

## Chapter 2

# Classical and Molecular Cytogenetic Analysis of Hematolymphoid Disorders

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**Keywords** Myelodysplastic/myeloproliferative disorders · Leukemia · Lymphoma · Fluorescence in situ hybridization · Array comparative genomic hybridization (array CGH) · WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues · Hematolymphoid neoplasms · Philadelphia chromosome · Chronic myelogenous leukemia (CML) · Burkitt lymphoma · *BCR/ABL* · *PML/RARA* · Acute promyelocytic leukemia · Chromosome microarray analysis · International System of Human Cytogenetic Nomenclature (2009) · Illegitimate V(D)J or switch recombination · *ALU* sequences · LINE elements · Error-prone non-homologous end joining · Translin-binding consensus sequences · Scaffold-associated regions · Centromere enumeration probes (CEPs) · Locus-specific identifier (LSI) probes · Whole-chromosome paint (WCP) probes · Dual-color · dual-fusion (DCDF) LSI probes · Interphase FISH · Paraffin FISH · Myeloproliferative neoplasms (MPNs) · JAK2 V617F mutation · