR. Allogeneic related donor hematopoietic stem cell transplantation for treatment of chronic lymphocytic leukemia. Bone Marrow Transplant. 2002;29:817–23.

1002. Pavletic ZS, Arrowsmith ER, Bierman PJ et al. Outcome of allogeneic stem cell transplantation for B cell chronic lymphocytic leukemia. Bone Marrow Transplant. 2000;25:717–22.

1003. Ritgen M, Stilgenbauer S, von Neuhoff N et al. Graft-versus-leukemia activity may overcome therapeutic resistance of chronic lymphocytic leukemia with unmutated immunoglobulin variable heavy-chain gene status: implications of minimal residual disease measurement with quantitative PCR. Blood. 2004;104:2600–2.

1004. Mattsson J, Uzunel M, Remberger M et al. Minimal residual disease is common after allogeneic stem cell transplantation in patients with B cell chronic lymphocytic leukemia and may be controlled by graft-versus-host disease. Leukemia. 2000;14:247–54.

1005. Rawstron AC, Kennedy B, Moreton P et al. Early prediction of outcome and response to alemtuzumab therapy in chronic lymphocytic leukemia. Blood. 2004;103:2027–31.

1006. Mandigers CM, Meijerink JP, Mensink EJ et al. Lack of correlation between numbers of circulating t(14;18)-positive cells and response to first-line treatment in follicular lymphoma. Blood. 2001;98:940–4.

1007. Lambrechts AC, Hupkes PE, Dorssers LC, van't Veer MB. Clinical significance of t(14; 18)-positive cells in the circulation of patients with stage III or IV follicular non-Hodgkin's lymphoma during first remission. J Clin Oncol. 1994;12:1541–6.

1008. Lopez-Guillermo A, Cabanillas F, McLaughlin P et al. The clinical significance of molecular response in indolent follicular lymphomas. Blood. 1998;91:2955–60.

1009. Zwicky CS, Maddocks AB, Andersen N, Gribben JG. Eradication of polymerase chain reaction detectable immunoglobulin gene rearrangement in non-Hodgkin's lymphoma is associated with decreased relapse after autologous bone marrow transplantation. Blood. 1996;88:3314–22.

1010. Jacquy C, Lambert F, Soree A et al. Peripheral blood stem cell contamination in mantle cell non-Hodgkin lymphoma: the case for purging? Bone Marrow Transplant. 1999;23:681–6.

1011. Andersen NS, Donovan JW, Borus JS et al. Failure of immunologic purging in mantle cell lymphoma assessed by polymerase chain reaction detection of minimal residual disease. Blood. 1997;90:4212–21.

1012. Howard OM, Gribben JG, Neuberg DS et al. Rituximab and CHOP induction therapy for newly diagnosed

mantle-cell lymphoma: molecular complete responses are not predictive of progression-free survival. J Clin Oncol. 2002;20:1288–94.

1013. Brugger W, Hirsch J, Grunebach F et al. Rituximab consolidation after high-dose chemotherapy and autologous blood stem cell transplantation in follicular and mantle cell lymphoma: a prospective, multicenter phase II study. Ann Oncol. 2004;15:1691–8.

1014. Lin P, Owens R, Tricot G, Wilson CS. Flow cytometric immunophenotypic analysis of 306 cases of multiple myeloma. Am J Clin Pathol. 2004;121:482–8.

1015. Barbui AM, Galli M, Dotti G et al. Negative selection of peripheral blood stem cells to support a tandem autologous transplantation programme in multiple myeloma. Br J Haematol. 2002;116:202–10.

1016. Corradini P, Voena C, Tarella C et al. Molecular and clinical remissions in multiple myeloma: role of autologous and allogeneic transplantation of hematopoietic cells. J Clin Oncol. 1999;17:208–15.

1017. Martinelli G, Terragna C, Zamagni E et al. Molecular remission after allogeneic or autologous transplantation of hematopoietic stem cells for multiple myeloma. J Clin Oncol. 2000;18:2273–81.

1018. Bjorkstrand B, Ljungman P, Bird JM, Samson D, Gahrton G. Double high-dose chemoradiotherapy with autologous stem cell transplantation can induce molecular remissions in multiple myeloma. Bone Marrow Transplant. 1995;15:367–71.

1019. Corradini P, Cavo M, Lokhorst H et al. Molecular remission after myeloablative allogeneic stem cell transplantation predicts a better relapse-free survival in patients with multiple myeloma. Blood. 2003;102:1927–9.

1020. Cavo M, Terragna C, Martinelli G et al. Molecular monitoring of minimal residual disease in patients in long-term complete remission after allogeneic stem cell transplantation for multiple myeloma. Blood. 2000;96:355–7.

1021. Martinelli G, Terragna C, Zamagni E et al. Polymerase chain reaction-based detection of minimal residual disease in multiple myeloma patients receiving allogeneic stem cell transplantation. Haematologica. 2000;85:930–4.

1022. Raab MS, Cremer FW, Breitkreutz IN et al. Molecular monitoring of tumour load kinetics predicts disease progression after non-myeloablative allogeneic stem cell transplantation in multiple myeloma. Ann Oncol. 2005;16:611–17.

1023. Matsushita M, Yamazaki R, Ikeda H, Kawakami Y. Preferentially expressed antigen of melanoma (PRAME) in the development of diagnostic and therapeutic methods for hematological malignancies. Leuk Lymphoma. 2003;44:439–44.

1024. Cazzaniga G, Dell'Oro MG, Mecucci C et al. Nucleophosmin mutations in childhood acute myelogenous leukemia with normal karyotype. Blood. 2005;106:1419–22.

1025. San Miguel JF, Vidriales MB, Lopez-Berges C et al. Early immunophenotypical evaluation of minimal residual disease in acute myeloid leukemia identifies different patient risk groups and may contribute to postinduction treatment stratification. Blood. 2001;98:1746–51.

1026. Feller N, van der Pol MA, van Stijn A et al. MRD parameters using immunophenotypic detection methods are highly reliable in predicting survival in acute myeloid leukaemia. Leukemia. 2004;18:1380–90.

1027. Kern W, Voskova D, Schnittger S, Schoch C, Hiddemann W, Haferlach T. Four-fold staining including CD45 gating improves the sensitivity of multiparameter flow cytometric assessment of minimal residual disease in patients with acute myeloid leukemia. Hematol J. 2004;5:410–18.

1028. Venditti A, Buccisano F, Del Poeta G et al. Level of minimal residual disease after consolidation therapy predicts outcome in acute myeloid leukemia. Blood. 2000; 96:3948–52.

1029. Lo-Coco F, Diverio D, Pandolfi PP et al. Molecular evaluation of residual disease as a predictor of relapse in acute promyelocytic leukaemia. Lancet. 1992;340:1437–38.

1030. Burnett AK, Grimwade D, Solomon E, Wheatley K, Goldstone AH. Presenting white blood cell count and kinetics of molecular remission predict prognosis in acute promyelocytic leukemia treated with all-trans retinoic acid: result of the Randomized MRC Trial. Blood. 1999;93:4131–43.

1031. Grimwade D. The pathogenesis of acute promyelocytic leukaemia: evaluation of the role of molecular diagnosis and monitoring in the management of the disease. Br J Haematol. 1999;106:591–613.

1032. Diverio D, Pandolfi PP, Rossi V, Biondi A, Pelicci PG, Lo Coco F. Monitoring of treatment outcome in acute promyelocytic leukemia by RT-PCR. Leukemia. 1994; 8:1105–7.

1033. Lo-Coco F, Breccia M, Diverio D. The importance of molecular monitoring in acute promyelocytic leukaemia. Best Pract Res Clin Haematol. 2003;16:503–20.

1034. Hu J, Shen ZX, Sun GL, Chen SJ, Wang ZY, Chen Z. Long-term survival and prognostic study in acute promyelocytic leukemia treated with all-trans-retinoic

acid, chemotherapy, and As2O3: an experience of 120 patients at a single institution. Int J Hematol. 1999; 70:248–60.

1035. Martinelli G, Remiddi C, Visani G et al. Molecular analysis of PML-RAR alpha fusion mRNA detected by reverse transcription-polymerase chain reaction assay in long-term disease-free acute promyelocytic leukaemia patients. Br J Haematol. 1995;90:966–8.

1036. Diverio D, Rossi V, Avvisati G et al. Early detection of relapse by prospective reverse transcriptase-polymerase chain reaction analysis of the PML/RARalpha fusion gene in patients with acute promyelocytic leukemia enrolled in the GIMEMA-AIEOP multicenter 'AIDA' trial. GIMEMA-AIEOP Multicenter 'AIDA' Trial. Blood. 1998;92:784–9.

1037. Mandelli F, Diverio D, Avvisati G et al. Molecular remission in PML/RAR alpha-positive acute promyelocytic leukemia by combined all-trans retinoic acid and idarubicin (AIDA) therapy. Gruppo Italiano-Malattie Ematologiche Maligne dell'Adulto and Associazione Italiana di Ematologia ed Oncologia Pediatrica Cooperative Groups. Blood. 1997;90:1014–21.

1038. Liu YF, Zhu YM, Shen SH et al. Molecular response in acute promyelocytic leukemia: a direct comparison of regular and real-time RT-PCR. Leukemia. 2006;20:1393–9.

1039. Mitterbauer M, Mitterbauer-Hohendanner G, Sperr WR et al. Molecular disease eradication is a prerequisite for long-term remission in patients with t(8;21) positive acute myeloid leukemia: a single center study. Leuk Lymphoma. 2004;45:971–7.

1040. Miyamoto T, Weissman IL, Akashi K. AML1/ETO-expressing nonleukemic stem cells in acute myelogenous leukemia with 8;21 chromosomal translocation. Proc Natl Acad Sci USA. 2000;97:7521–6.

1041. Perea G, Lasa A, Aventin A et al. Prognostic value of minimal residual disease (MRD) in acute myeloid leukemia (AML) with favorable cytogenetics [t(8;21) and inv(16)]. Leukemia. 2006;20:87–94.

1042. Chou WC, Tang JL, Wu SJ et al. Clinical implications of minimal residual disease monitoring by quantitative polymerase chain reaction in acute myeloid leukemia patients bearing nucleophosmin (NPM1) mutations. Leukemia. 2007;21:998–1004.

1043. Szczepanski T, Orfao A, van der Velden VH, San Miguel JF, van Dongen JJ. Minimal residual disease in leukaemia patients. Lancet Oncol. 2001;2:409–17.

1044. O'Reilly J, Meyer B, Baker D, Herrmann R, Cannell P, Davies J. Correlation of bone marrow minimal residual disease and apparent isolated extramedullary relapse in childhood acute lymphoblastic leukaemia. Leukemia. 1995;9:624–7.

1045. Neale GA, Coustan-Smith E, Stow P et al. Comparative analysis of flow cytometry and polymerase chain reaction for the detection of minimal residual disease in childhood acute lymphoblastic leukemia. Leukemia. 2004;18:934–8.

1046. Neale GA, Menarguez J, Kitchingman GR et al. Detection of minimal residual disease in T-cell acute lymphoblastic leukemia using polymerase chain reaction predicts impending relapse. Blood. 1991;78:739–47.

1047. Malec M, van der Velden VH, Bjorklund E et al. Analysis of minimal residual disease in childhood acute lymphoblastic leukemia: comparison between RQ-PCR analysis of Ig/TcR gene rearrangements and multicolor flow cytometric immunophenotyping. Leukemia. 2004;18:1630–6.

1048. Yamada M, Wasserman R, Lange B, Reichard BA, Womer RB, Rovera G. Minimal residual disease in childhood B-lineage lymphoblastic leukemia. Persistence of leukemic cells during the first 18 months of treatment. N Engl J Med. 1990;323:448–55.

1049. Gruhn B, Hongeng S, Yi H et al. Minimal residual disease after intensive induction therapy in childhood acute lymphoblastic leukemia predicts outcome. Leukemia. 1998;12:675–81.

1050. van Dongen JJ, Seriu T, Panzer-Grumayer ER et al. Prognostic value of minimal residual disease in acute lymphoblastic leukaemia in childhood. Lancet. 1998;352:1731–8.

1051. Brisco MJ, Condon J, Hughes E et al. Outcome prediction in childhood acute lymphoblastic leukaemia by molecular quantification of residual disease at the end of induction. Lancet. 1994;343:196–200.

1052. Cave H, van der Werff ten Bosch J, Suciu S et al. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia. European Organization for Research and Treatment of Cancer – Childhood Leukemia Cooperative Group. N Engl J Med. 1998;339:591–8.

1053. Nyvold C, Madsen HO, Ryder LP et al. Precise quantification of minimal residual disease at day 29 allows identification of children with acute lymphoblastic leukemia and an excellent outcome. Blood. 2002;99:1253–8.

1054. Knechtli CJ, Goulden NJ, Hancock JP et al. Minimal residual disease status before allogeneic bone marrow transplantation is an important determinant of successful outcome for children and adolescents with acute lymphoblastic leukemia. Blood. 1998;92:4072–9.

1055. Sanchez J, Serrano J, Gomez P et al. Clinical value of immunological monitoring of minimal residual disease in acute lymphoblastic leukaemia after allogeneic transplantation. Br J Haematol. 2002;116:686–94.

1056. Knechtli CJ, Goulden NJ, Hancock JP et al. Minimal residual disease status as a predictor of relapse after allogeneic bone marrow transplantation for children with acute lymphoblastic leukaemia. Br J Haematol. 1998;102:860–71.

1057. Hooijkaas H, Hahlen K, Adriaansen HJ, Dekker I, van Zanen GE, van Dongen JJ. Terminal deoxynucleotidyl transferase (TdT)-positive cells in cerebrospinal fluid and development of overt CNS leukemia: a 5-year follow-up study in 113 children with a TdT-positive leukemia or non-Hodgkin's lymphoma. Blood. 1989;74:416–22.

1058. Subira D, Castanon S, Roman A et al. Flow cytometry and the study of central nervous disease in patients with acute leukaemia. Br J Haematol. 2001;112:381–4.

1059. Foroni L, Hoffbrand AV. Molecular analysis of minimal residual disease in adult acute lymphoblastic leukaemia. Best Pract Res Clin Haematol. 2002;15:71–90.

1060. Druker BJ, Talpaz M, Resta DJ et al. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N Engl J Med. 2001; 344:1031–7.

1061. Kantarjian HM, Cortes JE, O'Brien S et al. Long-term survival benefit and improved complete cytogenetic and molecular response rates with imatinib mesylate in Philadelphia chromosome-positive chronic-phase chronic myeloid leukemia after failure of interferon-alpha. Blood. 2004;104:1979–88.

1062. Sawyers CL, Hochhaus A, Feldman E et al. Imatinib induces hematologic and cytogenetic responses in patients with chronic myelogenous leukemia in myeloid blast crisis: results of a phase II study. Blood. 2002;99:3530–9.

1063. Talpaz M, Silver RT, Druker BJ et al. Imatinib induces durable hematologic and cytogenetic responses in patients with accelerated phase chronic myeloid leukemia: results of a phase 2 study. Blood. 2002;99:1928–37.

1064. Rosti G, Martinelli G, Bassi S et al. Molecular response to imatinib in late chronic-phase chronic myeloid leukemia. Blood. 2004;103:2284–90.

1065. Kantarjian HM, Talpaz M, O'Brien S et al. Survival benefit with imatinib mesylate versus interferon-alpha-based regimens in newly diagnosed chronic-phase chronic myelogenous leukemia. Blood. 2006;108:1835–40.

1066. Druker BJ, Guilhot F, O'Brien SG et al. Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. N Engl J Med. 2006;355:2408–17.

1067. Marin D, Marktel S, Bua M et al. Prognostic factors for patients with chronic myeloid leukaemia in chronic phase treated with imatinib mesylate after failure of interferon alfa. Leukemia. 2003;17:1448–53.

1068. Amiel A, Yarkoni S, Fejgin M et al. Clinical detection of BCR-ABL fusion by in situ hybridization in chronic myelogenous leukemia. Cancer Genet Cytogenet. 1993;65:32–4.

1069. Bentz M, Cabot G, Moos M et al. Detection of chimeric BCR-ABL genes on bone marrow samples and blood smears in chronic myeloid and acute lymphoblastic leukemia by in situ hybridization. Blood. 1994;83:1922–8.

1070. San Miguel J, Gonzalez M, Orfao A. Detection of minimal residual disease in myeloid malignancies. In: Degos L, Linch, D. C., Lowenberg, B., eds. Textbook of Malignant Hematology, 2nd edn. London and New York: Taylor & Francis; 2005:308–25.

1071. Hughes T, Branford S. Molecular monitoring of chronic myeloid leukemia. Semin Hematol. 2003;40:62–8.

1072. Hughes T, Branford S. Molecular monitoring of BCR-ABL as a guide to clinical management in chronic myeloid leukaemia. Blood Rev. 2006;20:29–41.

1073. Hughes TP, Deininger MW, Hochhaus A et al. Monitoring CML patients responding to treatment with tyrosine kinase inhibitors – Review and recommendations for 'harmonizing' current methodology for detecting BCR-ABL transcripts and kinase domain mutations and for expressing results. Blood 2006;108:28–37.

1074. Press RD, Love Z, Tronnes AA et al. BCR-ABL mRNA levels at and after the time of a complete cytogenetic response (CCR) predict the duration of CCR in imatinib mesylate-treated patients with CML. Blood. 2006; 107:4250–6.

1075. Hughes TP, Kaeda J, Branford S et al. Frequency of major molecular responses to imatinib or interferon alfa plus cytarabine in newly diagnosed chronic myeloid leukemia. N Engl J Med. 2003;349:1423–32.

1076. Olavarria E, Kanfer E, Szydlo R et al. Early detection of BCR-ABL transcripts by quantitative reverse transcriptase-polymerase chain reaction predicts outcome after allogeneic stem cell transplantation for chronic myeloid leukemia. Blood. 2001;97:1560–5.

1077. Montillo M, Hamblin T, Hallek M, Montserrat E, Morra E. Chronic lymphocytic leukemia: novel prognostic factors and their relevance for risk-adapted therapeutic strategies. Haematologica. 2005;90:391–9.

1078. Giles FJ, Bekele BN, O'Brien S et al. A prognostic model for survival in chronic lymphocytic leukaemia based on p53 expression. Br J Haematol. 2003;121:578–85.

1079. Lin K, Sherrington PD, Dennis M, Matrai Z, Cawley JC, Pettitt AR. Relationship between p53 dysfunction, CD38 expression, and IgV(H) mutation in chronic lymphocytic leukemia. Blood. 2002;100:1404–9.

1080. Nola M, Pavletic SZ, Weisenburger DD et al. Prognostic factors influencing survival in patients with B-cell small lymphocytic lymphoma. Am J Hematol. 2004;77:31–5.

1081. Bea S, Lopez-Guillermo A, Ribas M et al. Genetic imbalances in progressed B-cell chronic lymphocytic leukemia and transformed large-cell lymphoma (Richter's syndrome). Am J Pathol. 2002;161:957–68.

1082. Criel A, Wlodarska I, Meeus P et al. Trisomy 12 is uncommon in typical chronic lymphocytic leukaemias. Br J Haematol. 1994;87:523–8.

1083. O'Connor SJ, Su'ut L, Morgan GJ, Jack AS. The relationship between typical and atypical B-cell chronic lymphocytic leukemia. A comparative genomic hybridization-based study. Am J Clin Pathol. 2000;114:448–58.

1084. Criel A, Michaux L, De Wolf-Peeters C. The concept of typical and atypical chronic lymphocytic leukaemia. Leuk Lymphoma. 1999;33:33–45.

1085. Bigoni R, Cuneo A, Roberti MG et al. Chromosome aberrations in atypical chronic lymphocytic leukemia: a cytogenetic and interphase cytogenetic study. Leukemia. 1997;11:1933–40.

1086. Stilgenbauer S, Lichter P, Dohner H. Genetic features of B-cell chronic lymphocytic leukemia. Rev Clin Exp Hematol. 2000;4:48–72.

1087. Hernandez JM, Mecucci C, Criel A et al. Cytogenetic analysis of B cell chronic lymphoid leukemias classified according to morphologic and immunophenotypic (FAB) criteria. Leukemia. 1995;9:2140–6.

1088. Karhu R, Tobin G, Thunberg U et al. More extensive genetic alterations in unmutated than in hypermutated cases of chronic lymphocytic leukemia. Genes Chromosomes Cancer. 2003;37:417–20.

1089. Oscier DG, Matutes E, Copplestone A et al. Atypical lymphocyte morphology: an adverse prognostic factor for disease progression in stage A CLL independent of trisomy 12. Br J Haematol. 1997;98:934–9.

1090. Criel A, Verhoef G, Vlietinck R et al. Further characterization of morphologically defined typical and atypical CLL: a clinical, immunophenotypic, cytogenetic and prognostic study on 390 cases. Br J Haematol. 1997;97:383–91.

1091. Matutes E. Trisomy 12 in chronic lymphocytic leukaemia. Leuk Res. 1996;20:375–7.

1092. Juliusson G, Gahrton G. Chromosome aberrations in B-cell chronic lymphocytic leukemia. Pathogenetic and clinical implications. Cancer Genet Cytogenet. 1990;45:143–60.

1093. Escudier SM, Pereira-Leahy JM, Drach JW et al. Fluorescent in situ hybridization and cytogenetic studies of trisomy 12 in chronic lymphocytic leukemia. Blood. 1993;81:2702–7.

1094. Auer RL, Bienz N, Neilson J et al. The sequential analysis of trisomy 12 in B-cell chronic lymphocytic leukaemia. Br J Haematol. 1999;104:742–4.

1095. Glassman AB, Harper-Allen EA, Hayes KJ, Hopwood VL, Gutterman EE, Zagryn SP. Chromosome 6 abnormalities associated with prolymphocytic acceleration in chronic lymphocytic leukemia. Ann Clin Lab Sci. 1998;28:24–9.

1096. Capello D, Vitolo U, Pasqualucci L et al. Distribution and pattern of BCL-6 mutations throughout the spectrum of B-cell neoplasia. Blood. 2000;95:651–9.

1097. Maloum K, Davi F, Merle-Beral H et al. Expression of unmutated VH genes is a detrimental prognostic factor in chronic lymphocytic leukemia. Blood. 2000;96:377–9.

1098. Fais F, Ghiotto F, Hashimoto S et al. Chronic lymphocytic leukemia B cells express restricted sets of mutated and unmutated antigen receptors. J Clin Invest. 1998;102:1515–25.

1099. Osuji NC, Del Giudice I, Matutes E, Wotherspoon AC, Dearden C, Catovsky D. The efficacy of alemtuzumab for refractory chronic lymphocytic leukemia in relation to cytogenetic abnormalities of p53. Haematologica. 2005;90:1435–6.

1100. Thornton PD, Gruszka-Westwood AM, Hamoudi RA et al. Characterisation of TP53 abnormalities in chronic lymphocytic leukaemia. Hematol J. 2004;5:47–54.

1101. Wang J, Coombes KR, Highsmith WE, Keating MJ, Abruzzo LV. Differences in gene expression between B-cell chronic lymphocytic leukemia and normal B cells: a meta-analysis of three microarray studies. Bioinformatics. 2004;20:3166–78.

1102. Rosenwald A, Alizadeh AA, Widhopf G et al. Relation of gene expression phenotype to immunoglobulin mutation genotype in B cell chronic lymphocytic leukemia. J Exp Med. 2001;194:1639–47.

1103. Rosenwald A, Staudt LM. Clinical translation of gene expression profiling in lymphomas and leukemias. Semin Oncol. 2002;29:258–63.

1104. Aalto Y, El-Rifa W, Vilpo L et al. Distinct gene expression profiling in chronic lymphocytic leukemia with 11q23 deletion. Leukemia. 2001;15:1721–8.

1105. Durig J, Nuckel H, Huttmann A et al. Expression of ribosomal and translation-associated genes is correlated

with a favorable clinical course in chronic lymphocytic leukemia. Blood. 2003;101:2748–55.

1106. Haslinger C, Schweifer N, Stilgenbauer S et al. Microarray gene expression profiling of B-cell chronic lymphocytic leukemia subgroups defined by genomic aberrations and VH mutation status. J Clin Oncol. 2004;22:3937–49.

1107. Hercher C, Robain M, Davi F et al. A multicentric study of 41 cases of B-prolymphocytic leukemia: two evolutive forms. Leuk Lymphoma. 2001;42:981–7.

1108. Shvidel L, Shtalrid M, Bassous L, Klepfish A, Vorst E, Berrebi A. B-cell prolymphocytic leukemia: a survey of 35 patients emphasizing heterogeneity, prognostic factors and evidence for a group with an indolent course. Leuk Lymphoma. 1999;33:169–79.

1109. Lens D, De Schouwer PJ, Hamoudi RA et al. p53 abnormalities in B-cell prolymphocytic leukemia. Blood. 1997;89:2015–23.

1110. Lens D, Matutes E, Catovsky D, Coignet LJ. Frequent deletions at 11q23 and 13q14 in B cell prolymphocytic leukemia (B-PLL). Leukemia. 2000;14:427–30.

1111. Crisostomo RH, Fernandez JA, Caceres W. Complex karyotype including chromosomal translocation (8;14)(q24;q32) in one case with B-cell prolymphocytic leukemia. Leuk Res.2007;31:699–701.

1112. Ruchlemer R, Parry-Jones N, Brito-Babapulle V et al. B-prolymphocytic leukaemia with t(11;14) revisited: a splenomegalic form of mantle cell lymphoma evolving with leukaemia. Br J Haematol. 2004;125:330–6.

1113. Kuriakose P, Perveen N, Maeda K, Wiktor A, Van Dyke DL. Translocation (8;14)(q24;q32) as the sole cytogenetic abnormality in B-cell prolymphocytic leukemia. Cancer Genet Cytogenet. 2004;150:156–8.

1114. Goodman GR, Bethel KJ, Saven A. Hairy cell leukemia: an update. Curr Opin Hematol. 2003;10:258–66.

1115. Sambani C, Trafalis DT, Mitsoulis-Mentzikoff C et al. Clonal chromosome rearrangements in hairy cell leukemia: personal experience and review of literature. Cancer Genet Cytogenet. 2001;129:138–44.

1116. Vallianatou K, Brito-Babapulle V, Matutes E, Atkinson S, Catovsky D. p53 gene deletion and trisomy 12 in hairy cell leukemia and its variant. Leuk Res. 1999;23:1041–5.

1117. Sole F, Woessner S, Florensa L et al. Cytogenetic findings in five patients with hairy cell leukemia. Cancer Genet Cytogenet. 1999;110:41–3.

1118. Ishida F, Kitano K, Ichikawa N et al. Hairy cell leukemia with translocation (11;20)(q13;q11) and overexpression of cyclin D1. Leuk Res. 1999;23:763–5.

1119. Sucak GT, Ogur G, Topal G, Ataoglu O, Cankus G, Haznedar R. del(17)(q25) in a patient with hairy cell leukemia: a new clonal chromosome abnormality. Cancer Genet Cytogenet. 1998;100:152–4.

1120. Cawley JC. The pathophysiology of the hairy cell. Hematol Oncol Clin North Am. 2006;20:1011–21.

1121. Dierlamm J, Stefanova M, Wlodarska I et al. Chromosomal gains and losses are uncommon in hairy cell leukemia: a study based on comparative genomic hybridization and interphase fluorescence in situ hybridization. Cancer Genet Cytogenet. 2001;128:164–7.

1122. Andersen CL, Gruszka-Westwood A, Ostergaard M et al. A narrow deletion of 7q is common to HCL, and SMZL, but not CLL. Eur J Haematol. 2004;72:390–402.

1123. Horning SJ. Natural history of and therapy for the indolent non-Hodgkin's lymphomas. Semin Oncol. 1993;20:75–88.

1124. Lee MS. Molecular aspects of chromosomal translocation t(14;18). Semin Hematol. 1993;30:297–305.

1125. Horsman DE, Okamoto I, Ludkovski O et al. Follicular lymphoma lacking the t(14;18)(q32;q21): identification of two disease subtypes. Br J Haematol. 2003;120:424–33.

1126. Jardin F, Gaulard P, Buchonnet G et al. Follicular lymphoma without t(14;18) and with BCL-6 rearrangement: a lymphoma subtype with distinct pathological, molecular and clinical characteristics. Leukemia. 2002;16:2309–17.

1127. Horsman DE, Connors JM, Pantzar T, Gascoyne RD. Analysis of secondary chromosomal alterations in 165 cases of follicular lymphoma with t(14;18). Genes Chromosomes Cancer. 2001;30:375–82.

1128. Lestou VS, Gascoyne RD, Sehn L et al. Multicolour fluorescence in situ hybridization analysis of t(14;18)-positive follicular lymphoma and correlation with gene expression data and clinical outcome. Br J Haematol. 2003;122:745–59.

1129. Martin AR, Weisenburger DD, Chan WC et al. Prognostic value of cellular proliferation and histologic grade in follicular lymphoma. Blood. 1995;85:3671–8.

1130. Glas AM, Kersten MJ, Delahaye LJ et al. Gene expression profiling in follicular lymphoma to assess clinical aggressiveness and to guide the choice of treatment. Blood. 2005;105:301–7.

1131. Dave SS, Wright G, Tan B et al. Prediction of survival in follicular lymphoma based on molecular features of tumor-infiltrating immune cells. N Engl J Med. 2004;351:2159–69.

1132. Hubbard SM, Chabner BA, DeVita VT, Jr et al. Histologic progression in non-Hodgkin's lymphoma. Blood. 1982;59:258–64.

1133. Horning SJ, Rosenberg SA. The natural history of initially untreated low-grade non-Hodgkin's lymphomas. N Engl J Med. 1984;311:1471–5.

1134. Bastion Y, Sebban C, Berger F et al. Incidence, predictive factors, and outcome of lymphoma transformation in follicular lymphoma patients. J Clin Oncol. 1997; 15:1587–94.

1135. Ersboll J, Schultz HB, Pedersen-Bjergaard J, Nissen NI. Follicular low-grade non-Hodgkin's lymphoma: long-term outcome with or without tumor progression. Eur J Haematol. 1989;42:155–63.

1136. Acker B, Hoppe RT, Colby TV, Cox RS, Kaplan HS, Rosenberg SA. Histologic conversion in the non-Hodgkin's lymphomas. J Clin Oncol. 1983;1:11–16.

1137. Feller AC, Diebold, J. Histopathology of Nodal and Extranodal non-Hodgkin's Lymphomas, 3rd edn. Berlin Heidelberg: Springer-Verlag, 2004.

1138. Lee JT, Innes DJ, Jr., Williams ME. Sequential bcl-2 and c-myc oncogene rearrangements associated with the clinical transformation of non-Hodgkin's lymphoma. J Clin Invest. 1989;84:1454–9.

1139. Natkunam Y, Warnke RA, Zehnder JL, Jones CD, Milatovich-Cherry A, Cornbleet PJ. Blastic/blastoid transformation of follicular lymphoma: immunohistologic and molecular analyses of five cases. Am J Surg Pathol. 2000;24:525–34.

1140. de Jong D, Voetdijk BM, Beverstock GC, van Ommen GJ, Willemze R, Kluin PM. Activation of the c-myc oncogene in a precursor-B-cell blast crisis of follicular lymphoma, presenting as composite lymphoma. N Engl J Med. 1988;318:1373–8.

1141. Kroft SH, Domiati-Saad R, Finn WG et al. Precursor B-lymphoblastic transformation of grade I follicle center lymphoma. Am J Clin Pathol. 2000;113:411–18.

1142. Nagy M, Balazs M, Adam Z et al. Genetic instability is associated with histological transformation of follicle center lymphoma. Leukemia. 2000;14:2142–8.

1143. Armitage JO, Sanger WG, Weisenburger DD et al. Correlation of secondary cytogenetic abnormalities with histologic appearance in non-Hodgkin's lymphomas bearing t(14;18)(q32;q21). J Natl Cancer Inst. 1988; 80:576–80.

1144. Richardson ME, Chen QG, Filippa DA et al. Intermediate- to high-grade histology of lymphomas carrying t(14;18) is associated with additional nonrandom chromosome changes. Blood. 1987;70:444–7.

1145. Yano T, Jaffe ES, Longo DL, Raffeld M. MYC rearrangements in histologically progressed follicular lymphomas. Blood. 1992;80:758–67.

1146. Lossos IS, Levy R. Higher grade transformation of follicular lymphoma: phenotypic tumor progression associated with diverse genetic lesions. Semin Cancer Biol. 2003;13:191–202.

1147. Lossos IS, Alizadeh AA, Diehn M et al. Transformation of follicular lymphoma to diffuse large-cell lymphoma: alternative patterns with increased or decreased expression of c-myc and its regulated genes. Proc Natl Acad Sci USA. 2002;99:8886–91.

1148. Lo Coco F, Gaidano G, Louie DC, Offit K, Chaganti RS, Dalla-Favera R. p53 mutations are associated with histologic transformation of follicular lymphoma. Blood. 1993;82:2289–95.

1149. Sander CA, Yano T, Clark HM et al. p53 mutation is associated with progression in follicular lymphomas. Blood. 1993;82:1994–2004.

1150. Matolcsy A. High-grade transformation of low-grade non-Hodgkin's lymphomas: mechanisms of tumor progression. Leuk Lymphoma. 1999;34:251–9.

1151. Matolcsy A, Casali P, Warnke RA, Knowles DM. Morphologic transformation of follicular lymphoma is associated with somatic mutation of the translocated Bcl-2 gene. Blood. 1996;88:3937–44.

1152. Lossos IS, Warnke R, Levy R. BCL-6 mRNA expression in higher grade transformation of follicle center lymphoma: correlation with somatic mutations in the 5' regulatory region of the BCL-6 gene. Leukemia. 2002;16:1857–62.

1153. Mohamed AN, Palutke M, Eisenberg L, Al-Katib A. Chromosomal analyses of 52 cases of follicular lymphoma with t(14;18), including blastic/blastoid variant. Cancer Genet Cytogenet. 2001;126:45–51.

1154. Martinez-Climent JA, Alizadeh AA, Segraves R et al. Transformation of follicular lymphoma to diffuse large cell lymphoma is associated with a heterogeneous set of DNA copy number and gene expression alterations. Blood. 2003;101:3109–17.

1155. Li L, Gupta S, Bashir T, Koduru PR, Brody J, Allen SL. Serial cytogenetic alterations resulting in transformation of a low-grade follicular lymphoma to Burkitt lymphoma. Cancer Genet Cytogenet. 2006;170: 140–6.

1156. Yuen AR, Kamel OW, Halpern J, Horning SJ. Long-term survival after histologic transformation of low-grade follicular lymphoma. J Clin Oncol. 1995;13:1726–33.

1157. Rohatiner A, Lister TA. Management of follicular lymphoma. Curr Opin Oncol. 1994;6:473–9.

1158. Natkunam Y, Soslow R, Matolcsy A et al. Immunophenotypic and genotypic characterization of progression in follicular lymphomas. Appl Immunohistochem Mol Morphol. 2004;12:97–104.

1159. Davies AJ, Rosenwald A, Wright G et al. Transformation of follicular lymphoma to diffuse large B-cell lymphoma proceeds by distinct oncogenic mechanisms. Br J Haematol. 2007;136:286–93.

1160. Pittaluga S, Wlodarska I, Stul MS et al. Mantle cell lymphoma: a clinicopathological study of 55 cases. Histopathology. 1995;26:17–24.

1161. Meusers P, Engelhard M, Bartels H et al. Multicentre randomized therapeutic trial for advanced centrocytic lymphoma: anthracycline does not improve the prognosis. Hematol Oncol. 1989;7:365–80.

1162. Bosch F, Lopez-Guillermo A, Campo E et al. Mantle cell lymphoma: presenting features, response to therapy, and prognostic factors. Cancer. 1998;82:567–75.

1163. Espinet B, Sole F, Woessner S et al. Translocation (11;14)(q13;q32) and preferential involvement of chromosomes 1, 2, 9, 13, and 17 in mantle cell lymphoma. Cancer Genet Cytogenet. 1999;111:92–8.

1164. Wlodarska I, Pittaluga S, Hagemeijer A, De Wolf-Peeters C, Van Den Berghe H. Secondary chromosome changes in mantle cell lymphoma. Haematologica. 1999;84:594–9.

1165. Bentz M, Plesch A, Bullinger L et al. t(11;14)-positive mantle cell lymphomas exhibit complex karyotypes and share similarities with B-cell chronic lymphocytic leukemia. Genes Chromosomes Cancer. 2000;27:285–94.

1166. Allen JE, Hough RE, Goepel JR et al. Identification of novel regions of amplification and deletion within mantle cell lymphoma DNA by comparative genomic hybridization. Br J Haematol. 2002;116:291–8.

1167. Parry-Jones N, Matutes E, Morilla R et al. Cytogenetic abnormalities additional to t(11;14) correlate with clinical features in leukaemic presentation of mantle cell lymphoma, and may influence prognosis: a study of 60 cases by FISH. Br J Haematol. 2007;137:117–24.

1168. Schraders M, Pfundt R, Straatman HM et al. Novel chromosomal imbalances in mantle cell lymphoma detected by genome-wide array-based comparative genomic hybridization. Blood. 2005;105:1686–93.

1169. Martinez-Climent JA, Vizcarra E, Sanchez D et al. Loss of a novel tumor suppressor gene locus at chromosome 8p is associated with leukemic mantle cell lymphoma. Blood. 2001;98:3479–82.

1170. Argatoff LH, Connors JM, Klasa RJ, Horsman DE, Gascoyne RD. Mantle cell lymphoma: a clinicopathologic study of 80 cases. Blood. 1997;89:2067–78.

1171. Norton AJ, Matthews J, Pappa V et al. Mantle cell lymphoma: natural history defined in a serially biopsied population over a 20-year period. Ann Oncol. 1995;6:249–56.

1172. Bernard M, Gressin R, Lefrere F et al. Blastic variant of mantle cell lymphoma: a rare but highly aggressive subtype. Leukemia. 2001;15:1785–91.

1173 Hernandez L, Fest T, Cazorla M et al. p53 gene mutations and protein overexpression are associated with aggressive variants of mantle cell lymphomas. Blood. 1996;87:3351–9.

1174. Ott G, Kalla J, Ott MM et al. Blastoid variants of mantle cell lymphoma: frequent bcl-1 rearrangements at the major translocation cluster region and tetraploid chromosome clones. Blood. 1997;89:1421–9.

1175. Bea S, Ribas M, Hernandez JM et al. Increased number of chromosomal imbalances and high-level DNA amplifications in mantle cell lymphoma are associated with blastoid variants. Blood. 1999;93:4365–74.

1176. Raty R, Franssila K, Jansson SE, Joensuu H, Wartiovaara-Kautto U, Elonen E. Predictive factors for blastoid transformation in the common variant of mantle cell lymphoma. Eur J Cancer. 2003;39:321–9.

1177. Pinyol M, Hernandez L, Cazorla M et al. Deletions and loss of expression of p16INK4a and p21Waf1 genes are associated with aggressive variants of mantle cell lymphomas. Blood. 1997;89:272–80.

1178. Pinyol M, Cobo F, Bea S et al. p16(INK4a) gene inactivation by deletions, mutations, and hypermethylation is associated with transformed and aggressive variants of non-Hodgkin's lymphomas. Blood. 1998;91:2977–84.

1179. Mansoor A, Medeiros LJ, Weber DM et al. Cytogenetic findings in lymphoplasmacytic lymphoma/Waldenstrom macroglobulinemia. Chromosomal abnormalities are associated with the polymorphous subtype and an aggressive clinical course. Am J Clin Pathol. 2001;116:543–9.

1180. Ackroyd S, O'Connor SJ, Owen RG. Rarity of IgH translocations in Waldenstrom macroglobulinemia. Cancer Genet Cytogenet. 2005;163:77–80.

1181. Chng WJ, Schop RF, Price-Troska T et al. Gene-expression profiling of Waldenstrom macroglobulinemia reveals a phenotype more similar to chronic lymphocytic leukemia than multiple myeloma. Blood. 2006;108:2755–63.

1182. Kuppers R. Mechanisms of B-cell lymphoma pathogenesis. Nat Rev Cancer. 2005;5:251–62.

1183. Isaacson PG. Gastric MALT lymphoma: from concept to cure. Ann Oncol. 1999;10:637–45.

1184. Isaacson PG. Gastric lymphoma and *Helicobacter pylori*. N Engl J Med. 1994;330:1310–11.

1185. Lecuit M, Abachin E, Martin A et al. Immunoproliferative small intestinal disease associated with *Campylobacter jejuni*. N Engl J Med. 2004;350:239–48.

1186. Yeung L, Tsao YP, Chen PY, Kuo TT, Lin KK, Lai LJ. Combination of adult inclusion conjunctivitis and mucosa-associated lymphoid tissue (MALT) lymphoma in a young adult. Cornea. 2004;23:71–5.

1187. de la Fouchardiere A, Vandenesch F, Berger F. Borrelia-associated primary cutaneous MALT lymphoma in a nonendemic region. Am J Surg Pathol. 2003;27:702–3.

1188. de la Fouchardiere A, Balme B, Chouvet B et al. Primary cutaneous marginal zone B-cell lymphoma: a report of 9 cases. J Am Acad Dermatol. 1999;41:181–8.

1189. Hyjek E, Isaacson PG. Primary B cell lymphoma of the thyroid and its relationship to Hashimoto's thyroiditis. Hum Pathol. 1988;19:1315–26.

1190. Arcaini L, Burcheri S, Rossi A et al. Nongastric marginal-zone B-cell MALT lymphoma: prognostic value of disease dissemination. Oncologist. 2006;11:285–91.

1191. Dierlamm J, Pittaluga S, Wlodarska I et al. Marginal zone B-cell lymphomas of different sites share similar cytogenetic and morphologic features. Blood. 1996;87:299–307.

1192. Farinha P, Gascoyne RD. Molecular pathogenesis of mucosa-associated lymphoid tissue lymphoma. J Clin Oncol. 2005;23:6370–8.

1193. Brynes RK, Almaguer PD, Leathery KE et al. Numerical cytogenetic abnormalities of chromosomes 3, 7, and 12 in marginal zone B-cell lymphomas. Mod Pathol. 1996;9:995–1000.

1194. Rosenwald A, Ott G, Stilgenbauer S et al. Exclusive detection of the t(11;18)(q21;q21) in extranodal marginal zone B cell lymphomas (MZBL) of MALT type in contrast to other MZBL and extranodal large B cell lymphomas. Am J Pathol. 1999;155:1817–21.

1195. Sanchez-Izquierdo D, Buchonnet G, Siebert R et al. MALT1 is deregulated by both chromosomal translocation and amplification in B-cell non-Hodgkin lymphoma. Blood. 2003;101:4539–46.

1196. Sagaert X, De Wolf-Peeters C, Noels H, Baens M. The pathogenesis of MALT lymphomas: where do we stand? Leukemia. 2007;21:389–96.

1197. Baens M, Steyls A, Dierlamm J, De Wolf-Peeters C, Marynen P. Structure of the MLT gene and molecular characterization of the genomic breakpoint junctions in the t(11;18)(q21;q21) of marginal zone B-cell lymphomas of MALT type. Genes Chromosomes Cancer. 2000;29:281–91.

1198. Remstein ED, Dogan A, Einerson RR et al. The incidence and anatomic site specificity of chromosomal translocations in primary extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) in North America. Am J Surg Pathol. 2006;30:1546–53.

1199. Joao C, Farinha P, da Silva MG, Martins C, Crespo M, Cabecadas J. Cytogenetic abnormalities in MALT lymphomas and their precursor lesions from different organs. A fluorescence in situ hybridization (FISH) study. Histopathology. 2007;50:217–24.

1200. Dierlamm J, Murga Penas EM, Daibata M et al. The novel t(11;12;18)(q21;q13;q21) represents a variant translocation of the t(11;18)(q21;q21) associated with MALT-type lymphoma. Leukemia. 2002;16:1863–4.

1201. Murga Penas EM, Hinz K, Roser K et al. Translocations t(11;18)(q21;q21) and t(14;18)(q32;q21) are the main chromosomal abnormalities involving MLT/MALT1 in MALT lymphomas. Leukemia. 2003;17:2225–9.

1202. Remstein ED, James CD, Kurtin PJ. Incidence and subtype specificity of API2–MALT1 fusion translocations in extranodal, nodal, and splenic marginal zone lymphomas. Am J Pathol. 2000;156:1183–8.

1203. Streubel B, Simonitsch-Klupp I, Mullauer L et al. Variable frequencies of MALT lymphoma-associated genetic aberrations in MALT lymphomas of different sites. Leukemia. 2004;18:1722–6.

1204. Oscier D, Owen, R, and S.Johnson. Splenic marginal zone lymphoma. Blood Rev. 2005;19:39–51.

1205. Parry-Jones N, Matutes E, Gruszka-Westwood AM, Swansbury GJ, Wotherspoon AC, Catovsky D. Prognostic features of splenic lymphoma with villous lymphocytes: a report on 129 patients. Br J Haematol. 2003;120:759–64.

1206. Mulligan SP, Matutes E, Dearden C, Catovsky D. Splenic lymphoma with villous lymphocytes: natural history and response to therapy in 50 cases. Br J Haematol. 1991;78:206–9.

1207. Troussard X, Valensi F, Duchayne E et al. Splenic lymphoma with villous lymphocytes: clinical presentation, biology and prognostic factors in a series of 100 patients. Groupe Francais d'Hematologie Cellulaire (GFHC). Br J Haematol. 1996;93:731–6.

1208. Chacon JI, Mollejo M, Munoz E et al. Splenic marginal zone lymphoma: clinical characteristics and prognostic factors in a series of 60 patients. Blood. 2002;100:1648–54.

1209. Arcaini L, Lazzarino M, Colombo N et al. Splenic marginal zone lymphoma: a prognostic model for clinical use. Blood. 2006;107:4643–9.

1210. Ott MM, Rosenwald A, Katzenberger T et al. Marginal zone B-cell lymphomas (MZBL) arising at different sites represent different biological entities. Genes Chromosomes Cancer. 2000;28:380–6.

1211. Camacho FI, Mollejo M, Mateo MS et al. Progression to large B-cell lymphoma in splenic marginal zone lymphoma: a description of a series of 12 cases. Am J Surg Pathol. 2001;25:1268–76.

1212. Martinez-Climent JA, Sanchez-Izquierdo D, Sarsotti E et al. Genomic abnormalities acquired in the blastic transformation of splenic marginal zone B-cell lymphoma. Leuk Lymphoma. 2003;44:459–64.

1213. Lloret E, Mollejo M, Mateo MS et al. Splenic marginal zone lymphoma with increased number of blasts: an aggressive variant? Hum Pathol. 1999;30:1153–60.

1214. Bertoni F, Cavalli F, Cotter FE, Zucca E. Genetic alterations underlying the pathogenesis of MALT lymphoma. Hematol J. 2002;3:10–13.

1215. Bertoni F, Cotter FE, Zucca E. Molecular genetics of extranodal marginal zone (MALT-type) B-cell lymphoma. Leuk Lymphoma. 1999;35:57–68.

1216. Zucca E, Bertoni F, Roggero E et al. Molecular analysis of the progression from *Helicobacter pylori*-associated chronic gastritis to mucosa-associated lymphoid-tissue lymphoma of the stomach. N Engl J Med. 1998;338:804–10.

1217. Clark HM, Jones DB, Wright DH. Cytogenetic and molecular studies of t(14;18) and t(14;19) in nodal and extranodal B-cell lymphoma. J Pathol. 1992;166:129–37.

1218. Du M, Peng H, Singh N, Isaacson PG, Pan L. The accumulation of p53 abnormalities is associated with progression of mucosa-associated lymphoid tissue lymphoma. Blood. 1995;86:4587–93.

1219. Dierlamm J, Baens M, Stefanova-Ouzounova M et al. Detection of t(11;18)(q21;q21) by interphase fluorescence in situ hybridization using API2 and MLT specific probes. Blood. 2000;96:2215–18.

1220. Neumeister P, Hoefler G, Beham-Schmid C et al. Deletion analysis of the p16 tumor suppressor gene in gastrointestinal mucosa-associated lymphoid tissue lymphomas. Gastroenterology. 1997;112:1871–5.

1221. Ott G, Kalla J, Steinhoff A et al. Trisomy 3 is not a common feature in malignant lymphomas of mucosa-associated lymphoid tissue type. Am J Pathol. 1998;153:689–94.

1222. Remstein ED, Kurtin PJ, Einerson RR, Paternoster SF, Dewald GW. Primary pulmonary MALT lymphomas show frequent and heterogeneous cytogenetic abnormalities, including aneuploidy and translocations involving API2 and MALT1 and IGH and MALT1. Leukemia. 2004;18:156–60.

1223. Zhou Y, Ye H, Martin-Subero JI et al. Distinct comparative genomic hybridisation profiles in gastric mucosa-associated lymphoid tissue lymphomas with and without t(11;18)(q21;q21). Br J Haematol. 2006;133:35–42.

1224. Isaacson PG, Du MQ. MALT lymphoma: from morphology to molecules. Nat Rev Cancer. 2004;4:644–53.

1225. Preudhomme C, Fenaux P. The clinical significance of mutations of the P53 tumour suppressor gene in haematological malignancies. Br J Haematol. 1997;98:502–11.

1226. de Jong D, Boot H, Taal B. Histological grading with clinical relevance in gastric mucosa-associated lymphoid tissue (MALT) lymphoma. Recent Results Cancer Res. 2000;156:27–32.

1227. de Jong D, Boot H, van Heerde P, Hart GA, Taal BG. Histological grading in gastric lymphoma: pretreatment criteria and clinical relevance. Gastroenterology. 1997;112:1466–74.

1228. Cavalli F, Isaacson PG, Gascoyne RD, Zucca E. MALT Lymphomas. Hematology (Am Soc Hematol Educ Program). 2001:241–58.

1229. Thieblemont C, Berger F, Dumontet C et al. Mucosa-associated lymphoid tissue lymphoma is a disseminated disease in one third of 158 patients analyzed. Blood. 2000;95:802–6.

1230. Zucca E, Bertoni F, Roggero E, Cavalli F. The gastric marginal zone B-cell lymphoma of MALT type. Blood. 2000;96:410–19.

1231. Zinzani PL, Magagnoli M, Galieni P et al. Nongastrointestinal low-grade mucosa-associated lymphoid tissue lymphoma: analysis of 75 patients. J Clin Oncol. 1999;17:1254.

1232. Akagi T, Motegi M, Tamura A et al. A novel gene, MALT1 at 18q21, is involved in t(11;18)(q21;q21) found in low-grade B-cell lymphoma of mucosa-associated lymphoid tissue. Oncogene. 1999;18:5785–94.

1233. Dierlamm J, Baens M, Wlodarska I et al. The apoptosis inhibitor gene API2 and a novel 18q gene, MLT, are recurrently rearranged in the t(11;18)(q21;q21) associated with mucosa-associated lymphoid tissue lymphomas. Blood. 1999;93:3601–9.

1234. Morgan JA, Yin Y, Borowsky AD et al. Breakpoints of the t(11;18)(q21;q21) in mucosa-associated lymphoid tissue (MALT) lymphoma lie within or near the

previously undescribed gene MALT1 in chromosome 18. Cancer Res. 1999;59:6205–13.

1235. Roy N, Deveraux QL, Takahashi R, Salvesen GS, Reed JC. The c-IAP-1 and c-IAP-2 proteins are direct inhibitors of specific caspases. Embo J. 1997;16:6914–25.

1236. Ott G, Katzenberger T, Greiner A et al. The t(11;18)(q21;q21) chromosome translocation is a frequent and specific aberration in low-grade but not high-grade malignant non-Hodgkin's lymphomas of the mucosa-associated lymphoid tissue (MALT-) type. Cancer Res. 1997;57:3944–8.

1237. Streubel B, Huber D, Wohrer S, Chott A, Raderer M. Frequency of chromosomal aberrations involving MALT1 in mucosa-associated lymphoid tissue lymphoma in patients with Sjogren's syndrome. Clin Cancer Res. 2004;10:476–80.

1238. Liu H, Ruskon-Fourmestraux A, Lavergne-Slove A et al. Resistance of t(11;18) positive gastric mucosa-associated lymphoid tissue lymphoma to *Helicobacter pylori* eradication therapy. Lancet. 2001;357:39–40.

1239. Inagaki H, Nakamura T, Li C et al. Gastric MALT lymphomas are divided into three groups based on responsiveness to *Helicobacter pylori* eradication and detection of API2-MALT1 fusion. Am J Surg Pathol. 2004;28:1560–7.

1240. Starostik P, Patzner J, Greiner A et al. Gastric marginal zone B-cell lymphomas of MALT type develop along 2 distinct pathogenetic pathways. Blood. 2002;99:3–9.

1241. Remstein ED, Kurtin PJ, James CD, Wang XY, Meyer RG, Dewald GW. Mucosa-associated lymphoid tissue lymphomas with t(11;18)(q21;q21) and mucosa-associated lymphoid tissue lymphomas with aneuploidy develop along different pathogenetic pathways. Am J Pathol. 2002;161:63–71.

1242. Arcaini L, Paulli M, Burcheri S et al. Primary nodal marginal zone B-cell lymphoma: clinical features and prognostic assessment of a rare disease. Br J Haematol. 2007;136:301–4.

1243. Traverse-Glehen A, Felman P, Callet-Bauchu E et al. A clinicopathological study of nodal marginal zone B-cell lymphoma. A report on 21 cases. Histopathology. 2006;48:162–73.

1244. Nathwani BN, Drachenberg MR, Hernandez AM, Levine AM, Sheibani K. Nodal monocytoid B-cell lymphoma (nodal marginal-zone B-cell lymphoma). Semin Hematol. 1999;36:128–38.

1245. Callet-Bauchu E, Baseggio L, Felman P et al. Cytogenetic analysis delineates a spectrum of chromosomal changes that can distinguish non-MALT marginal zone B-cell lymphomas among mature B-cell entities: a description of 103 cases. Leukemia. 2005;19:1818–23.

1246. Tomaszewski MM, Abbondanzo SL, Lupton GP. Extranodal marginal zone B-cell lymphoma of the skin: a morphologic and immunophenotypic study of 11 cases. Am J Dermatopathol. 2000;22:205–11.

1247. Bailey EM, Ferry JA, Harris NL, Mihm MC, Jr, Jacobson JO, Duncan LM. Marginal zone lymphoma (low-grade B-cell lymphoma of mucosa-associated lymphoid tissue type) of skin and subcutaneous tissue: a study of 15 patients. Am J Surg Pathol. 1996;20:1011–23.

1248. Gallardo F, Bellosillo B, Espinet B et al. Aberrant nuclear BCL10 expression and lack of t(11;18)(q21;q21) in primary cutaneous marginal zone B-cell lymphoma. Hum Pathol. 2006;37:867–73.

1249. Coiffier B. Diffuse large cell lymphoma. Curr Opin Oncol. 2001;13:325–34.

1250. Coiffier B. Treatment of diffuse large B-cell lymphoma. Curr Hematol Rep. 2005;4:7–14.

1251. Chang CC, McClintock S, Cleveland RP et al. Immunohistochemical expression patterns of germinal center and activation B-cell markers correlate with prognosis in diffuse large B-cell lymphoma. Am J Surg Pathol. 2004;28:464–70.

1252. Muramatsu M, Akasaka T, Kadowaki N et al. Rearrangement of the BCL6 gene in B-cell lymphoid neoplasms: comparison with lymphomas associated with BCL2 rearrangement. Br J Haematol. 1996;93:911–20.

1253. Leroy K, Haioun C, Lepage E et al. p53 gene mutations are associated with poor survival in low and low-intermediate risk diffuse large B-cell lymphomas. Ann Oncol. 2002;13:1108–15.

1254. Jaffe ES, Wilson WH. Lymphomatoid granulomatosis: pathogenesis, pathology and clinical implications. Cancer Surv. 1997;30:233–48.

1255. Zhang A, Ohshima K, Sato K et al. Prognostic clinicopathologic factors, including immunologic expression in diffuse large B-cell lymphomas. Pathol Int. 1999; 49:1043–52.

1256. Gascoyne RD. Emerging prognostic factors in diffuse large B cell lymphoma. Curr Opin Oncol. 2004; 16:436–41.

1257. Lossos IS, Alizadeh AA, Eisen MB et al. Ongoing immunoglobulin somatic mutation in germinal center B cell-like but not in activated B cell-like diffuse large cell lymphomas. Proc Natl Acad Sci USA. 2000;97:10209–13.

1258. Huang JZ, Sanger WG, Greiner TC et al. The t(14;18) defines a unique subset of diffuse large B-cell lymphoma

with a germinal center B-cell gene expression profile. Blood. 2002;99:2285–90.

1259. Poulsen CB, Borup R, Nielsen FC et al. Microarray-based classification of diffuse large B-cell lymphoma. Eur J Haematol. 2005;74:453–65.

1260. Iqbal J, Sanger WG, Horsman DE et al. BCL2 translocation defines a unique tumor subset within the germinal center B-cell-like diffuse large B-cell lymphoma. Am J Pathol. 2004;165:159–66.

1261. Lossos IS, Czerwinski DK, Alizadeh AA et al. Prediction of survival in diffuse large-B-cell lymphoma based on the expression of six genes. N Engl J Med. 2004;350:1828–37.

1262. Ramuz O, Bouabdallah R, Devilard E et al. Identification of TCL1A as an immunohistochemical marker of adverse outcome in diffuse large B-cell lymphomas. Int J Oncol. 2005;26:151–7.

1263. Moller MB, Pedersen NT, Christensen BE. Diffuse large B-cell lymphoma: clinical implications of extranodal versus nodal presentation – a population-based study of 1575 cases. Br J Haematol. 2004;124:151–9.

1264. Murase T, Yamaguchi M, Suzuki R et al. Intravascular large B-cell lymphoma (IVLBCL): a clinicopathologic study of 96 cases with special reference to the immunophenotypic heterogeneity of CD5. Blood. 2007;109:478–85.

1265. Ferreri AJ, Campo E, Seymour JF et al. Intravascular lymphoma: clinical presentation, natural history, management and prognostic factors in a series of 38 cases, with special emphasis on the 'cutaneous variant'. Br J Haematol. 2004;127:173–83.

1266. Ponzoni M, Ferreri AJ. Intravascular lymphoma: a neoplasm of 'homeless' lymphocytes? Hematol Oncol. 2006;24:105–12.

1267. Willemze R, Beljaards RC. Spectrum of primary cutaneous CD30 (Ki-1)-positive lymphoproliferative disorders. A proposal for classification and guidelines for management and treatment. J Am Acad Dermatol. 1993;28:973–80.

1268. Willemze R, Kerl H, Sterry W et al. EORTC classification for primary cutaneous lymphomas: a proposal from the Cutaneous Lymphoma Study Group of the European Organization for Research and Treatment of Cancer. Blood. 1997;90:354–71.

1269. Willemze R, Meijer CJ, Sentis HJ et al. Primary cutaneous large cell lymphomas of follicular center cell origin. A clinical follow-up study of nineteen patients. J Am Acad Dermatol. 1987;16:518–26.

1270. Pimpinelli N, Santucci M, Carli P et al. Primary cutaneous follicular center cell lymphoma: clinical and histological aspects. Curr Probl Dermatol. 1990;19:203–20.

1271. Pimpinelli N, Santucci M, Bosi A et al. Primary cutaneous follicular centre-cell lymphoma – a lymphoproliferative disease with favourable prognosis. Clin Exp Dermatol. 1989;14:12–19.

1272. Santucci M, Pimpinelli N, Arganini L. Primary cutaneous B-cell lymphoma: a unique type of low-grade lymphoma. Clinicopathologic and immunologic study of 83 cases. Cancer. 1991;67:2311–26.

1273. Grange F, Bekkenk MW, Wechsler J et al. Prognostic factors in primary cutaneous large B-cell lymphomas: a European multicenter study. J Clin Oncol. 2001; 19:3602–10.

1274. Grange F, Hedelin G, Joly P et al. Prognostic factors in primary cutaneous lymphomas other than mycosis fungoides and the Sezary syndrome. The French Study Group on Cutaneous Lymphomas. Blood. 1999;93:3637–42.

1275. Vermeer MH, Geelen FA, van Haselen CW et al. Primary cutaneous large B-cell lymphomas of the legs. A distinct type of cutaneous B-cell lymphoma with an intermediate prognosis. Dutch Cutaneous Lymphoma Working Group. Arch Dermatol. 1996;132:1304–8.

1276. Zinzani PL, Quaglino P, Pimpinelli N et al. Prognostic factors in primary cutaneous B-cell lymphoma: The Italian Study Group for Cutaneous Lymphomas. J Clin Oncol. 2006;24:1376–82

1277. Goodlad JR, Krajewski AS, Batstone PJ et al. Primary cutaneous diffuse large B-cell lymphoma: prognostic significance of clinicopathological subtypes. Am J Surg Pathol. 2003;27:1538–45.

1278. Touroutoglou N, Dimopoulos MA, Younes A et al. Testicular lymphoma: late relapses and poor outcome despite doxorubicin-based therapy. J Clin Oncol. 1995; 13:1361–7.

1279. Darby S, Hancock BW. Localised non-Hodgkin lymphoma of the testis: The Sheffield Lymphoma Group experience. Int J Oncol. 2005;26:1093–9.

1280. Gianelli U, Patriarca C, Moro A et al. Lymphomas of the bone: a pathological and clinical study of 54 cases. Int J Surg Pathol. 2002;10:257–66.

1281. Kitsoulis P, Vlychou M, Papoudou-Bai A et al. Primary lymphomas of bone. Anticancer Res. 2006;26:325–37.

1282. Desai S, Jambhekar NA, Soman CS, Advani SH. Primary lymphoma of bone: a clinicopathologic study of 25 cases reported over 10 years. J Surg Oncol. 1991;46:265–9.

1283. Dubey P, Ha CS, Besa PC et al. Localized primary malignant lymphoma of bone. Int J Radiat Oncol Biol Phys. 1997;37:1087–93.

1284. Huebner-Chan D, Fernandes B, Yang G, Lim MS. An immunophenotypic and molecular study of primary large B-cell lymphoma of bone. Mod Pathol. 2001;14:1000–7.

1285. Zhao XF, Young KH, Frank D et al. Pediatric primary bone lymphoma-diffuse large B-cell lymphoma: morphologic and immunohistochemical characteristics of 10 cases. Am J Clin Pathol. 2007;127:47–54.

1286. Larocca LM, Capello D, Rinelli A et al. The molecular and phenotypic profile of primary central nervous system lymphoma identifies distinct categories of the disease and is consistent with histogenetic derivation from germinal center-related B cells. Blood. 1998;92:1011–19.

1287. Thompsett AR, Ellison DW, Stevenson FK, Zhu D. V(H) gene sequences from primary central nervous system lymphomas indicate derivation from highly mutated germinal center B cells with ongoing mutational activity. Blood. 1999;94:1738–46.

1288. Montesinos-Rongen M, Kuppers R, Schluter D et al. Primary central nervous system lymphomas are derived from germinal-center B cells and show a preferential usage of the V4-34 gene segment. Am J Pathol. 1999;155:2077–86.

1289. Hoang-Xuan K, Camilleri-Broet S, Soussain C. Recent advances in primary CNS lymphoma. Curr Opin Oncol. 2004;16:601–6.

1290. DeAngelis LM, Seiferheld W, Schold SC, Fisher B, Schultz CJ. Combination chemotherapy and radiotherapy for primary central nervous system lymphoma: Radiation Therapy Oncology Group Study 93-10. J Clin Oncol. 2002;20:4643–8.

1291. Braaten KM, Betensky RA, de Leval L et al. BCL-6 expression predicts improved survival in patients with primary central nervous system lymphoma. Clin Cancer Res. 2003;9:1063–9.

1292. Camilleri-Broet S, Criniere E, Broet P et al. A uniform activated B-cell-like immunophenotype might explain the poor prognosis of primary central nervous system lymphomas: analysis of 83 cases. Blood. 2006; 107:190–6.

1293. Chang CC, Kampalath B, Schultz C et al. Expression of p53, c-Myc, or Bcl-6 suggests a poor prognosis in primary central nervous system diffuse large B-cell lymphoma among immunocompetent individuals. Arch Pathol Lab Med. 2003;127:208–12.

1294. Cazals-Hatem D, Lepage E, Brice P et al. Primary mediastinal large B-cell lymphoma. A clinicopathologic study of 141 cases compared with 916 nonmediastinal large B-cell lymphomas, a GELA ('Groupe d'Etude des Lymphomes de l'Adulte') study. Am J Surg Pathol. 1996;20:877–88.

1295. Savage KJ, Monti S, Kutok JL et al. The molecular signature of mediastinal large B-cell lymphoma differs from that of other diffuse large B-cell lymphomas and shares features with classical Hodgkin's lymphoma. Blood 2003;102:3871–9.

1296. Rosenwald A, Wright G, Leroy K et al. Molecular diagnosis of primary mediastinal B cell lymphoma identifies a clinically favorable subgroup of diffuse large B cell lymphoma related to Hodgkin lymphoma. J Exp Med. 2003;198:851–62.

1297. Malpeli G, Barbi S, Moore PS et al. Primary mediastinal B-cell lymphoma: hypermutation of the BCL6 gene targets motifs different from those in diffuse large B-cell and follicular lymphomas. Haematologica. 2004; 89:1091–9.

1298. Yamaguchi M, Ohno T, Oka K et al. De novo CD5-positive diffuse large B-cell lymphoma: clinical characteristics and therapeutic outcome. Br J Haematol. 1999; 105:1133–9.

1299. Yamaguchi M, Seto M, Okamoto M et al. De novo CD5+ diffuse large B-cell lymphoma: a clinicopathologic study of 109 patients. Blood. 2002;99:815–21.

1300. Harada S, Suzuki R, Uehira K et al. Molecular and immunological dissection of diffuse large B cell lymphoma: CD5+, and CD5– with CD10+ groups may constitute clinically relevant subtypes. Leukemia. 1999; 13:1441–7.

1301. Kroft SH, Howard MS, Picker LJ, Ansari MQ, Aquino DB, McKenna RW. De novo CD5+ diffuse large B-cell lymphomas. A heterogeneous group containing an unusual form of splenic lymphoma. Am J Clin Pathol. 2000;114:523–33.

1302. Tagawa H, Tsuzuki S, Suzuki R et al. Genome-wide array-based comparative genomic hybridization of diffuse large B-cell lymphoma: comparison between CD5-positive and CD5-negative cases. Cancer Res. 2004;64:5948–55.

1303. Yoshioka T, Miura I, Kume M et al. Cytogenetic features of de novo CD5-positive diffuse large B-cell lymphoma: chromosome aberrations affecting 8p21 and 11q13 constitute major subgroups with different overall survival. Genes Chromosomes Cancer. 2005;42:149–57.

1304. Akasaka T, Akasaka H, Ueda C et al. Molecular and clinical features of non-Burkitt's, diffuse large-cell lymphoma of B-cell type associated with the c-MYC/immunoglobulin heavy-chain fusion gene. J Clin Oncol. 2000;18:510–18.

1305. Nakamura N, Nakamine H, Tamaru J et al. The distinction between Burkitt lymphoma and diffuse large B-cell lymphoma with c-myc rearrangement. Mod Pathol. 2002;15:771–6.

1306. Haralambieva E, Boerma EJ, van Imhoff GW et al. Clinical, immunophenotypic, and genetic analysis of adult lymphomas with morphologic features of Burkitt lymphoma. Am J Surg Pathol. 2005;29:1086–94.

1307. Patte C, Auperin A, Michon J et al. The Societe Francaise d'Oncologie Pediatrique LMB89 protocol: highly effective multiagent chemotherapy tailored to the tumor burden and initial response in 561 unselected children with B-cell lymphomas and L3 leukemia. Blood. 2001;97:3370–9.

1308. Laver JH, Kraveka JM, Hutchison RE et al. Advanced-stage large-cell lymphoma in children and adolescents: results of a randomized trial incorporating intermediate-dose methotrexate and high-dose cytarabine in the maintenance phase of the APO regimen: a Pediatric Oncology Group phase III trial. J Clin Oncol. 2005;23:541–7.

1309. Woessmann W, Seidemann K, Mann G et al. The impact of the methotrexate administration schedule and dose in the treatment of children and adolescents with B-cell neoplasms: a report of the BFM Group Study NHL-BFM95. Blood. 2005;105:948–58.

1310. Oschlies I, Klapper W, Zimmermann M et al. Diffuse large B-cell lymphoma in pediatric patients belongs predominantly to the germinal-center type B-cell lymphomas: a clinicopathologic analysis of cases included in the German BFM (Berlin–Frankfurt–Munster) Multicenter Trial. Blood. 2006;107:4047–52.

1311. Simonitsch-Klupp I, Hauser I, Ott G et al. Diffuse large B-cell lymphomas with plasmablastic/plasmacytoid features are associated with TP53 deletions and poor clinical outcome. Leukemia. 2004;18:146–55.

1312. Murphy SB, Bowman WP, Abromowitch M et al. Results of treatment of advanced-stage Burkitt's lymphoma and B cell (SIg+) acute lymphoblastic leukemia with high-dose fractionated cyclophosphamide and coordinated high-dose methotrexate and cytarabine. J Clin Oncol. 1986;4:1732–9.

1313. Patte C, Michon J, Frappaz D et al. Therapy of Burkitt and other B-cell acute lymphoblastic leukaemia and lymphoma: experience with the LMB protocols of the SFOP (French Paediatric Oncology Society) in children and adults. Baillieres Clin Haematol. 1994; 7:339–48.

1314. Blum KA, Lozanski G, Byrd JC. Adult Burkitt leukemia and lymphoma. Blood. 2004;104:3009–20.

1315. Kornblau SM, Goodacre A, Cabanillas F. Chromosomal abnormalities in adult non-endemic Burkitt's lymphoma and leukemia:22 new reports and a review of 148 cases from the literature. Hematol Oncol. 1991;9:63–78.

1316. Fenaux P, Lai JL, Miaux O, Zandecki M, Jouet JP, Bauters F. Burkitt cell acute leukaemia (L3 ALL) in adults: a report of 18 cases. Br J Haematol. 1989; 71:371–6.

1317. Barth TF, Muller S, Pawlita M et al. Homogeneous immunophenotype and paucity of secondary genomic aberrations are distinctive features of endemic but not of sporadic Burkitt's lymphoma and diffuse large B-cell lymphoma with MYC rearrangement. J Pathol. 2004;203:940–5.

1318. Onciu M, Schlette E, Zhou Y et al. Secondary chromosomal abnormalities predict outcome in pediatric and adult high-stage Burkitt lymphoma. Cancer. 2006; 107:1084–92.

1319. Berger R, Le Coniat M, Derre J, Vecchione D. Secondary nonrandom chromosomal abnormalities of band 13q34 in Burkitt lymphoma-leukemia. Genes Chromosomes Cancer. 1989;1:115–18.

1320. Barin C, Valtat C, Briault S et al. Structural rearrangements of chromosome 13 as additional abnormalities in Burkitt lymphoma and type 3 acute lymphoblastic leukemia. Cancer Genet Cytogenet. 1992;60:206–9.

1321. Valnet-Rabier MB, Challier B, Thiebault S et al. c-Flip protein expression in Burkitt's lymphomas is associated with a poor clinical outcome. Br J Haematol. 2005; 128:767–73.

1322. Sigaux F, Berger R, Bernheim A, Valensi F, Daniel MT, Flandrin G. Malignant lymphomas with band 8q24 chromosome abnormality: a morphologic continuum extending from Burkitt's to immunoblastic lymphoma. Br J Haematol. 1984;57:393–405.

1323. Nishida K, Tamura A, Nakazawa N et al. The Ig heavy chain gene is frequently involved in chromosomal translocations in multiple myeloma and plasma cell leukemia as detected by in situ hybridization. Blood. 1997;90:526–34.

1324. Stewart AK, Bergsagel PL, Greipp PR et al. A practical guide to defining high-risk myeloma for clinical trials, patient counseling and choice of therapy. Leukemia. 2007;21:529–34.

1325. Drach J, Schuster J, Nowotny H et al. Multiple myeloma: high incidence of chromosomal aneuploidy as detected by interphase fluorescence in situ hybridization. Cancer Res. 1995;55:3854–9.

1326. Seong C, Delasalle K, Hayes K et al. Prognostic value of cytogenetics in multiple myeloma. Br J Haematol. 1998; 101:189–94.

1327. Calasanz MJ, Cigudosa JC, Odero MD et al. Hypodiploidy and 22q11 rearrangements at diagnosis are associated with poor prognosis in patients with multiple myeloma. Br J Haematol. 1997;98:418–25.

1328. Lai JL, Zandecki M, Mary JY et al. Improved cytogenetics in multiple myeloma: a study of 151 patients including 117 patients at diagnosis. Blood. 1995;85:2490–7.

1329. Lloveras E, Granada I, Zamora L et al. Cytogenetic and fluorescence in situ hybridization studies in 60 patients with multiple myeloma and plasma cell leukemia. Cancer Genet Cytogenet. 2004;148:71–6.

1330. Smadja NV, Fruchart C, Isnard F et al. Chromosomal analysis in multiple myeloma: cytogenetic evidence of two different diseases. Leukemia. 1998;12:960–9.

1331. Wuilleme S, Robillard N, Lode L et al. Ploidy, as detected by fluorescence in situ hybridization, defines different subgroups in multiple myeloma. Leukemia. 2005;19:275–8.

1332. Cremer FW, Bila J, Buck I et al. Delineation of distinct subgroups of multiple myeloma and a model for clonal evolution based on interphase cytogenetics. Genes Chromosomes Cancer. 2005;44:194–203.

1333. Rasillo A, Tabernero MD, Sanchez ML et al. Fluorescence in situ hybridization analysis of aneuploidization patterns in monoclonal gammopathy of undetermined significance versus multiple myeloma and plasma cell leukemia. Cancer. 2003;97:601–9.

1334. Sawyer JR, Tricot G, Mattox S, Jagannath S, Barlogie B. Jumping translocations of chromosome 1q in multiple myeloma: evidence for a mechanism involving decondensation of pericentromeric heterochromatin. Blood. 1998;91:1732–41.

1335. Taniwaki M, Nishida K, Ueda Y, Takashima T. Nonrandom chromosomal rearrangements and their implications in clinical features and outcome of multiple myeloma and plasma cell leukemia. Leuk Lymphoma. 1996;21:25–30.

1336. Hanamura I, Stewart JP, Huang Y et al. Frequent gain of chromosome band 1q21 in plasma-cell dyscrasias detected by fluorescence in situ hybridization: incidence increases from MGUS to relapsed myeloma and is related to prognosis and disease progression following tandem stem-cell transplantation. Blood. 2006;108:1724–32.

1337. Shaughnessy JD, Jr., Zhan F, Burington BE et al. A validated gene expression model of high-risk multiple myeloma is defined by deregulated expression of genes mapping to chromosome 1. Blood. 2007;109:2276–84.

1338. Carrasco DR, Tonon G, Huang Y et al. High-resolution genomic profiles define distinct clinico-pathogenetic subgroups of multiple myeloma patients. Cancer Cell. 2006;9:313–25.

1339. Kroger N, Schilling G, Einsele H et al. Deletion of chromosome band 13q14 as detected by fluorescence in situ hybridization is a prognostic factor in patients with multiple myeloma who are receiving allogeneic dose-reduced stem cell transplantation. Blood. 2004;103:4056–61.

1340. Avet-Loiseau H, Li JY, Morineau N et al. Monosomy 13 is associated with the transition of monoclonal gammopathy of undetermined significance to multiple myeloma. Intergroupe Francophone du Myelome. Blood. 1999;94:2583–9.

1341. Shaughnessy J, Jacobson J, Sawyer J et al. Continuous absence of metaphase-defined cytogenetic abnormalities, especially of chromosome 13 and hypodiploidy, ensures long-term survival in multiple myeloma treated with Total Therapy I: interpretation in the context of global gene expression. Blood. 2003;101: 3849–56.

1342. Jagannath S, Richardson PG, Sonneveld P et al. Bortezomib appears to overcome the poor prognosis conferred by chromosome 13 deletion in phase 2 and 3 trials. Leukemia. 2007;21:151–7.

1343. Garand R, Avet-Loiseau H, Accard F, Moreau P, Harousseau JL, Bataille R. t(11;14) and t(4;14) translocations correlated with mature lymphoplasmacytoid and immature morphology, respectively, in multiple myeloma. Leukemia. 2003;17:2032–5.

1344. Bergsagel PL, Nardini E, Brents L, Chesi M, Kuehl WM. IgH translocations in multiple myeloma: a nearly universal event that rarely involves c-myc. Curr Top Microbiol Immunol. 1997;224:283–7.

1345. Lai JL, Michaux L, Dastugue N et al. Cytogenetics in multiple myeloma: a multicenter study of 24 patients with t(11;14)(q13;q32) or its variant. Cancer Genet Cytogenet. 1998;104:133–8.

1346. Bergsagel PL, Kuehl WM. Chromosome translocations in multiple myeloma. Oncogene. 2001;20:5611–22.

1347. Hideshima T, Bergsagel PL, Kuehl WM, Anderson KC. Advances in biology of multiple myeloma: clinical applications. Blood. 2004;104:607–18.

1348. Kastrinakis NG, Gorgoulis VG, Foukas PG, Dimopoulos MA, Kittas C. Molecular aspects of multiple myeloma. Ann Oncol. 2000;11:1217–28.

1349. Bergsagel PL, Kuehl WM. Molecular pathogenesis and a consequent classification of multiple myeloma. J Clin Oncol. 2005;23:6333–8.

1350. Facon T, Avet-Loiseau H, Guillerm G et al. Chromosome 13 abnormalities identified by FISH analysis and serum beta2-microglobulin produce a powerful myeloma staging system for patients receiving high-dose therapy. Blood. 2001;97:1566–71.

1351. Chiecchio L, Protheroe RK, Ibrahim AH et al. Deletion of chromosome 13 detected by conventional cytogenetics is a critical prognostic factor in myeloma. Leukemia. 2006;20:1610–17.

1352. Robillard N, Avet-Loiseau H, Garand R et al. CD20 is associated with a small mature plasma cell morphology and t(11;14) in multiple myeloma. Blood. 2003;102:1070–1.

1353. Smadja NV, Bastard C, Brigaudeau C, Leroux D, Fruchart C. Hypodiploidy is a major prognostic factor in multiple myeloma. Blood. 2001;98:2229–38.

1354. Tricot G, Barlogie B, Van Rhee F. Treatment advances in multiple myeloma. Br J Haematol. 2004;125:24–30.

1355. Bergsagel PL, Kuehl WM, Zhan F, Sawyer J, Barlogie B, Shaughnessy J Jr. Cyclin D dysregulation: an early and unifying pathogenic event in multiple myeloma. Blood 2005;106:296–303.

1356. De Vos J, Thykjaer T, Tarte K et al. Comparison of gene expression profiling between malignant and normal plasma cells with oligonucleotide arrays. Oncogene. 2002;21:6848–57.

1357. Kuehl WM, Bergsagel PL. Multiple myeloma: evolving genetic events and host interactions. Nat Rev Cancer. 2002;2:175–87.

1358. Gutierrez NC, Hernandez JM, Garcia JL et al. Differences in genetic changes between multiple myeloma and plasma cell leukemia demonstrated by comparative genomic hybridization. Leukemia. 2001;15:840–5.

1359. Liu P, Leong T, Quam L et al. Activating mutations of N- and K-ras in multiple myeloma show different clinical associations: analysis of the Eastern Cooperative Oncology Group Phase III Trial. Blood. 1996;88:2699–706.

1360. Rajkumar SV, Fonseca R, Dewald GW et al. Cytogenetic abnormalities correlate with the plasma cell labeling index and extent of bone marrow involvement in myeloma. Cancer Genet Cytogenet. 1999;113:73–7.

1361. Fonseca R, Debes-Marun CS, Picken EB et al. The recurrent IgH translocations are highly associated with nonhyperdiploid variant multiple myeloma. Blood. 2003;102:2562–7.

1362. Zhan F, Hardin J, Kordsmeier B et al. Global gene expression profiling of multiple myeloma, monoclonal gammopathy of undetermined significance, and normal bone marrow plasma cells. Blood. 2002;99:1745–57.

1363. Lin P, Mahdavy M, Zhan F, Zhang HZ, Katz RL, Shaughnessy JD. Expression of PAX5 in CD20-positive multiple myeloma assessed by immunohistochemistry and oligonucleotide microarray. Mod Pathol. 2004;17:1217–22.

1364. Savage KJ, Chhanabhai M, Gascoyne RD, Connors JM. Characterization of peripheral T-cell lymphomas in a single North American institution by the WHO classification. Ann Oncol. 2004;15:1467–75.

1365. Kluin PM, Feller A, Gaulard P et al. Peripheral T/NK-cell lymphoma: a report of the IXth Workshop of the European Association for Haematopathology. Histopathology. 2001;38:250–70.

1366. Jaffe ES, Krenacs L, Raffeld M. Classification of cytotoxic T-cell and natural killer cell lymphomas. Semin Hematol. 2003;40:175–84.

1367. Jaffe ES, Krenacs L, Raffeld M. Classification of T-cell and NK-cell neoplasms based on the REAL classification. Ann Oncol. 1997;8(Suppl 2):17–24.

1368. Gorczyca W, Weisberger J, Liu Z et al. An approach to diagnosis of T-cell lymphoproliferative disorders by flow cytometry. Cytometry. 2002;50:177–90.

1369. Arrowsmith ER, Macon WR, Kinney MC et al. Peripheral T-cell lymphomas: clinical features and prognostic factors of 92 cases defined by the revised European American lymphoma classification. Leuk Lymphoma. 2003;44:241–9.

1370. Coiffier B, Berger F, Bryon PA, Magaud JP. T-cell lymphomas: immunologic, histologic, clinical, and therapeutic analysis of 63 cases. J Clin Oncol. 1988;6:1584–9.

1371. Gallamini A, Stelitano C, Calvi R et al. Peripheral T-cell lymphoma unspecified (PTCL-U): a new prognostic model from a retrospective multicentric clinical study. Blood. 2004;103:2474–9.

1372. Dearden CE, Foss FM. Peripheral T-cell lymphomas: diagnosis and management. Hematol Oncol Clin North Am. 2003;17:1351–66.

1373. Armitage JO, Weisenburger DD. New approach to classifying non-Hodgkin's lymphomas: clinical features of the major histologic subtypes. Non-Hodgkin's Lymphoma Classification Project. J Clin Oncol. 1998;16:2780–95.

1374. Armitage JO, Vose JM, Linder J et al. Clinical significance of immunophenotype in diffuse aggressive non-Hodgkin's lymphoma. J Clin Oncol. 1989;7:1783–90.

1375. Brown DC, Heryet A, Gatter KC, Mason DY. The prognostic significance of immunophenotype in high-grade non-Hodgkin's lymphoma. Histopathology. 1989;14:621–7.

1376. Cheng AL, Chen YC, Wang CH et al. Direct comparisons of peripheral T-cell lymphoma with diffuse B-cell lymphoma of comparable histological grades – should peripheral T-cell lymphoma be considered separately? J Clin Oncol. 1989;7:725–31.

1377. Lippman SM, Miller TP, Spier CM, Slymen DJ, Grogan TM. The prognostic significance of the immunotype in diffuse large-cell lymphoma: a comparative study of the T-cell and B-cell phenotype. Blood. 1988;72:436–41.

1378. Bartlett NL, Longo DL. T-small lymphocyte disorders. Semin Hematol. 1999;36:164–70.

1379. Matutes E, Brito-Babapulle V, Worner I, Sainati L, Foroni L, Catovsky D. T-cell chronic lymphocytic leukaemia: the spectrum of mature T-cell disorders. Nouv Rev Fr Hematol. 1988;30:347–51.

1380. Matutes E. T-cell prolymphocytic leukemia. Cancer Control. 1998;5:19–24.

1381. Matutes E. Chronic T-cell lymphoproliferative disorders. Rev Clin Exp Hematol. 2002;6:401–420; discussion 449-450.

1382. Garand R, Goasguen J, Brizard A et al. Indolent course as a relatively frequent presentation in T-prolymphocytic leukaemia. Groupe Francais d'Hematologie Cellulaire. Br J Haematol. 1998;103:488–94.

1383. Costa D, Queralt R, Aymerich M et al. High levels of chromosomal imbalances in typical and small-cell variants of T-cell prolymphocytic leukemia. Cancer Genet Cytogenet. 2003;147:36–43.

1384. Sandberg Y, Almeida J, Gonzalez M et al. TCRgammadelta+ large granular lymphocyte leukemias reflect the spectrum of normal antigen-selected TCRgammadelta+ T-cells. Leukemia. 2006;20:505–13.

1385. Dhodapkar MV, Li CY, Lust JA, Tefferi A, Phyliky RL. Clinical spectrum of clonal proliferations of T-large granular lymphocytes: a T-cell clonopathy of undetermined significance? Blood. 1994;84:1620–7.

1386. Lamy T, Loughran TP, Jr. Clinical features of large granular lymphocyte leukemia. Semin Hematol. 2003; 40:185–95.

1387. Loughran TP, Jr. Clonal diseases of large granular lymphocytes. Blood. 1993;82:1–14.

1388. Morice WG, Kurtin PJ, Tefferi A, Hanson CA. Distinct bone marrow findings in T-cell granular lymphocytic leukemia revealed by paraffin section immunoperoxidase stains for CD8, TIA-1, and granzyme B. Blood. 2002;99:268–74.

1389. Sood R, Stewart CC, Aplan PD et al. Neutropenia associated with T-cell large granular lymphocyte leukemia: long-term response to cyclosporine therapy despite persistence of abnormal cells. Blood. 1998;91:3372–8.

1390. Osuji N, Matutes E, Tjonnfjord G et al. T-cell large granular lymphocyte leukemia: a report on the treatment of 29 patients and a review of the literature. Cancer. 2006;107:570–8.

1391. Wong KF, Chan JC, Liu HS, Man C, Kwong YL. Chromosomal abnormalities in T-cell large granular lymphocyte leukaemia: report of two cases and review of the literature. Br J Haematol. 2002;116:598–600.

1392. Man C, Au WY, Pang A, Kwong YL. Deletion 6q as a recurrent chromosomal aberration in T-cell large granular lymphocyte leukemia. Cancer Genet Cytogenet. 2002;139:71–4.

1393. Salido M, Sole F, Espinet B, Zamora L, Woessner S, Florensa L. New t(11;12)(q12;q11) characterized by RxFISH in a patient with T-cell large granular lymphocyte leukemia. Cancer Genet Cytogenet. 2001;125:70–3.

1394. Brito-Babapulle V, Matutes E, Foroni L, Pomfret M, Catovsky D. A t(8;14)(q24;q32) in a T-lymphoma/leukemia of CD8+ large granular lymphocytes. Leukemia. 1987;1:789–94.

1395. Schmidt HH, Pirc-Danoewinata H, Panzer-Grumayer ER et al. Translocation (3;5)(p26;q13) in a patient with chronic T-cell lymphoproliferative disorder. Cancer Genet Cytogenet. 1998;104:82–5.

1396. Poiesz BJ, Ruscetti FW, Gazdar AF, Bunn PA, Minna JD, Gallo RC. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. Proc Natl Acad Sci USA. 1980;77:7415–19.

1397. Blattner WA, Kalyanaraman VS, Robert-Guroff M et al. The human type-C retrovirus, HTLV, in Blacks from the Caribbean region, and relationship to adult T-cell leukemia/lymphoma. Int J Cancer. 1982; 30:257–64.

1398. Shimoyama M. Diagnostic criteria and classification of clinical subtypes of adult T-cell leukaemia-lymphoma. A report from the Lymphoma Study Group (1984–87). Br J Haematol. 1991;79:428–37.

1399. Takatsuki K, Yamaguchi K, Kawano F et al. Clinical diversity in adult T-cell leukemia-lymphoma. Cancer Res. 1985;45:4644s-4645s.

1400. Kawano F, Yamaguchi K, Nishimura H, Tsuda H, Takatsuki K. Variation in the clinical courses of adult T-cell leukemia. Cancer. 1985;55:851–6.

1401. Bazarbachi A, Ghez D, Lepelletier Y et al. New therapeutic approaches for adult T-cell leukaemia. Lancet Oncol. 2004;5:664–72.

1402. Yamaguchi K, Watanabe T. Human T lymphotropic virus type-I and adult T-cell leukemia in Japan. Int J Hematol. 2002;76(Suppl 2):240–5.

1403. Shimoyama M. Treatment of patients with adult T-cell leukemia-lymphoma: an overview. In: Takatsuki K, Hinuma, Y., Yoshida, M., eds. Advances in Adult T-Cell Leukemia and HTLV-1 Research. Tokyo: Japan Scientific Societies Press; 1992:43–6.

1404. Shimoyama M. Chemotherapy of ATL. In: Takatsuki K, ed. Adult T-Cell Leukemia. London: Oxford University Press; 1994:221–36.

1405. Jaffe ES, Blattner WA, Blayney DW et al. The pathologic spectrum of adult T-cell leukemia/lymphoma in the United States. Human T-cell leukemia/lymphoma virus-associated lymphoid malignancies. Am J Surg Pathol. 1984;8:263–75.

1406. Tsukasaki K, Krebs J, Nagai K et al. Comparative genomic hybridization analysis in adult T-cell leukemia/lymphoma: correlation with clinical course. Blood. 2001;97:3875–81.

1407. Oshiro A, Tagawa H, Ohshima K et al. Identification of subtype-specific genomic alterations in aggressive adult T-cell leukemia/lymphoma. Blood. 2006;107:4500–7.

1408. Ascani S, Zinzani PL, Gherlinzoni F et al. Peripheral T-cell lymphomas. Clinico-pathologic study of 168 cases diagnosed according to the R.E.A.L. Classification. Ann Oncol. 1997;8:583–92.

1409. Gisselbrecht C, Gaulard P, Lepage E et al. Prognostic significance of T-cell phenotype in aggressive non-Hodgkin's lymphomas. Groupe d'Etudes des Lymphomes de l'Adulte (GELA). Blood. 1998;92:76–82.

1410. Kim K, Kim WS, Jung CW et al. Clinical features of peripheral T-cell lymphomas in 78 patients diagnosed according to the Revised European-American lymphoma (REAL) classification. Eur J Cancer. 2002;38:75–81.

1411. Lopez-Guillermo A, Cid J, Salar A et al. Peripheral T-cell lymphomas: initial features, natural history, and prognostic factors in a series of 174 patients diagnosed according to the R.E.A.L. Classification. Ann Oncol. 1998;9:849–55.

1412. Ansell SM, Habermann TM, Kurtin PJ et al. Predictive capacity of the International Prognostic Factor Index in patients with peripheral T-cell lymphoma. J Clin Oncol. 1997;15:2296–301.

1413. Thorns C, Bastian B, Pinkel D et al. Chromosomal aberrations in angioimmunoblastic T-cell lymphoma and peripheral T-cell lymphoma unspecified: A matrix-based CGH approach. Genes Chromosomes Cancer. 2007;46:37–44.

1414. Streubel B, Vinatzer U, Willheim M, Raderer M, Chott A. Novel t(5;9)(q33;q22) fuses ITK to SYK in unspecified peripheral T-cell lymphoma. Leukemia. 2006;20:313–18.

1415. Lakkala-Paranko T, Franssila K, Lappalainen K et al. Chromosome abnormalities in peripheral T-cell lymphoma. Br J Haematol. 1987;66:451–60.

1416. Lepretre S, Buchonnet G, Stamatoullas A et al. Chromosome abnormalities in peripheral T-cell lymphoma. Cancer Genet Cytogenet. 2000;117:71–9.

1417. Schlegelberger B, Himmler A, Godde E, Grote W, Feller AC, Lennert K. Cytogenetic findings in peripheral T-cell lymphomas as a basis for distinguishing low-grade and high-grade lymphomas. Blood. 1994;83:505–11.

1418. Kaneko Y, Maseki N, Sakurai M et al. Characteristic karyotypic pattern in T-cell lymphoproliferative disorders with reactive 'angioimmunoblastic lymphadenopathy with dysproteinema-type' features. Blood. 1988;72:413–21.

1419. Schlegelberger B, Zhang Y, Weber-Matthiesen K, Grote W. Detection of aberrant clones in nearly all cases of angioimmunoblastic lymphadenopathy with dysproteinemia-type T-cell lymphoma by combined interphase and metaphase cytogenetics. Blood. 1994;84:2640–8.

1420. O'Connor NT, Crick JA, Wainscoat JS et al. Evidence for monoclonal T lymphocyte proliferation in angioimmunoblastic lymphadenopathy. J Clin Pathol. 1986;39:1229–32.

1421. Feller AC, Griesser H, Schilling CV et al. Clonal gene rearrangement patterns correlate with immunophenotype and clinical parameters in patients with angioimmunoblastic lymphadenopathy. Am J Pathol. 1988;133:549–56.

1422. Weiss LM, Strickler JG, Dorfman RF, Horning SJ, Warnke RA, Sklar J. Clonal T-cell populations in angioimmunoblastic lymphadenopathy and angioimmunoblastic lymphadenopathy-like lymphoma. Am J Pathol. 1986;122:392–7.

1423. Lipford EH, Smith HR, Pittaluga S, Jaffe ES, Steinberg AD, Cossman J. Clonality of angioimmunoblastic lymphadenopathy and implications for its evolution to malignant lymphoma. J Clin Invest. 1987;79:637–42.

1424. Tan BT, Warnke RA, Arber DA. The frequency of B- and T-cell gene rearrangements and Epstein–Barr virus in T-cell lymphomas: a comparison between angioimmunoblastic T-cell lymphoma and peripheral T-cell lymphoma, unspecified with and without associated B-cell proliferations. J Mol Diagn. 2006;8:466–475; quiz 527.

1425. Attygalle AD, Chuang SS, Diss TC, Du MQ, Isaacson PG, Dogan A. Distinguishing angioimmunoblastic T-cell lymphoma from peripheral T-cell lymphoma, unspecified, using morphology, immunophenotype and molecular genetics. Histopathology. 2007;50:498–508.

1426. Cooke CB, Krenacs L, Stetler-Stevenson M et al. Hepatosplenic T-cell lymphoma: a distinct clinicopathologic entity of cytotoxic gamma delta T-cell origin. Blood. 1996;88:4265–74.

1427. Farcet JP, Gaulard P, Marolleau JP et al. Hepatosplenic T-cell lymphoma: sinusal/sinusoidal localization of malignant cells expressing the T-cell receptor gamma delta. Blood. 1990;75:2213–19.

1428. Przybylski GK, Wu H, Macon WR et al. Hepatosplenic and subcutaneous panniculitis-like gamma/delta T cell lymphomas are derived from different Vdelta subsets of gamma/delta T lymphocytes. J Mol Diagn. 2000;2:11–19.

1429. Vega F, Medeiros LJ, Gaulard P. Hepatosplenic and other gammadelta T-cell lymphomas. Am J Clin Pathol. 2007;127:869–80.

1430. Wong KF, Chan JK, Matutes E et al. Hepatosplenic gamma delta T-cell lymphoma. A distinctive aggressive lymphoma type. Am J Surg Pathol. 1995;19:718–26.

1431. Kumar S, Lawlor C, Jaffe ES. Hepatosplenic T-cell lymphoma of alphabeta lineage. Am J Surg Pathol. 2001;25:970–1.

1432. Lai R, Larratt LM, Etches W et al. Hepatosplenic T-cell lymphoma of alphabeta lineage in a 16-year-old boy presenting with hemolytic anemia and thrombocytopenia. Am J Surg Pathol. 2000;24:459–63.

1433. Macon WR, Levy NB, Kurtin PJ et al. Hepatosplenic alphabeta T-cell lymphomas: a report of 14 cases and comparison with hepatosplenic gammadelta T-cell lymphomas. Am J Surg Pathol. 2001;25:285–96.

1434. Suarez F, Wlodarska I, Rigal-Huguet F et al. Hepatosplenic alphabeta T-cell lymphoma: an unusual case with clinical, histologic, and cytogenetic features of gammadelta hepatosplenic T-cell lymphoma. Am J Surg Pathol. 2000;24:1027–32.

1435. Tamaska J, Adam E, Kozma A et al. Hepatosplenic gammadelta T-cell lymphoma with ring chromosome 7, an isochromosome 7q equivalent clonal chromosomal aberration. Virchows Arch. 2006;449:479–83.

1436. Zettl A, deLeeuw R, Haralambieva E, Mueller-Hermelink HK. Enteropathy-type T-cell lymphoma. Am J Clin Pathol. 2007;127:701–6.

1437. Verkarre V, Romana SP, Cellier C et al. Recurrent partial trisomy 1q22–q44 in clonal intraepithelial lymphocytes in refractory celiac sprue. Gastroenterology. 2003;125:40–6.

1438. Deleeuw RJ, Zettl A, Klinker E et al. Whole-genome analysis and HLA genotyping of enteropathy-type T-cell lymphoma reveals 2 distinct lymphoma subtypes. Gastroenterology. 2007;132:1902–11.

1439. Zettl A, Ott G, Makulik A et al. Chromosomal gains at 9q characterize enteropathy-type T-cell lymphoma. Am J Pathol. 2002;161:1635–45.

1440. Massone C, Chott A, Metze D et al. Subcutaneous, blastic natural killer (NK), NK/T-cell, and other cytotoxic lymphomas of the skin: a morphologic, immunophenotypic, and molecular study of 50 patients. Am J Surg Pathol. 2004;28:719–35.

1441. Al-Hakeem DA, Fedele S, Carlos R, Porter S. Extranodal NK/T-cell lymphoma, nasal type. Oral Oncol. 2007; 43:4–14.

1442. Takahara M, Kishibe K, Bandoh N, Nonaka S, Harabuchi Y. P53, N- and K-Ras, and beta-catenin gene mutations and prognostic factors in nasal NK/T-cell lymphoma from Hokkaido, Japan. Hum Pathol. 2004;35:86–95.

1443. Wong KF, Zhang YM, Chan JK. Cytogenetic abnormalities in natural killer cell lymphoma/leukaemia – is there a consistent pattern? Leuk Lymphoma. 1999;34:241–50.

1444. Nakashima Y, Tagawa H, Suzuki R et al. Genome-wide array-based comparative genomic hybridization of natural killer cell lymphoma/leukemia: different genomic alteration patterns of aggressive NK-cell leukemia and extranodal Nk/T-cell lymphoma, nasal type. Genes Chromosomes Cancer. 2005;44:247–55.

1445. Diamandidou E, Cohen PR, Kurzrock R. Mycosis fungoides and Sezary syndrome. Blood. 1996;88:2385–409.

1446. Hoppe RT, Wood GS, Abel EA. Mycosis fungoides and the Sezary syndrome: pathology, staging, and treatment. Curr Probl Cancer. 1990;14:293–371.

1447. Kim YH, Hoppe RT. Mycosis fungoides and the Sezary syndrome. Semin Oncol. 1999;26:276–89.

1448. Kim YH, Liu HL, Mraz-Gernhard S, Varghese A, Hoppe RT. Long-term outcome of 525 patients with mycosis fungoides and Sezary syndrome: clinical prognostic factors and risk for disease progression. Arch Dermatol. 2003;139:857–66.

1449. van Doorn R, Van Haselen CW, van Voorst Vader PC, et al. Mycosis fungoides: disease evolution and prognosis

of 309 Dutch patients. Arch Dermatol. 2000;136: 504–10.

1450. Batista DA, Vonderheid EC, Hawkins A et al. Multicolor fluorescence in situ hybridization (SKY) in mycosis fungoides and Sezary syndrome: search for recurrent chromosome abnormalities. Genes Chromosomes Cancer. 2006;45:383–91.

1451. Thangavelu M, Finn WG, Yelavarthi KK et al. Recurring structural chromosome abnormalities in peripheral blood lymphocytes of patients with mycosis fungoides/Sezary syndrome. Blood. 1997;89:3371–7.

1452. Wood GS, Tung RM, Haeffner AC et al. Detection of clonal T-cell receptor gamma gene rearrangements in early mycosis fungoides/Sezary syndrome by polymerase chain reaction and denaturing gradient gel electrophoresis (PCR/DGGE). J Invest Dermatol. 1994;103:34–41.

1453. Trainor KJ, Brisco MJ, Wan JH, Neoh S, Grist S, Morley AA. Gene rearrangement in B- and T-lymphoproliferative disease detected by the polymerase chain reaction. Blood. 1991;78:192–6.

1454. Bottaro M, Berti E, Biondi A, Migone N, Crosti L. Heteroduplex analysis of T-cell receptor gamma gene rearrangements for diagnosis and monitoring of cutaneous T-cell lymphomas. Blood. 1994;83:3271–8.

1455. Delfau-Larue MH, Dalac S, Lepage E et al. Prognostic significance of a polymerase chain reaction-detectable dominant T-lymphocyte clone in cutaneous lesions of patients with mycosis fungoides. Blood. 1998;92:3376–80.

1456. Costa C, Gallardo F, Pujol RM et al. Comparative analysis of TCR-gamma gene rearrangements by Genescan and polyacrylamide gel-electrophoresis in cutaneous T-cell lymphoma. Acta Derm Venereol. 2004;84:6–11.

1457. Siegel RS, Pandolfino T, Guitart J, Rosen S, Kuzel TM. Primary cutaneous T-cell lymphoma: review and current concepts. J Clin Oncol. 2000;18:2908–25.

1458. Fraser-Andrews EA, Mitchell T, Ferreira S et al. Molecular staging of lymph nodes from 60 patients with mycosis fungoides and Sezary syndrome: correlation with histopathology and outcome suggests prognostic relevance in mycosis fungoides. Br J Dermatol. 2006;155:756–62.

1459. Lynch JW, Jr., Linoilla I, Sausville EA et al. Prognostic implications of evaluation for lymph node involvement by T-cell antigen receptor gene rearrangement in mycosis fungoides. Blood. 1992;79:3293–9.

1460. Theodorou I, Delfau-Larue MH, Bigorgne C et al. Cutaneous T-cell infiltrates: analysis of T-cell receptor gamma gene rearrangement by polymerase chain reaction and denaturing gradient gel electrophoresis. Blood. 1995;86:305–10.

1461. Muche JM, Lukowsky A, Asadullah K, Gellrich S, Sterry W. Demonstration of frequent occurrence of clonal T- cells in the peripheral blood of patients with primary cutaneous T-cell lymphoma. Blood. 1997;90:1636–42.

1462. Fraser-Andrews EA, Woolford AJ, Russell-Jones R, Seed PT, Whittaker SJ. Detection of a peripheral blood T-cell clone is an independent prognostic marker in mycosis fungoides. J Invest Dermatol. 2000;114:117–21.

1463. Beylot-Barry M, Sibaud V, Thiebaut R et al. Evidence that an identical T-cell clone in skin and peripheral blood lymphocytes is an independent prognostic factor in primary cutaneous T-cell lymphomas. J Invest Dermatol. 2001;117:920–6.

1464. Muche JM, Lukowsky A, Ahnhudt C, Gellrich S, Sterry W. Peripheral blood T-cell clonality in mycosis fungoides – an independent prognostic marker? J Invest Dermatol. 2000;115:504–5.

1465. Muche JM, Sterry W, Gellrich S, Rzany B, Audring H, Lukowsky A. Peripheral blood T-cell clonality in mycosis fungoides and nonlymphoma controls. Diagn Mol Pathol. 2003;12:142–50.

1466. Laetsch B, Haffner AC, Dobbeling U et al. CD4 + /CD7– T cell frequency and polymerase chain reaction-based clonality assay correlate with stage in cutaneous T-cell lymphomas. J Invest Dermatol. 2000;114:107–11.

1467. Vega F, Luthra R, Medeiros LJ et al. Clonal heterogeneity in mycosis fungoides and its relationship to clinical course. Blood. 2002;100:3369–73.

1468. Stein H, Foss HD, Durkop H et al. CD30(+) anaplastic large cell lymphoma: a review of its histopathologic, genetic, and clinical features. Blood. 2000;96:3681–95.

1469. Falini B, Pileri S, Zinzani PL et al. ALK+ lymphoma: clinico-pathological findings and outcome. Blood. 1999;93:2697–706.

1470. Mason DY, Pulford KA, Bischof D et al. Nucleolar localization of the nucleophosmin-anaplastic lymphoma kinase is not required for malignant transformation. Cancer Res. 1998;58:1057–62.

1471. Liang X, Meech SJ, Odom LF et al. Assessment of t(2;5)(p23;q35) translocation and variants in pediatric ALK+ anaplastic large cell lymphoma. Am J Clin Pathol. 2004;121:496–506.

1472. Shiota M, Mori S. Anaplastic large cell lymphomas expressing the novel chimeric protein p80NPM/ALK: a distinct clinicopathologic entity. Leukemia. 1997;11(Suppl 3): 538–40.

1473. Gascoyne RD, Aoun P, Wu D et al. Prognostic significance of anaplastic lymphoma kinase (ALK) protein expression in adults with anaplastic large cell lymphoma. Blood. 1999;93:3913–21.

1474. Benharroch D, Meguerian-Bedoyan Z, Lamant L et al. ALK-positive lymphoma: a single disease with a broad spectrum of morphology. Blood. 1998;91:2076–84.

1475. Falini B, Bigerna B, Fizzotti M et al. ALK expression defines a distinct group of T/null lymphomas ('ALK lymphomas') with a wide morphological spectrum. Am J Pathol. 1998;153:875–86.

1476. Lones MA, Heerema NA, Le Beau MM et al. Chromosome abnormalities in advanced stage lymphoblastic lymphoma of children and adolescents: a report from CCG-E08. Cancer Genet Cytogenet. 2007;172:1–11.

1477. Kansal R, Sait SN, Block AW et al. Extra copies of chromosome 2 are a recurring aberration in ALK-negative lymphomas with anaplastic morphology. Mod Pathol. 2005;18:235–43.

1478. Mao X, Orchard G, Lillington DM, Russell-Jones R, Young BD, Whittaker S. Genetic alterations in primary cutaneous CD30+ anaplastic large cell lymphoma. Genes Chromosomes Cancer. 2003;37:176–85.

1479. Garcia JF, Camacho FI, Morente M et al. Hodgkin and Reed–Sternberg cells harbor alterations in the major tumor suppressor pathways and cell-cycle checkpoints: analyses using tissue microarrays. Blood. 2003;101:681–9.

1480. Colby TV, Hoppe RT, Warnke RA. Hodgkin's disease: a clinicopathologic study of 659 cases. Cancer. 1982;49:1848–58.

1481. Wlodarska I, Nooyen P, Maes B et al. Frequent occurrence of BCL6 rearrangements in nodular lymphocyte predominance Hodgkin lymphoma but not in classical Hodgkin lymphoma. Blood. 2003;101:706–10.

1482. Wlodarska I, Stul M, De Wolf-Peeters C, Hagemeijer A. Heterogeneity of BCL6 rearrangements in nodular lymphocyte predominant Hodgkin's lymphoma. Haematologica. 2004;89:965–72.

1483. Renne C, Martin-Subero JI, Hansmann ML, Siebert R. Molecular cytogenetic analyses of immunoglobulin loci in nodular lymphocyte predominant Hodgkin's lymphoma reveal a recurrent IGH-BCL6 juxtaposition. J Mol Diagn. 2005;7:352–6.

1484. Appelbaum FR, Rowe JM, Radich J, Dick JE. Acute myeloid leukemia. Hematology (Am Soc Hematol Educ Program). 2001:62–86.

1485. Lowenberg B, Downing JR, Burnett A. Acute myeloid leukemia. N Engl J Med. 1999;341:1051–62.

1486. Rathnasabapathy R, Lancet JE. Management of acute myelogenous leukemia in the elderly. Cancer Control. 2003;10:469–77.

1487. Farag SS, Archer KJ, Mrozek K et al. Pretreatment cytogenetics add to other prognostic factors predicting complete remission and long-term outcome in patients 60 years of age or older with acute myeloid leukemia: results from Cancer and Leukemia Group B 8461. Blood. 2006;108:63–73.

1488. Dastugue N, Payen C, Lafage-Pochitaloff M et al. Prognostic significance of karyotype in de novo adult acute myeloid leukemia. The BGMT group. Leukemia. 1995;9:1491–8.

1489. Slovak ML, Kopecky KJ, Cassileth PA et al. Karyotypic analysis predicts outcome of preremission and postremission therapy in adult acute myeloid leukemia: a Southwest Oncology Group/Eastern Cooperative Oncology Group Study. Blood. 2000;96:4075–83.

1490. Tallman MS. Acute promyelocytic leukemia as a paradigm for targeted therapy. Semin Hematol. 2004;41:27–32.

1491. Fenaux P, Chevret S, de Botton S. Treatment of older adults with acute promyelocytic leukaemia. Best Pract Res Clin Haematol. 2003;16:495–501.

1492. Fenaux P, Chomienne C, Degos L. Acute promyelocytic leukemia: biology and treatment. Semin Oncol. 1997;24:92–102.

1493. Fenaux P, Degos L. Differentiation therapy for acute promyelocytic leukemia. N Engl J Med. 1997;337:1076–7.

1494. Schlenk RF, Benner A, Hartmann F et al. Risk-adapted postremission therapy in acute myeloid leukemia: results of the German multicenter AML HD93 treatment trial. Leukemia. 2003;17:1521–8.

1495. Mrozek K, Heinonen K, Lawrence D et al. Adult patients with de novo acute myeloid leukemia and t(9; 11)(p22; q23) have a superior outcome to patients with other translocations involving band 11q23: a cancer and leukemia group B study. Blood. 1997;90:4532–8.

1496. Buchner T, Hiddemann W, Wormann B et al. Double induction strategy for acute myeloid leukemia: the effect of high-dose cytarabine with mitoxantrone instead of standard-dose cytarabine with daunorubicin and 6-thioguanine: a randomized trial by the German AML Cooperative Group. Blood. 1999;93:4116–24.

1497. Pompetti F, Spadano A, Sau A et al. Long-term remission in BCR/ABL-positive AML-M6 patient treated with Imatinib Mesylate. Leuk Res. 2007;31:563–7.

1498. Soupir CP, Vergilio JA, Dal Cin P et al. Philadelphia chromosome-positive acute myeloid leukemia: a rare

aggressive leukemia with clinicopathologic features distinct from chronic myeloid leukemia in myeloid blast crisis. Am J Clin Pathol. 2007;127:642–50.

1499. Baldus CD, Mrozek K, Marcucci G, Bloomfield CD. Clinical outcome of de novo acute myeloid leukaemia patients with normal cytogenetics is affected by molecular genetic alterations: a concise review. Br J Haematol. 2007;137:387–400.

1500. Metzgeroth G, Walz C, Score J et al. Recurrent finding of the FIP1L1-PDGFRA fusion gene in eosinophilia-associated acute myeloid leukemia and lymphoblastic T-cell lymphoma. Leukemia. 2007;21:1183–8.

1501. Kottaridis PD, Gale RE, Frew ME et al. The presence of a FLT3 internal tandem duplication in patients with acute myeloid leukemia (AML) adds important prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy: analysis of 854 patients from the United Kingdom Medical Research Council AML 10 and 12 trials. Blood. 2001;98:1752–9.

1502. Kiyoi H, Naoe T, Nakano Y et al. Prognostic implication of FLT3 and N-RAS gene mutations in acute myeloid leukemia. Blood. 1999;93:3074–80.

1503. Kottaridis PD, Gale RE, Linch DC. Prognostic implications of the presence of FLT3 mutations in patients with acute myeloid leukemia. Leuk Lymphoma. 2003;44: 905–13.

1504. Beaupre DM, Kurzrock R. RAS and leukemia: from basic mechanisms to gene-directed therapy. J Clin Oncol. 1999;17:1071–9.

1505. Valk PJ, Verhaak RG, Beijen MA et al. Prognostically useful gene-expression profiles in acute myeloid leukemia. N Engl J Med. 2004;350:1617–28.

1506. Steudel C, Wermke M, Schaich M et al. Comparative analysis of MLL partial tandem duplication and FLT3 internal tandem duplication mutations in 956 adult patients with acute myeloid leukemia. Genes Chromosomes Cancer. 2003;37:237–51.

1507. Falini B, Nicoletti I, Martelli MF, Mecucci C. Acute myeloid leukemia carrying cytoplasmic/mutated nucleophosmin (NPMc+ AML): biologic and clinical features. Blood. 2007;109:874–85.

1508. Nakano Y, Naoe T, Kiyoi H et al. Prognostic value of p53 gene mutations and the product expression in de novo acute myeloid leukemia. Eur J Haematol. 2000;65:23–31.

1509. Karakas T, Miething CC, Maurer U et al. The coexpression of the apoptosis-related genes bcl-2 and wt1 in predicting survival in adult acute myeloid leukemia. Leukemia. 2002;16:846–54.

1510. List AF. Role of multidrug resistance and its pharmacological modulation in acute myeloid leukemia. Leukemia. 1996;10:937–42.

1511. Murren JR. Modulating multidrug resistance: can we target this therapy? Clin Cancer Res. 2002;8:633–5.

1512. Avvisati G, ten Cate JW, Mandelli F. Acute promyelocytic leukaemia. Br J Haematol. 1992;81:315–20.

1513. Avvisati G, Lo Coco F, Mandelli F. Acute promyelocytic leukemia: clinical and morphologic features and prognostic factors. Semin Hematol. 2001;38:4–12.

1514. Brunel V, Lafage-Pochitaloff M, Alcalay M, Pelicci PG, Birg F. Variant and masked translocations in acute promyelocytic leukemia. Leuk Lymphoma. 1996;22: 221–8.

1515. Corey SJ, Locker J, Oliveri DR et al. A non-classical translocation involving 17q12 (retinoic acid receptor alpha) in acute promyelocytic leukemia (APML) with atypical features. Leukemia. 1994;8:1350–3.

1516. Bloomfield CD, Shuma C, Regal L et al. Long-term survival of patients with acute myeloid leukemia: a third follow-up of the Fourth International Workshop on Chromosomes in Leukemia. Cancer. 1997;80:2191–8.

1517. Sainty D, Liso V, Cantu-Rajnoldi A et al. A new morphologic classification system for acute promyelocytic leukemia distinguishes cases with underlying PLZF/RARA gene rearrangements. Group Francais de Cytogenetique Hematologique, UK Cancer Cytogenetics Group and BIOMED 1 European Community-Concerted Action Molecular Cytogenetic Diagnosis in Haematological Malignancies. Blood. 2000;96:1287–96.

1518. Haferlach T, Schoch C, Schnittger S, Kern W, Loffler H, Hiddemann W. Distinct genetic patterns can be identified in acute monoblastic and acute monocytic leukaemia (FAB AML M5a and M5b): a study of 124 patients. Br J Haematol. 2002;118:426–31.

1519. Sun T, Wu E. Acute monoblastic leukemia with t(8;16): a distinct clinicopathologic entity; report of a case and review of the literature. Am J Hematol. 2001;66:207–12.

1520. Murati A, Adelaide J, Popovici C et al. A further case of acute myelomonocytic leukemia with inv(8) chromosomal rearrangement and MOZ-NCOA2 gene fusion. Int J Mol Med. 2003;12:423–8.

1521. Lessard M, Struski S, Leymarie V et al. Cytogenetic study of 75 erythroleukemias. Cancer Genet Cytogenet. 2005;163:113–22.

1522. Preiss BS, Kerndrup GB, Schmidt KG et al. Cytogenetic findings in adult de novo acute myeloid leukaemia.

A population-based study of 303/337 patients. Br J Haematol. 2003;123:219–34.

1523. Cuneo A, Van Orshoven A, Michaux JL et al. Morphologic, immunologic and cytogenetic studies in erythroleukaemia: evidence for multilineage involvement and identification of two distinct cytogenetic-clinicopathological types. Br J Haematol. 1990;75: 346–54.

1524. Olopade OI, Thangavelu M, Larson RA et al. Clinical, morphologic, and cytogenetic characteristics of 26 patients with acute erythroblastic leukemia. Blood. 1992;80: 2873–82.

1525. Garand R, Duchayne E, Blanchard D et al. Minimally differentiated erythroleukaemia (AML M6 'variant'): a rare subset of AML distinct from AML M6. Groupe Francais d'Hematologie Cellulaire. Br J Haematol. 1995;90:868–75.

1526. Park S, Picard F, Azgui Z et al. Erythroleukemia: a comparison between the previous FAB approach and the WHO classification. Leuk Res. 2002;26:423–9.

1527. Cuneo A, Ferrant A, Michaux JL et al. Philadelphia chromosome-positive acute myeloid leukemia: cytoimmunologic and cytogenetic features. Haematologica. 1996;81:423–7.

1528. Yamamoto K, Nakamura Y, Arai H, Saito K, Furusawa S. Triple Philadelphia chromosomes with major-bcr rearrangement in hypotriploid erythroleukaemia. Eur J Haematol. 2000;65:182–7.

1529. Dastugue N, Lafage-Pochitaloff M, Pages MP et al. Cytogenetic profile of childhood and adult megakaryoblastic leukemia (M7): a study of the Groupe Francais de Cytogenetique Hematologique (GFCH). Blood. 2002;100:618–26.

1530. Cuneo A, Mecucci C, Kerim S et al. Multipotent stem cell involvement in megakaryoblastic leukemia: cytologic and cytogenetic evidence in 15 patients. Blood. 1989; 74:1781–90.

1531. Tallman MS, Neuberg D, Bennett JM et al. Acute megakaryocytic leukemia: the Eastern Cooperative Oncology Group experience. Blood. 2000;96: 2405–11.

1532. Ribeiro RC, Oliveira MS, Fairclough D et al. Acute megakaryoblastic leukemia in children and adolescents: a retrospective analysis of 24 cases. Leuk Lymphoma. 1993;10:299–306.

1533. Athale UH, Razzouk BI, Raimondi SC et al. Biology and outcome of childhood acute megakaryoblastic leukemia: a single institution's experience. Blood. 2001;97:3727–32.

1534. Chan WC, Carroll A, Alvarado CS et al. Acute megakaryoblastic leukemia in infants with t(1;22)(p13;q13) abnormality. Am J Clin Pathol. 1992;98:214–21.

1535. Tosi S, Ballabio E, Teigler-Schlegel A, Boultwood J, Bruch J, Harbott J. Characterization of 6q abnormalities in childhood acute myeloid leukemia and identification of a novel t(6;11)(q24.1;p15.5) resulting in a NUP98-C6orf80 fusion in a case of acute megakaryoblastic leukemia. Genes Chromosomes Cancer. 2005;44:225–32.

1536. Ohyashiki K, Ohyashiki JH, Hojo H et al. Cytogenetic findings in adult acute leukemia and myeloproliferative disorders with an involvement of megakaryocyte lineage. Cancer. 1990;65:940–8.

1537. Alvarez S, MacGrogan D, Calasanz MJ, Nimer SD, Jhanwar SC. Frequent gain of chromosome 19 in megakaryoblastic leukemias detected by comparative genomic hybridization. Genes Chromosomes Cancer. 2001;32:285–93.

1538. Chitlur MB, Bhambhani K, Mohamed AN, Ravindranath Y, Savasan S. Acute megakaryoblastic leukemia with t(17;22)(q21;q13) and liver dysfunction. Cancer Genet Cytogenet. 2004;154:167–8.

1539. Ascani S, Piccaluga PP, Pileri SA. Granulocytic sarcoma of main biliary ducts. Br J Haematol. 2003;121:534.

1540. Pileri SA, Ascani S, Cox MC et al. Myeloid sarcoma: clinico-pathologic, phenotypic and cytogenetic analysis of 92 adult patients. Leukemia. 2007;21:340–50.

1541. Gorczyca W, Weisberger J, Seiter K. Colonic adenomas with extramedullary myeloid tumor (granulocytic sarcoma). Leuk Lymphoma. 1999;34:621–4.

1542. Pui CH. Childhood leukemias. N Engl J Med. 1995;332:1618–30.

1543. Pui CH. Recent advances in childhood acute lymphoblastic leukemia. J Formos Med Assoc. 2004;103:85–95.

1544. Pui CH, Evans WE. Acute lymphoblastic leukemia. N Engl J Med. 1998;339:605–15.

1545. Pui CH, Evans WE. Treatment of acute lymphoblastic leukemia. N Engl J Med. 2006;354:166–78.

1546. Pui CH, Relling MV, Downing JR. Acute lymphoblastic leukemia. N Engl J Med. 2004;350:1535–48.

1547. Greaves MF, Janossy G, Peto J, Kay H. Immunologically defined subclasses of acute lymphoblastic leukaemia in children: their relationship to presentation features and prognosis. Br J Haematol. 1981;48:179–97.

1548. Baccarani M, Corbelli G, Amadori S et al. Adolescent and adult acute lymphoblastic leukemia: prognostic features and outcome of therapy. A study of 293 patients. Blood. 1982;60:677–84.

1549. Amadori S, Meloni G, Baccarani M et al. Long-term survival in adolescent and adult acute lymphoblastic leukemia. Cancer. 1983;52:30–4.

1550. Pui CH, Campana D, Evans WE. Childhood acute lymphoblastic leukaemia – current status and future perspectives. Lancet Oncol. 2001;2:597–607.

1551. Gustafsson G, Kreuger A. Sex and other prognostic factors in acute lymphoblastic leukemia in childhood. Am J Pediatr Hematol Oncol. 1983;5:243–50.

1552. Miller DR, Leikin S, Albo V, Sather H, Karon M, Hammond D. Prognostic factors and therapy in acute lymphoblastic leukemia of childhood: CCG-141. A report from childrens cancer study group. Cancer. 1983;51:1041–9.

1553. Kurec AS, Belair P, Stefanu C, Barrett DM, Dubowy RL, Davey FR. Significance of aberrant immunophenotypes in childhood acute lymphoid leukemia. Cancer. 1991;67:3081–6.

1554. Look AT, Roberson PK, Williams DL et al. Prognostic importance of blast cell DNA content in childhood acute lymphoblastic leukemia. Blood. 1985;65:1079–86.

1555. Consolini R, Legitimo A, Rondelli R et al. Clinical relevance of CD10 expression in childhood ALL. The Italian Association for Pediatric Hematology and Oncology (AIEOP). Haematologica. 1998;83: 967–73.

1556. Pui CH, Rivera GK, Hancock ML et al. Clinical significance of CD10 expression in childhood acute lymphoblastic leukemia. Leukemia. 1993;7:35–40.

1557. Pullen DJ, Boyett JM, Crist WM et al. Pediatric oncology group utilization of immunologic markers in the designation of acute lymphocytic leukemia subgroups: influence on treatment response. Ann NY Acad Sci. 1984;428:26–48.

1558. Crist W, Boyett J, Jackson J et al. Prognostic importance of the pre-B-cell immunophenotype and other presenting features in B-lineage childhood acute lymphoblastic leukemia: a Pediatric Oncology Group study. Blood. 1989;74:1252–9.

1559. Williams DL, Tsiatis A, Brodeur GM et al. Prognostic importance of chromosome number in 136 untreated children with acute lymphoblastic leukemia. Blood. 1982;60:864–71.

1560. Kaspers GJ, Smets LA, Pieters R, Van Zantwijk CH, Van Wering ER, Veerman AJ. Favorable prognosis of hyperdiploid common acute lymphoblastic leukemia may be explained by sensitivity to antimetabolites and other drugs: results of an in vitro study. Blood. 1995;85:751–56.

1561. Jackson JF, Boyett J, Pullen J et al. Favorable prognosis associated with hyperdiploidy in children with acute lymphocytic leukemia correlates with extra chromosome 6. A Pediatric Oncology Group study. Cancer. 1990; 66:1183–9.

1562. Harris MB, Shuster JJ, Carroll A et al. Trisomy of leukemic cell chromosomes 4 and 10 identifies children with B-progenitor cell acute lymphoblastic leukemia with a very low risk of treatment failure: a Pediatric Oncology Group study. Blood. 1992;79:3316–24.

1563. Heerema NA, Sather HN, Sensel MG et al. Prognostic impact of trisomies of chromosomes 10, 17, and 5 among children with acute lymphoblastic leukemia and high hyperdiploidy (>50 chromosomes). J Clin Oncol. 2000;18:1876–87.

1564. Pui CH, Carroll AJ, Head D et al. Near-triploid and near-tetraploid acute lymphoblastic leukemia of childhood. Blood. 1990;76:590–96.

1565. Heerema NA, Nachman JB, Sather HN et al. Hypodiploidy with less than 45 chromosomes confers adverse risk in childhood acute lymphoblastic leukemia: a report from the children's cancer group. Blood. 1999; 94:4036–45.

1566. Raimondi SC, Zhou Y, Mathew S et al. Reassessment of the prognostic significance of hypodiploidy in pediatric patients with acute lymphoblastic leukemia. Cancer. 2003;98:2715–22.

1567. Forestier E, Johansson B, Gustafsson G et al. Prognostic impact of karyotypic findings in childhood acute lymphoblastic leukaemia: a Nordic series comparing two treatment periods. For the Nordic Society of Paediatric Haematology and Oncology (NOPHO) Leukaemia Cytogenetic Study Group. Br J Haematol. 2000; 110:147–53.

1568. Pui CH, Williams DL, Raimondi SC et al. Hypodiploidy is associated with a poor prognosis in childhood acute lymphoblastic leukemia. Blood. 1987; 70:247–53.

1569. Crist W, Carroll A, Shuster J et al. Philadelphia chromosome positive childhood acute lymphoblastic leukemia: clinical and cytogenetic characteristics and treatment outcome. A Pediatric Oncology Group study. Blood. 1990;76:489–94.

1570. Ribeiro RC, Abromowitch M, Raimondi SC, Murphy SB, Behm F, Williams DL. Clinical and biologic hallmarks of the Philadelphia chromosome in childhood acute lymphoblastic leukemia. Blood. 1987;70:948–53.

1571. Arico M, Valsecchi MG, Camitta B et al. Outcome of treatment in children with Philadelphia

chromosome-positive acute lymphoblastic leukemia. N Engl J Med. 2000;342:998–1006.

1572. Fletcher JA, Lynch EA, Kimball VM, Donnelly M, Tantravahi R, Sallan SE. Translocation (9;22) is associated with extremely poor prognosis in intensively treated children with acute lymphoblastic leukemia. Blood. 1991;77:435–9.

1573. Carroll AJ, Crist WM, Parmley RT, Roper M, Cooper MD, Finley WH. Pre-B cell leukemia associated with chromosome translocation 1;19. Blood. 1984;63:721–4.

1574. Arthur DC, Bloomfield CD, Lindquist LL, Nesbit ME, Jr. Translocation 4;11 in acute lymphoblastic leukemia: clinical characteristics and prognostic significance. Blood. 1982;59:96–9.

1575. Pui CH, Frankel LS, Carroll AJ et al. Clinical characteristics and treatment outcome of childhood acute lymphoblastic leukemia with the t(4;11)(q21;q23): a collaborative study of 40 cases. Blood. 1991;77:440–7.

1576. Hrusak O, Porwit-MacDonald A. Antigen expression patterns reflecting genotype of acute leukemias. Leukemia. 2002;16:1233–58.

1577. Seeger K, Buchwald D, Taube T et al. TEL-AML1 positivity in relapsed B cell precursor acute lymphoblastic leukemia in childhood. Berlin–Frankfurt–Munster Study Group. Leukemia. 1999;13:1469–70.

1578. Harbott J, Viehmann S, Borkhardt A, Henze G, Lampert F. Incidence of TEL/AML1 fusion gene analyzed consecutively in children with acute lymphoblastic leukemia in relapse. Blood. 1997;90:4933–7.

1579. Kanerva J, Saarinen-Pihkala UM, Niini T et al. Favorable outcome in 20-year follow-up of children with very-low-risk ALL and minimal standard therapy, with special reference to TEL-AML1 fusion. Pediatr Blood Cancer. 2004;42:30–5.

1580. Takahashi Y, Horibe K, Kiyoi H et al. Prognostic significance of TEL/AML1 fusion transcript in childhood B-precursor acute lymphoblastic leukemia. J Pediatr Hematol Oncol. 1998;20:190–5.

1581. Avigad S, Kuperstein G, Zilberstein J et al. TEL-AML1 fusion transcript designates a favorable outcome with an intensified protocol in childhood acute lymphoblastic leukemia. Leukemia. 1999;13:481–3.

1582. Moorman AV, Richards SM, Robinson HM et al. Prognosis of children with acute lymphoblastic leukemia (ALL) and intrachromosomal amplification of chromosome 21 (iAMP21). Blood. 2007;109:2327–30.

1583. Patte C, Philip T, Rodary C et al. High survival rate in advanced-stage B-cell lymphomas and leukemias without CNS involvement with a short intensive polychemotherapy: results from the French Pediatric Oncology Society of a randomized trial of 216 children. J Clin Oncol. 1991;9:123–32.

1584. Schwenn MR, Blattner SR, Lynch E, Weinstein HJ. HiC-COM: a 2-month intensive chemotherapy regimen for children with stage III and IV Burkitt's lymphoma and B-cell acute lymphoblastic leukemia. J Clin Oncol. 1991;9:133–8.

1585. Reiter A, Schrappe M, Ludwig WD et al. Favorable outcome of B-cell acute lymphoblastic leukemia in childhood: a report of three consecutive studies of the BFM group. Blood. 1992;80:2471–8.

1586. Yeoh EJ, Ross ME, Shurtleff SA et al. Classification, subtype discovery, and prediction of outcome in pediatric acute lymphoblastic leukemia by gene expression profiling. Cancer Cell. 2002;1:133–43.

1587. Ross ME, Zhou X, Song G et al. Classification of pediatric acute lymphoblastic leukemia by gene expression profiling. Blood. 2003;102:2951–59.

1588. Holleman A, Cheok MH, den Boer ML et al. Gene-expression patterns in drug-resistant acute lymphoblastic leukemia cells and response to treatment. N Engl J Med. 2004;351:533–42.

1589. Den Boer ML, Harms DO, Pieters R et al. Patient stratification based on prednisolone–vincristine–asparaginase resistance profiles in children with acute lymphoblastic leukemia. J Clin Oncol. 2003;21:3262–8.

1590. Pieters R, Huismans DR, Loonen AH et al. Relation of cellular drug resistance to long-term clinical outcome in childhood acute lymphoblastic leukaemia. Lancet. 1991;338:399–403.

1591. Hongo T, Okada S, Inoue N et al. Two groups of Philadelphia chromosome-positive childhood acute lymphoblastic leukemia classified by pretreatment multidrug sensitivity or resistance in in vitro testing. Int J Hematol. 2002;76:251–9.

1592. Prokop A, Wieder T, Sturm I et al. Relapse in childhood acute lymphoblastic leukemia is associated with a decrease of the Bax/Bcl-2 ratio and loss of spontaneous caspase-3 processing in vivo. Leukemia. 2000;14:1606–13.

1593. Foroni L, Harrison CJ, Hoffbrand AV, Potter MN. Investigation of minimal residual disease in childhood and adult acute lymphoblastic leukaemia by molecular analysis. Br J Haematol. 1999;105:7–24.

1594. Coustan-Smith E, Sancho J, Behm FG et al. Prognostic importance of measuring early clearance of leukemic cells by flow cytometry in childhood acute lymphoblastic leukemia. Blood. 2002;100:52–8.

1595. Coustan-Smith E, Sancho J, Hancock ML et al. Use of peripheral blood instead of bone marrow to monitor residual disease in children with acute lymphoblastic leukemia. Blood. 2002;100:2399–402.

1596. Hoelzer D, Thiel E, Loffler H et al. Prognostic factors in a multicenter study for treatment of acute lymphoblastic leukemia in adults. Blood. 1988;71:123–31.

1597. Gokbuget N, Hoelzer D, Arnold R et al. Treatment of Adult ALL according to protocols of the German Multicenter Study Group for Adult ALL (GMALL). Hematol Oncol Clin North Am. 2000;14:1307–1325, ix.

1598. Gaynor J, Chapman D, Little C et al. A cause-specific hazard rate analysis of prognostic factors among 199 adults with acute lymphoblastic leukemia: the Memorial Hospital experience since 1969. J Clin Oncol. 1988;6:1014–30.

1599. Larson RA, Dodge RK, Burns CP et al. A five-drug remission induction regimen with intensive consolidation for adults with acute lymphoblastic leukemia: cancer and leukemia group B study 8811. Blood. 1995;85:2025–37.

1600. Bassan R, Gatta G, Tondini C, Willemze R. Adult acute lymphoblastic leukaemia. Crit Rev Oncol Hematol. 2004;50:223–61.

1601. Kantarjian HM, O'Brien S, Smith T et al. Acute lymphocytic leukaemia in the elderly: characteristics and outcome with the vincristine–adriamycin–dexamethasone (VAD) regimen. Br J Haematol. 1994;88:94–100.

1602. Ferrari A, Annino L, Crescenzi S, Romani C, Mandelli F. Acute lymphoblastic leukemia in the elderly: results of two different treatment approaches in 49 patients during a 25-year period. Leukemia. 1995;9:1643–7.

1603. Pagano L, Mele L, Casorelli I, Fianchi L, Di Febo A, Leone G. Acute lymphoblastic leukemia in the elderly. A twelve-year retrospective, single center study. Haematologica. 2000;85:1327–9.

1604. Khalidi HS, O'Donnell MR, Slovak ML, Arber DA. Adult precursor-B acute lymphoblastic leukemia with translocations involving chromosome band 19p13 is associated with poor prognosis. Cancer Genet Cytogenet. 1999;109:58–65.

1605. Mancini M, Scappaticci D, Cimino G et al. A comprehensive genetic classification of adult Acute Lymphoblastic Leukemia (ALL): Analysis of the GIMEMA 0496 protocol. Blood. 2005.

1606. Gokbuget N, Raff R, Brugge-Mann M et al. Risk/MRD adapted GMALL trials in adult ALL. Ann Hematol. 2004;83 (Suppl 1):S129-131.

1607. Gokbuget N, Kneba M, Raff T et al. Risk-adapted treatment according to minimal residual disease in adult ALL. Best Pract Res Clin Haematol. 2002;15: 639–52.

1608. Garand R, Bene MC. A new approach of acute lymphoblastic leukemia immunophenotypic classification:1984–1994 the GEIL experience. Groupe d'Etude Immunologique des Leucemies. Leuk Lymphoma. 1994;13 (Suppl 1):1–5.

1609. Schneider NR, Carroll AJ, Shuster JJ et al. New recurring cytogenetic abnormalities and association of blast cell karyotypes with prognosis in childhood T-cell acute lymphoblastic leukemia: a pediatric oncology group report of 343 cases. Blood. 2000; 96:2543–9.

1610. Chiaretti S, Li X, Gentleman R et al. Gene expression profile of adult T-cell acute lymphocytic leukemia identifies distinct subsets of patients with different response to therapy and survival. Blood. 2004;103:2771–8.

1611. Chiaretti S, Li X, Gentleman R et al. Gene expression profiles of B-lineage adult acute lymphocytic leukemia reveal genetic patterns that identify lineage derivation and distinct mechanisms of transformation. Clin Cancer Res. 2005;11:7209–19.

1612. Gassmann W, Loffler H, Thiel E et al. Morphological and cytochemical findings in 150 cases of T-lineage acute lymphoblastic leukaemia in adults. German Multicentre ALL Study Group (GMALL). Br J Haematol. 1997;97:372–82.

1613. Ferrando AA, Look AT. Gene expression profiling in T-cell acute lymphoblastic leukemia. Semin Hematol. 2003;40:274–80.

1614. Khalidi HS, Chang KL, Medeiros LJ et al. Acute lymphoblastic leukemia. Survey of immunophenotype, French–American–British classification, frequency of myeloid antigen expression, and karyotypic abnormalities in 210 pediatric and adult cases. Am J Clin Pathol. 1999;111:467–76.

1615. Shuster JJ, Falletta JM, Pullen DJ et al. Prognostic factors in childhood T-cell acute lymphoblastic leukemia: a Pediatric Oncology Group study. Blood. 1990;75:166–73.

1616. Vitale A, Guarini A, Ariola C et al. Adult T-cell acute lymphoblastic leukemia: biologic profile at presentation and correlation with response to induction treatment in patients enrolled in the GIMEMA LAL 0496 protocol. Blood. 2006;107:473–9.

1617. Wetzler M, Dodge RK, Mrozek K et al. Prospective karyotype analysis in adult acute lymphoblastic leukemia: the cancer and leukemia Group B experience. Blood. 1999;93:3983–93.

1618. Harrison CJ, Foroni L. Cytogenetics and molecular genetics of acute lymphoblastic leukemia. Rev Clin Exp Hematol. 2002;6:91–113.

1619. Quentmeier H, Cools J, Macleod RA, Marynen P, Uphoff CC, Drexler HG. e6-a2 BCR-ABL1 fusion in T-cell acute lymphoblastic leukemia. Leukemia. 2005;19:295–6.

1620. Asnafi V, Radford-Weiss I, Dastugue N et al. CALM-AF10 is a common fusion transcript in T-ALL and is specific to the TCRgammadelta lineage. Blood. 2003;102:1000–6.

1621. Ferrando AA, Armstrong SA, Neuberg DS et al. Gene expression signatures in MLL-rearranged T-lineage and B-precursor acute leukemias: dominance of HOX dysregulation. Blood. 2003;102:262–8.

1622. Lucio P, Parreira A, Orfao A. CD123hi dendritic cell lymphoma: an unusual case of non-Hodgkin lymphoma. Ann Intern Med. 1999;131:549–50.

1623. Chaperot L, Bendriss N, Manches O et al. Identification of a leukemic counterpart of the plasmacytoid dendritic cells. Blood. 2001;97:3210–17.

1624. Feuillard J, Jacob MC, Valensi F et al. Clinical and biologic features of CD4(+)CD56(+) malignancies. Blood. 2002;99:1556–63.

1625. Petrella T, Comeau MR, Maynadie M et al. 'Agranular CD4+ CD56+ hematodermic neoplasm' (blastic NK-cell lymphoma) originates from a population of CD56+ precursor cells related to plasmacytoid monocytes. Am J Surg Pathol. 2002;26:852–62.

1626. Slater DN. The new World Health Organization-European Organization for Research and Treatment of Cancer classification for cutaneous lymphomas: a practical marriage of two giants. Br J Dermatol. 2005;153:874–80.

1627. Willemze R, Jaffe ES, Burg G et al. WHO-EORTC classification for cutaneous lymphomas. Blood. 2005;105:3768–85.

1628. Dijkman R, van Doorn R, Szuhai K, Willemze R, Vermeer MH, Tensen CP. Gene-expression profiling and array-based CGH classify CD4+CD56+ hematodermic neoplasm and cutaneous myelomonocytic leukemia as distinct disease entities. Blood. 2007;109:1720–7.

1629. Leroux D, Mugneret F, Callanan M et al. CD4(+), CD56(+) DC2 acute leukemia is characterized by recurrent clonal chromosomal changes affecting 6 major targets: a study of 21 cases by the Groupe Francais de Cytogenetique Hematologique. Blood. 2002;99:4154–9.

1630. Chaperot L, Perrot I, Jacob MC et al. Leukemic plasmacytoid dendritic cells share phenotypic and functional features with their normal counterparts. Eur J Immunol. 2004;34:418–26.

1631. Garnache-Ottou F, Chaperot L, Biichle S et al. Expression of the myeloid-associated marker CD33 is not an exclusive factor for leukemic plasmacytoid dendritic cells. Blood. 2005;105:1256–64.

1632. Gopcsa L, Banyai A, Jakab K et al. Extensive flow cytometric characterization of plasmacytoid dendritic cell leukemia cells. Eur J Haematol. 2005;75:346–51.

1633. Ng AP, Lade S, Rutherford T, McCormack C, Prince HM, Westerman DA. Primary cutaneous CD4+/CD56+ hematodermic neoplasm (blastic NK-cell lymphoma): a report of five cases. Haematologica. 2006;91:143–4.

1634. Apperley JF, Gardembas M, Melo JV et al. Response to imatinib mesylate in patients with chronic myeloproliferative diseases with rearrangements of the platelet-derived growth factor receptor beta. N Engl J Med. 2002;347:481–7.

1635. Jones AV, Kreil S, Zoi K et al. Widespread occurrence of the JAK2 V617F mutation in chronic myeloproliferative disorders. Blood. 2005;106:2162–8.

1636. Tefferi A, Sirhan S, Lasho TL et al. Concomitant neutrophil JAK2 mutation screening and PRV-1 expression analysis in myeloproliferative disorders and secondary polycythaemia. Br J Haematol. 2005;131:166–71.

1637. Passamonti F, Rumi E, Pietra D et al. Relation between JAK2 (V617F) mutation status, granulocyte activation, and constitutive mobilization of CD34+ cells into peripheral blood in myeloproliferative disorders. Blood. 2006;107:3676–82.

1638. Scott LM, Scott MA, Campbell PJ, Green AR. Progenitors homozygous for the V617F mutation occur in most patients with Polycythemia vera, but not essential thrombocythemia. Blood. 2006;108:2435–7.

1639. Bartram CR, de Klein A, Hagemeijer A et al. Translocation of c-ab1 oncogene correlates with the presence of a Philadelphia chromosome in chronic myelocytic leukaemia. Nature. 1983;306:277–80.

1640. Groffen J, Stephenson JR, Heisterkamp N, de Klein A, Bartram CR, Grosveld G. Philadelphia chromosomal breakpoints are clustered within a limited region, bcr, on chromosome 22. Cell. 1984;36:93–9.

1641. Georgii A, Buesche G, Kreft A. The histopathology of chronic myeloproliferative diseases. Baillieres Clin Haematol. 1998;11:721–49.

1642. Buhr T, Choritz H, Georgii A. The impact of megakaryocyte proliferation of the evolution of myelofibrosis. Histological follow-up study in 186 patients with

chronic myeloid leukaemia. Virchows Arch A Pathol Anat Histopathol. 1992;420:473–8.

1643. Buhr T, Georgii A, Choritz H. Myelofibrosis in chronic myeloproliferative disorders. Incidence among subtypes according to the Hannover Classification. Pathol Res Pract. 1993;189:121–32.

1644. Thiele J, Kvasnicka HM, Facchetti F, Franco V, van der Walt J, Orazi A. European consensus on grading bone marrow fibrosis and assessment of cellularity. Haematologica. 2005;90:1128–32.

1645. Thiele J, Kvasnicka HM, Schmitt-Graeff A et al. Bone marrow features and clinical findings in chronic myeloid leukemia — a comparative, multicenter, immunohistological and morphometric study on 614 patients. Leuk Lymphoma. 2000;36:295–308.

1646. Thiele J, Thienel C, Zankovich R, Fischer R. Prognostic features at diagnosis of chronic myeloid leukaemia with special emphasis on histological parameters. Med Oncol Tumor Pharmacother. 1988;5:49–60.

1647. Savage DG, Antman KH. Imatinib mesylate — a new oral targeted therapy. N Engl J Med. 2002;346:683–93.

1648. Thiele J, Kvasnicka HM. A critical reappraisal of the WHO classification of the chronic myeloproliferative disorders. Leuk Lymphoma. 2006;47:381–96.

1649. Thiele J, Kvasnicka HM, Fischer R. Bone marrow histopathology in chronic myelogenous leukemia (CML) — evaluation of distinctive features with clinical impact. Histol Histopathol. 1999;14:1241–56.

1650. Thiele J, Kvasnicka HM, Orazi A. Bone marrow histopathology in myeloproliferative disorders — current diagnostic approach. Semin Hematol. 2005;42:184–95.

1651. Thiele J, Kvasnicka HM, Vardiman J. Bone marrow histopathology in the diagnosis of chronic myeloproliferative disorders: a forgotten pearl. Best Pract Res Clin Haematol. 2006;19:413–37.

1652. Georgii A, Vykoupil KF, Buhr T et al. Chronic myeloproliferative disorders in bone marrow biopsies. Pathol Res Pract. 1990;186:3–27.

1653. Sawyers CL. Chronic myeloid leukemia. N Engl J Med. 1999;340:1330–40.

1654. Landstrom AP, Tefferi A. Fluorescent in situ hybridization in the diagnosis, prognosis, and treatment monitoring of chronic myeloid leukemia. Leuk Lymphoma. 2006;47:397–402.

1655. Sinclair PB, Nacheva EP, Leversha M et al. Large deletions at the t(9;22) breakpoint are common and may identify a poor-prognosis subgroup of patients with chronic myeloid leukemia. Blood. 2000;95:738–43.

1656. Yoong Y, VanDeWalker TJ, Carlson RO, Dewald GW, Tefferi A. Clinical correlates of submicroscopic deletions involving the ABL-BCR translocation region in chronic myeloid leukemia. Eur J Haematol. 2005;74:124–7.

1657. Quintas-Cardama A, Kantarjian H, Talpaz M et al. Imatinib mesylate therapy may overcome the poor prognostic significance of deletions of derivative chromosome 9 in patients with chronic myelogenous leukemia. Blood. 2005;105:2281–6.

1658. Cortes J. Natural history and staging of chronic myelogenous leukemia. Hematol Oncol Clin North Am. 2004;18:569–584, viii.

1659. Handa H, Hegde UP, Kotelnikov VM et al. Bcl-2 and c-myc expression, cell cycle kinetics and apoptosis during the progression of chronic myelogenous leukemia from diagnosis to blastic phase. Leuk Res. 1997;21:479–89.

1660. Shet AS, Jahagirdar BN, Verfaillie CM. Chronic myelogenous leukemia: mechanisms underlying disease progression. Leukemia. 2002;16:1402–11.

1661. Hirsch-Ginsberg C, LeMaistre AC, Kantarjian H et al. RAS mutations are rare events in Philadelphia chromosome-negative/bcr gene rearrangement-negative chronic myelogenous leukemia, but are prevalent in chronic myelomonocytic leukemia. Blood. 1990;76:1214–19.

1662. Bi S, Lanza F, Goldman JM. The involvement of "tumor suppressor" p53 in normal and chronic myelogenous leukemia hemopoiesis. Cancer Res. 1994;54:582–6.

1663. Griesshammer M, Heinze B, Bangerter M, Heimpel H, Fliedner TM. Karyotype abnormalities and their clinical significance in blast crisis of chronic myeloid leukemia. J Mol Med. 1997;75:836–8.

1664. Furukawa Y, DeCaprio JA, Belvin M, Griffin JD. Heterogeneous expression of the product of the retinoblastoma susceptibility gene in primary human leukemia cells. Oncogene. 1991;6:1343–6.

1665. Bernstein R. Cytogenetics of chronic myelogenous leukemia. Semin Hematol. 1988;25:20–34.

1666. Kardinal CG, Bateman JR, Weiner J. Chronic granulocytic leukemia. Review of 536 cases. Arch Intern Med. 1976;136:305–13.

1667. Sokal JE. Prognosis in chronic myeloid leukaemia: biology of the disease vs. treatment. Baillieres Clin Haematol. 1987;1:907–29.

1668. Gadzicki D, von Neuhoff N, Steinemann D et al. BCR-ABL gene amplification and overexpression in a patient with chronic myeloid leukemia treated with imatinib. Cancer Genet Cytogenet. 2005;159:164–7.

1669. Schoch C, Haferlach T, Kern W et al. Occurrence of additional chromosome aberrations in chronic myeloid leukemia patients treated with imatinib mesylate. Leukemia. 2003;17:461–3.

1670. O'Dwyer ME, Mauro MJ, Kurilik G et al. The impact of clonal evolution on response to imatinib mesylate (STI571) in accelerated phase CML. Blood. 2002;100:1628–33.

1671. Faderl S, Talpaz M, Estrov Z, Kantarjian HM. Chronic myelogenous leukemia: biology and therapy. Ann Intern Med. 1999;131:207–19.

1672. Kantarjian HM, Talpaz M, Kontoyiannis D et al. Treatment of chronic myelogenous leukemia in accelerated and blastic phases with daunorubicin, high-dose cytarabine, and granulocyte-macrophage colony-stimulating factor. J Clin Oncol. 1992;10:398–405.

1673. Beard MD, Durrant J, Catovsky D et al. Blast crisis of chronic myeloid leukaemia (CML). I. Presentation simulating acute lymphoid leukaemia (ALL). Br J Haematol. 1976;34:167–78.

1674. Cervantes F, Villamor N, Esteve J et al. 'Lymphoid' blast crisis of chronic myeloid leukaemia is associated with distinct clinicohaematological features. Br J Haematol. 1998;100:123–8.

1675. Marin D, Marktel S, Szydlo R et al. Survival of patients with chronic-phase chronic myeloid leukaemia on imatinib after failure on interferon alfa. Lancet. 2003;362:617–19.

1676. Marktel S, Marin D, Foot N et al. Chronic myeloid leukemia in chronic phase responding to imatinib: the occurrence of additional cytogenetic abnormalities predicts disease progression. Haematologica. 2003;88:260–7.

1677. Lange T, Deininger M, Brand R et al. BCR-ABL transcripts are early predictors for hematological relapse in chronic myeloid leukemia after hematopoietic cell transplantation with reduced intensity conditioning. Leukemia. 2004;18:1468–75.

1678. Passweg JR, Walker I, Sobocinski KA, Klein JP, Horowitz MM, Giralt SA. Validation and extension of the EBMT Risk Score for patients with chronic myeloid leukaemia (CML) receiving allogeneic haematopoietic stem cell transplants. Br J Haematol. 2004;125:613–20.

1679. Olavarria E, Ottmann OG, Deininger M et al. Response to imatinib in patients who relapse after allogeneic stem cell transplantation for chronic myeloid leukemia. Leukemia. 2003;17:1707–12.

1680. Muller MC, Gattermann N, Lahaye T et al. Dynamics of BCR-ABL mRNA expression in first-line therapy of chronic myelogenous leukemia patients with imatinib or interferon alpha/ara-C. Leukemia. 2003;17:2392–400.

1681. Merx K, Muller MC, Kreil S et al. Early reduction of BCR-ABL mRNA transcript levels predicts cytogenetic response in chronic phase CML patients treated with imatinib after failure of interferon alpha. Leukemia. 2002;16:1579–83.

1682. Branford S, Rudzki Z, Harper A et al. Imatinib produces significantly superior molecular responses compared to interferon alfa plus cytarabine in patients with newly diagnosed chronic myeloid leukemia in chronic phase. Leukemia. 2003;17:2401–9.

1683. Wang L, Pearson K, Ferguson JE, Clark RE. The early molecular response to imatinib predicts cytogenetic and clinical outcome in chronic myeloid leukaemia. Br J Haematol. 2003;120:990–9.

1684. Donato NJ, Wu JY, Stapley J et al. BCR-ABL independence and LYN kinase overexpression in chronic myelogenous leukemia cells selected for resistance to STI571. Blood. 2003;101:690–8.

1685. Gorre ME, Mohammed M, Ellwood K et al. Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. Science. 2001;293:876–80.

1686. Cowan-Jacob SW, Guez V, Fendrich G et al. Imatinib (STI571) resistance in chronic myelogenous leukemia: molecular basis of the underlying mechanisms and potential strategies for treatment. Mini Rev Med Chem. 2004;4:285–99.

1687. Chu S, Xu H, Shah NP et al. Detection of BCR-ABL kinase mutations in CD34+ cells from chronic myelogenous leukemia patients in complete cytogenetic remission on imatinib mesylate treatment. Blood. 2005;105:2093–8.

1688. Jabbour E, Kantarjian H, Jones D et al. Frequency and clinical significance of BCR-ABL mutations in patients with chronic myeloid leukemia treated with imatinib mesylate. Leukemia. 2006;20:1767–73.

1689. Goldman JM. Chronic myeloid leukemia – still a few questions. Exp Hematol. 2004;32:2–10.

1690. Campbell LJ, Patsouris C, Rayeroux KC, Somana K, Januszewicz EH, Szer J. BCR/ABL amplification in chronic myelocytic leukemia blast crisis following imatinib mesylate administration. Cancer Genet Cytogenet. 2002;139:30–3.

1691. Branford S, Rudzki Z, Parkinson I et al. Real-time quantitative PCR analysis can be used as a primary screen to identify patients with CML treated with imatinib who have BCR-ABL kinase domain mutations. Blood. 2004;104:2926–32.

1692. von Bubnoff N, Schneller F, Peschel C, Duyster J. BCR-ABL gene mutations in relation to clinical resistance of Philadelphia-chromosome-positive leukaemia to STI571: a prospective study. Lancet. 2002;359:487–91.

1693. Talpaz M, Shah NP, Kantarjian H et al. Dasatinib in imatinib-resistant Philadelphia chromosome-positive leukemias. N Engl J Med. 2006;354:2531–41.

1694. Tauchi T, Ohyashiki K. The second generation of BCR-ABL tyrosine kinase inhibitors. Int J Hematol. 2006;83:294–300.

1695. Quintas-Cardama A, Kantarjian H, Jones D et al. Dasatinib (BMS-354825) is active in Philadelphia chromosome-positive chronic myelogenous leukemia after imatinib and nilotinib (AMN107) therapy failure. Blood. 2007;109:497–9.

1696. Giles FJ, Cortes J, Jones D, Bergstrom D, Kantarjian H, Freedman SJ. MK-0457, a novel kinase inhibitor, is active in patients with chronic myeloid leukemia or acute lymphocytic leukemia with the T315I BCR-ABL mutation. Blood. 2007;109:500–2.

1697. Yokota A, Kimura S, Masuda S et al. INNO-406, a novel BCR-ABL/Lyn dual tyrosine kinase inhibitor, suppresses the growth of Ph+ leukemia cells in the central nervous system, and cyclosporine A augments its in vivo activity. Blood. 2007;109:306–14.

1698. Cortes J, Rousselot P, Kim DW et al. Dasatinib induces complete hematologic and cytogenetic responses in patients with imatinib-resistant or -intolerant chronic myeloid leukemia in blast crisis. Blood. 2007;109:3207–13.

1699. Kantarjian H, Giles F, Wunderle L et al. Nilotinib in imatinib-resistant CML and Philadelphia chromosome-positive ALL. N Engl J Med. 2006;354:2542–51.

1700. Jabbour E, Cortes J, O'Brien S, Giles F, Kantarjian H. New targeted therapies for chronic myelogenous leukemia: opportunities to overcome imatinib resistance. Semin Hematol. 2007;44:S25-31.

1701. Michiels JJ, Thiele J. Clinical and pathological criteria for the diagnosis of essential thrombocythemia, Polycythemia vera, and idiopathic myelofibrosis (agnogenic myeloid metaplasia). Int J Hematol. 2002;76:133–45.

1702. Pearson TC, Messinezy M. The diagnostic criteria of Polycythaemia rubra vera. Leuk Lymphoma. 1996;22 (Suppl 1):87–93.

1703. Tefferi A, Thiele J, Orazi A et al. Proposals and rationale for revision of the World Health Organization diagnostic criteria for Polycythemia vera, essential thrombocythemia, and primary myelofibrosis: recommendations from an ad hoc international expert panel. Blood. 2007;110:1092–7.

1704. Diez-Martin JL, Graham DL, Petitt RM, Dewald GW. Chromosome studies in 104 patients with Polycythemia vera. Mayo Clin Proc. 1991;66:287–99.

1705. Swolin B, Weinfeld A, Westin J. A prospective long-term cytogenetic study in Polycythemia vera in relation to treatment and clinical course. Blood. 1988;72:386–95.

1706. Rege-Cambrin G, Mecucci C, Tricot G et al. A chromosomal profile of Polycythemia vera. Cancer Genet Cytogenet. 1987;25:233–45.

1707. Zamora L, Espinet B, Florensa L et al. Is fluorescence in situ hybridization a useful method in diagnosis of Polycythemia vera patients? Cancer Genet Cytogenet. 2004;151:139–45.

1708. Berk PD, Goldberg JD, Silverstein MN et al. Increased incidence of acute leukemia in Polycythemia vera associated with chlorambucil therapy. N Engl J Med. 1981;304:441–7.

1709. Najean Y, Rain JD. The very long-term evolution of polycythemia vera: an analysis of 318 patients initially treated by phlebotomy or 32P between 1969 and 1981. Semin Hematol. 1997;34:6–16.

1710. Fruchtman SM, Mack K, Kaplan ME, Peterson P, Berk PD, Wasserman LR. From efficacy to safety: a Polycythemia Vera Study Group report on hydroxyurea in patients with polycythemia vera. Semin Hematol. 1997;34:17–23.

1711. Kiladjian JJ, Gardin C, Renoux M, Bruno F, Bernard JF. Long-term outcomes of polycythemia vera patients treated with pipobroman as initial therapy. Hematol J. 2003;4:198–207.

1712. Fruchtman SM, Petitt RM, Gilbert HS, Fiddler G, Lyne A. Anagrelide: analysis of long-term efficacy, safety and leukemogenic potential in myeloproliferative disorders. Leuk Res. 2005;29:481–91.

1713. Finazzi G, Caruso V, Marchioli R et al. Acute leukemia in Polycythemia vera: an analysis of 1638 patients enrolled in a prospective observational study. Blood. 2005;105:2664–70.

1714. Harrison CN, Green AR. Essential thrombocythemia. Hematol Oncol Clin North Am. 2003;17: 1175–90, vii.

1715. Kutti J, Wadenvik H. Diagnostic and differential criteria of essential thrombocythemia and reactive thrombocytosis. Leuk Lymphoma. 1996;22 (Suppl 1): 41–5.

1716. Murphy S, Iland H, Rosenthal D, Laszlo J. Essential thrombocythemia: an interim report from the Polycythemia Vera Study Group. Semin Hematol. 1986; 23:177–82.

1717. Murphy S, Peterson P, Iland H, Laszlo J. Experience of the Polycythemia Vera Study Group with essential thrombocythemia: a final report on diagnostic criteria, survival, and leukemic transition by treatment. Semin Hematol. 1997;34:29–39.

1718. Sanchez S, Ewton A. Essential thrombocythemia: a review of diagnostic and pathologic features. Arch Pathol Lab Med. 2006;130:1144–50.

1719. Thiele J, Kvasnicka HM, Schmitt-Graeff A, Zankovich R, Diehl V. Follow-up examinations including sequential bone marrow biopsies in essential thrombocythemia (ET): a retrospective clinicopathological study of 120 patients. Am J Hematol. 2002;70:283–91.

1720. Finazzi G, Harrison C. Essential thrombocythemia. Semin Hematol. 2005;42:230–8.

1721. Gianelli U, Vener C, Raviele PR et al. Essential thrombocythemia or chronic idiopathic myelofibrosis? A single-center study based on hematopoietic bone marrow histology. Leuk Lymphoma. 2006;47:1774–81.

1722 Griesshammer M, Bangerter M, Sauer T, Wennauer R, Bergmann L, Heimpel H. Aetiology and clinical significance of thrombocytosis: analysis of 732 patients with an elevated platelet count. J Intern Med. 1999;245:295–300.

1723. Santhosh-Kumar CR, Yohannan MD, Higgy KE, al-Mashhadani SA. Thrombocytosis in adults: analysis of 777 patients. J Intern Med. 1991;229:493–5.

1724. Schafer AI. Thrombocytosis. N Engl J Med. 2004;350:1211–19.

1725. Wolanskyj AP, Lasho TL, Schwager SM et al. JAK2 mutation in essential thrombocythaemia: clinical associations and long-term prognostic relevance. Br J Haematol. 2005;131:208–13.

1726. Campbell PJ, Baxter EJ, Beer PA et al. Mutation of JAK2 in the myeloproliferative disorders: timing, clonality studies, cytogenetic associations, and role in leukemic transformation. Blood. 2006;108:3548–55.

1727. Gangat N, Wolanskyj AP, McClure RF et al. Risk stratification for survival and leukemic transformation in essential thrombocythemia: a single institutional study of 605 patients. Leukemia. 2007;21:270–6.

1728. Panani AD. Cytogenetic findings in untreated patients with essential thrombocythemia. In Vivo. 2006;20:381–4.

1729. Elis A, Amiel A, Manor Y, Tangi I, Fejgin M, Lishner M. The detection of trisomies 8 and 9 in patients with essential thrombocytosis by fluorescence in situ hybridization. Cancer Genet Cytogenet. 1996;92:14–17.

1730. Wolanskyj AP, Schwager SM, McClure RF, Larson DR, Tefferi A. Essential thrombocythemia beyond the first decade: life expectancy, long-term complication rates, and prognostic factors. Mayo Clin Proc. 2006;81:159–66.

1731. Cervantes F, Tassies D, Salgado C, Rovira M, Pereira A, Rozman C. Acute transformation in nonleukemic chronic myeloproliferative disorders: actuarial probability and main characteristics in a series of 218 patients. Acta Haematol. 1991;85:124–7.

1732. Chim CS, Kwong YL, Lie AK et al. Long-term outcome of 231 patients with essential thrombocythemia: prognostic factors for thrombosis, bleeding, myelofibrosis, and leukemia. Arch Intern Med. 2005;165:2651–8.

1733. De Sanctis V, Mazzucconi MG, Spadea A et al. Long-term evaluation of 164 patients with essential thrombocythaemia treated with pipobroman: occurrence of leukaemic evolution. Br J Haematol. 2003; 123:517–21.

1734. Rios R, Sole F, del Mar Perez M, Gascon F, Garcia F, Gonzalez PM. t(2;3) in a case of blastic transformation of essential thrombocythaemia. Br J Haematol. 1996;92:769–70.

1735. Paolini R, Bonaldi L, Bianchini E, Ramazzina E, Cella G. Spontaneous evolution of essential thrombocythaemia into acute megakaryoblastic leukaemia with trisomy 8, trisomy 21 and cutaneous involvement. Eur J Haematol. 2003;71:466–9.

1736. Hirose Y, Masaki Y, Sugai S. Leukemic transformation with trisomy 8 in essential thrombocythemia: a report of four cases. Eur J Haematol. 2002;68:112–16.

1737. Hsiao HH, Ito Y, Sashida G, Ohyashiki JH, Ohyashiki K. De novo appearance of der(1;7)(q10;p10) is associated with leukemic transformation and unfavorable prognosis in essential thrombocythemia. Leuk Res. 2005;29:1247–52.

1738. Sterkers Y, Preudhomme C, Lai JL et al. Acute myeloid leukemia and myelodysplastic syndromes following essential thrombocythemia treated with hydroxyurea: high proportion of cases with 17p deletion. Blood. 1998;91:616–22.

1739. Lazarevic V, Tomin D, Jankovic GM et al. A novel t(2;17) in transformation of essential thrombocythemia to acute myelocytic leukemia. Cancer Genet Cytogenet. 2004;148:77–9.

1740. Emilia G, Sacchi S, Temperani P, Longo R, Vecchi A. Progression of essential thrombocythemia to blastic crisis via idiopathic myelofibrosis. Leuk Lymphoma. 1993;9:423–6.

1741. Gaidano G, Pastore C, Santini V et al. Genetic lesions associated with blastic transformation of Polycythemia vera and essential thrombocythemia. Genes Chromosomes Cancer. 1997;19:250–5.

1742. Barosi G. Myelofibrosis with myeloid metaplasia: diagnostic definition and prognostic classification for clinical studies and treatment guidelines. J Clin Oncol. 1999; 17:2954–70.

1743. Barosi G. Myelofibrosis with myeloid metaplasia. Hematol Oncol Clin North Am. 2003;17:1211–26.

1744. Cervantes F, Barosi G, Demory JL et al. Myelofibrosis with myeloid metaplasia in young individuals: disease characteristics, prognostic factors and identification of risk groups. Br J Haematol. 1998;102:684–90.

1745. Cervantes F, Barosi G, Hernandez-Boluda JC, Marchetti M, Montserrat E. Myelofibrosis with myeloid metaplasia in adult individuals 30 years old or younger: presenting features, evolution and survival. Eur J Haematol. 2001;66:324–7.

1746. Cervantes F, Pereira A, Esteve J, Cobo F, Rozman C, Montserrat E. The changing profile of idiopathic myelofibrosis: a comparison of the presenting features of patients diagnosed in two different decades. Eur J Haematol. 1998;60:101–5.

1747. Dupriez B, Demory JL, Lai JL, Fenaux P, Bauters F. Prognostic classification of myelofibrosis with myeloid metaplasia. Br J Haematol. 1989;73:136–7.

1748. Dupriez B, Morel P, Demory JL et al. Prognostic factors in agnogenic myeloid metaplasia: a report on 195 cases with a new scoring system. Blood. 1996;88:1013–18.

1749. Thiele J, Kvasnicka HM, Dietrich H et al. Dynamics of bone marrow changes in patients with chronic idiopathic myelofibrosis following allogeneic stem cell transplantation. Histol Histopathol. 2005;20:879–89.

1750. Deeg HJ, Gooley TA, Flowers ME et al. Allogeneic hematopoietic stem cell transplantation for myelofibrosis. Blood. 2003;102:3912–18.

1751. Rondelli D, Barosi G, Bacigalupo A et al. Allogeneic hematopoietic stem-cell transplantation with reduced-intensity conditioning in intermediate- or high-risk patients with myelofibrosis with myeloid metaplasia. Blood. 2005;105:4115–19.

1752. Kroger N, Badbaran A, Holler E et al. Monitoring of the JAK2-V617F mutation by highly sensitive quantitative real-time PCR after allogeneic stem cell transplantation in patients with myelofibrosis. Blood. 2007;109: 1316–21.

1753. Cervantes F, Barosi G. Myelofibrosis with myeloid metaplasia: diagnosis, prognostic factors, and staging. Semin Oncol. 2005;32:395–402.

1754. Tefferi A, Dingli D, Li CY, Dewald GW. Prognostic diversity among cytogenetic abnormalities in myelofibrosis with myeloid metaplasia. Cancer. 2005;104:1656–60.

1755. Strasser-Weippl K, Steurer M, Kees M et al. Prognostic relevance of cytogenetics determined by fluorescent in situ hybridization in patients having myelofibrosis with myeloid metaplasia. Cancer. 2006;107:2801–6.

1756. Mark HF, Sotomayor EA, Nelson M et al. Chronic idiopathic myelofibrosis (CIMF) resulting from a unique 3;9 translocation disrupting the janus kinase 2 (JAK2) gene. Exp Mol Pathol. 2006;81:217–23.

1757. Elliott MA. Chronic neutrophilic leukemia: a contemporary review. Curr Hematol Rep. 2004;3:210–17.

1758. Elliott MA. Chronic neutrophilic leukemia and chronic myelomonocytic leukemia: WHO defined. Best Pract Res Clin Haematol. 2006;19:571–93.

1759. Bohm J, Schaefer HE. Chronic neutrophilic leukaemia:14 new cases of an uncommon myeloproliferative disease. J Clin Pathol. 2002;55:862–4.

1760. Elliott MA, Dewald GW, Tefferi A, Hanson CA. Chronic neutrophilic leukemia (CNL): a clinical, pathologic and cytogenetic study. Leukemia. 2001;15:35–40.

1761. Reilly JT. Chronic neutrophilic leukaemia: a distinct clinical entity? Br J Haematol. 2002;116:10–18.

1762. Steensma DP, Dewald GW, Lasho TL et al. The JAK2 V617F activating tyrosine kinase mutation is an infrequent event in both 'atypical' myeloproliferative disorders and myelodysplastic syndromes. Blood. 2005;106:1207–9.

1763. Kako S, Kanda Y, Sato T et al. Early relapse of JAK2 V617F-positive chronic neutrophilic leukemia with central nervous system infiltration after unrelated bone marrow transplantation. Am J Hematol. 2007;82:386–90.

1764. Froberg MK, Brunning RD, Dorion P, Litz CE, Torlakovic E. Demonstration of clonality in neutrophils using FISH in a case of chronic neutrophilic leukemia. Leukemia. 1998;12:623–6.

1765. Bain BJ. Eosinophilic leukaemias and the idiopathic hypereosinophilic syndrome. Br J Haematol. 1996; 95:2–9.

1766. Martinelli G, Rondoni M, Ottaviani E, Paolini S, Baccarani M. Hypereosinophilic syndrome and molecularly targeted therapy. Semin Hematol. 2007;44:S4–S16.

1767. Wilkins HJ, Crane MM, Copeland K, Williams WV. Hypereosinophilic syndrome: an update. Am J Hematol. 2005;80:148–57.

1768. Roufosse F, Cogan E, Goldman M. The hypereosinophilic syndrome revisited. Annu Rev Med. 2003;54:169–84.

1769. Cools J. The hypereosinophilic syndrome: idiopathic or not, that is the question. Haematologica. 2005; 90:582–4.

1770. Gotlib V, Darji J, Bloomfield K, Chadburn A, Patel A, Braunschweig I. Eosinophilic variant of chronic myeloid leukemia with vascular complications. Leuk Lymphoma. 2003;44:1609–13.

1771. Griffin JH, Leung J, Bruner RJ, Caligiuri MA, Briesewitz R. Discovery of a fusion kinase in EOL-1 cells and idiopathic hypereosinophilic syndrome. Proc Natl Acad Sci U S A. 2003;100:7830–5.

1772. Vandenberghe P, Wlodarska I, Michaux L et al. Clinical and molecular features of FIP1L1-PDFGRA (+) chronic eosinophilic leukemias. Leukemia. 2004; 18:734–42.

1773. La Starza R, Specchia G, Cuneo A et al. The hypereosinophilic syndrome: fluorescence in situ hybridization detects the del(4)(q12)-FIP1L1/PDGFRA but not genomic rearrangements of other tyrosine kinases. Haematologica. 2005;90:596–601.

1774. Roche-Lestienne C, Lepers S, Soenen-Cornu V et al. Molecular characterization of the idiopathic hypereosinophilic syndrome (HES) in 35 French patients with normal conventional cytogenetics. Leukemia. 2005;19:792–8.

1775. Bain BJ. Systemic mastocytosis and other mast cell neoplasms. Br J Haematol. 1999;106:9–17.

1776. Horny HP, Ruck P, Krober S, Kaiserling E. Systemic mast cell disease (mastocytosis). General aspects and histopathological diagnosis. Histol Histopathol. 1997;12:1081–9.

1777. Valent P, Akin C, Sperr WR, Horny HP, Metcalfe DD. Mast cell proliferative disorders: current view on variants recognized by the World Health Organization. Hematol Oncol Clin North Am. 2003;17:1227–41.

1778. Wilkins BS, Bain BJ. Normal and neoplastic mast cells. Br J Haematol. 1999;106:1.

1779. Austen KF. Systemic mastocytosis. N Engl J Med. 1992;326:639–40.

1780. Barbie DA, Deangelo DJ. Systemic mastocytosis: current classification and novel therapeutic options. Clin Adv Hematol Oncol. 2006;4:768–75.

1781. Horan RF, Austen KF. Systemic mastocytosis: retrospective review of a decade's clinical experience at the Brigham and Women's Hospital. J Invest Dermatol. 1991;96:5S-13S; discussion 13S-14S.

1782. Valent P, Akin C, Sperr WR et al. Diagnosis and treatment of systemic mastocytosis: state of the art. Br J Haematol. 2003;122:695–717.

1783. Valent P, Horny HP, Escribano L et al. Diagnostic criteria and classification of mastocytosis: a consensus proposal. Leuk Res. 2001;25:603–25.

1784. Gupta R, Bain BJ, Knight CL. Cytogenetic and molecular genetic abnormalities in systemic mastocytosis. Acta Haematol. 2002;107:123–8.

1785. Swolin B, Rodjer S, Roupe G. Cytogenetic studies in patients with mastocytosis. Cancer Genet Cytogenet. 2000;120:131–5.

1786. Metcalfe DD, Akin C. Mastocytosis: molecular mechanisms and clinical disease heterogeneity. Leuk Res. 2001;25:577–82.

1787. Nagata H, Worobec AS, Oh CK et al. Identification of a point mutation in the catalytic domain of the protooncogene c-kit in peripheral blood mononuclear cells of patients who have mastocytosis with an associated hematologic disorder. Proc Natl Acad Sci USA. 1995;92:10560–4.

1788. Valent P, Akin C, Sperr WR et al. Mastocytosis: pathology, genetics, and current options for therapy. Leuk Lymphoma. 2005;46:35–48.

1789. Van Etten RA, Shannon KM. Focus on myeloproliferative diseases and myelodysplastic syndromes. Cancer Cell. 2004;6:547–52.

1790. Szpurka H, Tiu R, Murugesan G et al. Refractory anemia with ringed sideroblasts associated with marked thrombocytosis (RARS-T), another myeloproliferative condition characterized by JAK2 V617F mutation. Blood. 2006;108:2173–81.

1791. Levine RL, Loriaux M, Huntly BJ et al. The JAK2V617F activating mutation occurs in chronic myelomonocytic leukemia and acute myeloid leukemia, but not in acute lymphoblastic leukemia or chronic lymphocytic leukemia. Blood. 2005;106:3377–9.

1792. Jelinek J, Oki Y, Gharibyan V et al. JAK2 mutation 1849G>T is rare in acute leukemias but can be found in CMML, Philadelphia chromosome-negative CML, and megakaryocytic leukemia. Blood. 2005;106:3370–3.

1793. Onida F, Kantarjian HM, Smith TL et al. Prognostic factors and scoring systems in chronic myelomonocytic leukemia: a retrospective analysis of 213 patients. Blood. 2002;99:840–9.

1794. Solal-Celigny P, Desaint B, Herrera A et al. Chronic myelomonocytic leukemia according to FAB classification: analysis of 35 cases. Blood. 1984;63:634–8.

1795. Fenaux P, Beuscart R, Lai JL, Jouet JP, Bauters F. Prognostic factors in adult chronic myelomonocytic leukemia: an analysis of 107 cases. J Clin Oncol. 1988; 6:1417–24.

1796. Fenaux P, Jouet JP, Zandecki M et al. Chronic and subacute myelomonocytic leukaemia in the adult: a report of 60 cases with special reference to prognostic factors. Br J Haematol. 1987;65:101–6.

1797. Storniolo AM, Moloney WC, Rosenthal DS, Cox C, Bennett JM. Chronic myelomonocytic leukemia. Leukemia. 1990;4:766–70.

1798. Tefferi A, Hoagland HC, Therneau TM, Pierre RV. Chronic myelomonocytic leukemia: natural history and prognostic determinants. Mayo Clin Proc. 1989; 64:1246–54.

1799. Germing U, Kundgen A, Gattermann N. Risk assessment in chronic myelomonocytic leukemia (CMML). Leuk Lymphoma. 2004;45:1311–18.

1800. Anonymous. Chronic myelomonocytic leukemia: single entity or heterogeneous disorder? A prospective multicenter study of 100 patients. Groupe Francais de Cytogenetique Hematologique. Cancer Genet Cytogenet. 1991;55:57–65.

1801. Germing U, Gattermann N, Minning H, Heyll A, Aul C. Problems in the classification of CMML — dysplastic versus proliferative type. Leuk Res. 1998;22:871–8.

1802. Haase D, Fonatsch C, Freund M et al. Cytogenetic findings in 179 patients with myelodysplastic syndromes. Ann Hematol. 1995;70:171–87.

1803. Oo TH, Kenney L. Therapy-related chronic myelomonocytic leukemia with unique chromosomal abnormalities: monosomy 7 and t(12;17)(p13;q11.2). Am J Hematol. 2007;82:248–9.

1804. Golub TR, Barker GF, Lovett M, Gilliland DG. Fusion of PDGF receptor beta to a novel ets-like gene, tel, in chronic myelomonocytic leukemia with t(5;12) chromosomal translocation. Cell. 1994;77:307–16.

1805. Wlodarska I, Mecucci C, Marynen P et al. TEL gene is involved in myelodysplastic syndromes with either the typical t(5;12)(q33;p13) translocation or its variant t(10;12)(q24;p13). Blood. 1995; 85:2848–52.

1806. Baranger L, Szapiro N, Gardais J et al. Translocation t(5;12)(q31-q33;p12-p13): a non-random translocation associated with a myeloid disorder with eosinophilia. Br J Haematol. 1994;88:343–7.

1807. Tefferi A, Gilliland DG. Oncogenes in myeloproliferative disorders. Cell Cycle. 2007;6:550–66.

1808. Bennett JM, Catovsky D, Daniel MT et al. Proposals for the classification of the myelodysplastic syndromes. Br J Haematol. 1982;51:189–99.

1809. Aul C, Germing U, Gattermann N, Minning H. Increasing incidence of myelodysplastic syndromes: real or fictitious? Leuk Res. 1998;22:93–100.

1810. Aul C, Giagounidis A, Germing U. Epidemiological features of myelodysplastic syndromes: results from regional cancer surveys and hospital-based statistics. Int J Hematol. 2001;73:405–10.

1811. Williamson PJ, Kruger AR, Reynolds PJ, Hamblin TJ, Oscier DG. Establishing the incidence of myelodysplastic syndrome. Br J Haematol. 1994;87:743–5.

1812. Raza A, Gezer S, Mundle S et al. Apoptosis in bone marrow biopsy samples involving stromal and hematopoietic cells in 50 patients with myelodysplastic syndromes. Blood. 1995;86:268–76.

1813. Lepelley P, Campergue L, Grardel N, Preudhomme C, Cosson A, Fenaux P. Is apoptosis a massive process in myelodysplastic syndromes? Br J Haematol. 1996; 95:368–71.

1814. Shetty V, Hussaini S, Broady-Robinson L et al. Intramedullary apoptosis of hematopoietic cells in myelodysplastic syndrome patients can be massive: apoptotic cells recovered from high-density fraction of bone marrow aspirates. Blood. 2000;96:1388–92.

1815. Rosati S, Mick R, Xu F et al. Refractory cytopenia with multilineage dysplasia: further characterization of an 'unclassifiable' myelodysplastic syndrome. Leukemia. 1996;10:20–6.

1816. Germing U, Gattermann N, Strupp C, Aivado M, Aul C. Validation of the WHO proposals for a new classification of primary myelodysplastic syndromes: a retrospective analysis of 1600 patients. Leuk Res. 2000;24:983–92.

1817. Fenaux P. Myelodysplastic syndromes. Hematol Cell Ther. 1996;38:363–80.

1818. Morel P, Declercq C, Hebbar M, Bauters F, Fenaux P. Prognostic factors in myelodysplastic syndromes: critical analysis of the impact of age and gender and failure to identify a very-low-risk group using standard mortality ratio techniques. Br J Haematol. 1996;94:116–19.

1819. Schiffer CA, Lee EJ, Tomiyasu T, Wiernik PH, Testa JR. Prognostic impact of cytogenetic abnormalities in patients with de novo acute nonlymphocytic leukemia. Blood. 1989;73:263–70.

1820. Berger R, Bernheim A, Ochoa-Noguera ME et al. Prognostic significance of chromosomal abnormalities in

acute nonlymphocytic leukemia: a study of 343 patients. Cancer Genet Cytogenet. 1987;28:293–9.

1821. Bernasconi P, Klersy C, Boni M et al. World Health Organization classification in combination with cytogenetic markers improves the prognostic stratification of patients with de novo primary myelodysplastic syndromes. Br J Haematol. 2007;137:193–205.

1822. Bernasconi P, Klersy C, Boni M et al. Incidence and prognostic significance of karyotype abnormalities in de novo primary myelodysplastic syndromes: a study on 331 patients from a single institution. Leukemia. 2005;19:1424–31.

1823. Olney HJ, Le Beau MM. Evaluation of recurring cytogenetic abnormalities in the treatment of myelodysplastic syndromes. Leuk Res. 2007;31:427–34.

1824. Steensma DP, List AF. Genetic testing in the myelodysplastic syndromes: molecular insights into hematologic diversity. Mayo Clin Proc. 2005;80: 681–98.

1825. List AF, Sandberg AA, Doll DC. Myelodysplastic syndromes. In: Greer JP, Rodgers GM et al, ed. Wintrobe's Clinical Hematology 11th ed. Philadelphia: Lippincott Williams and Wilkins, 2004:2207–34.

1826. Mauritzson N, Johansson B, Rylander L et al. The prognostic impact of karyotypic subgroups in myelodysplastic syndromes is strongly modified by sex. Br J Haematol. 2001;113:347–56.

1827. Rossi G, Pelizzari AM, Bellotti D, Tonelli M, Barlati S. Cytogenetic analogy between myelodysplastic syndrome and acute myeloid leukemia of elderly patients. Leukemia. 2000;14:636–41.

1828. Maciejewski JP, Risitano A, Sloand EM, Nunez O, Young NS. Distinct clinical outcomes for cytogenetic abnormalities evolving from aplastic anemia. Blood. 2002;99:3129–35.

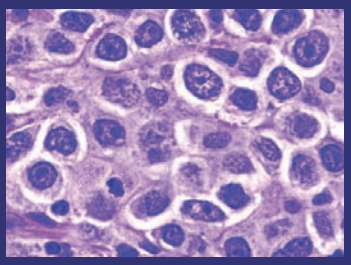
1829. Ridge SA, Worwood M, Oscier D, Jacobs A, Padua RA. FMS mutations in myelodysplastic, leukemic, and normal subjects. Proc Natl Acad Sci USA. 1990;87: 1377–80.

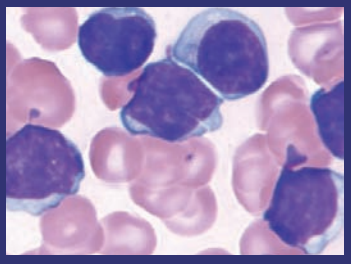
1830. Horiike S, Yokota S, Nakao M et al. Tandem duplications of the FLT3 receptor gene are associated with leukemic transformation of myelodysplasia. Leukemia. 1997;11:1442–6.

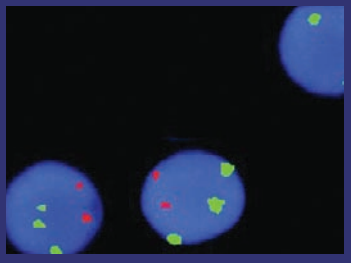
Index

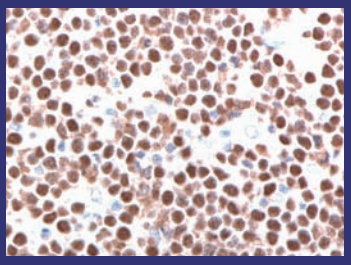
Page numbers in *italics* refer to figures or tables. Individual chromosomal abnormalities can be found under deletions, inversions, translocations and trisomy, as appropriate.

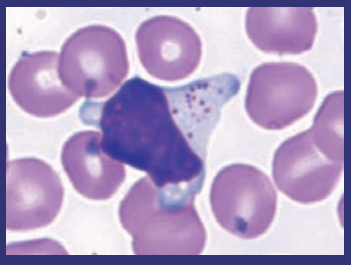
Cytogenetics, FISH and molecular testing in hematologic malignancies











Cytogenetics, fluorescence in situ hybridization (FISH) and molecular tests, especially polymerase chain reaction (PCR), play an important role in the management of patients with hematologic malignancies by helping to establish the diagnosis, as well as predict prognosis, response to treatment and disease progression. Chromosomal and molecular abnormalities provide the most reliable criteria for classification of hematopoietic tumors and often comprise the basis for targeted therapy.

Cytogenetics, FISH and Molecular Testing in Hematologic Malignancies provides a review of chromosomal and molecular changes in hematologic malignancies and correlates the karyotypic and genetic abnormalities with morphology, immunophenotype and clinical data. With over 180 figures and diagnostic algorithms, this text is essential reading for all pathologists, hematopathologists, hematologic oncologists, cytogenetists, cytogenetic technologists and cell biologists.

Wojciech Gorczyca MD PhD
Director of Hematopathology/Oncology Services, Genzyme Genetics, New York, NY, USA

Also available

Gorczyca, Weisberger & Emmons: *Atlas of Differential Diagnosis in Neoplastic Hematopathology*
(ISBN: 9781842142479)
Gorczyca: *Prognostic Markers in Hematologic Oncology*
(ISBN: 9781841845548)
Gorczyca: *Flow Cytometry in Neoplastic Hematology*
(ISBN: 9781841845654)
Mughal & Goldman: *Chronic Myeloproliferative Disorders*
(ISBN: 9780415415989)
Rozenberg: *Microscopic Haematology, 2nd edition*
(ISBN: 9781841842332)

9 780415 420099

informa
healthcare
www.informahealthcare.com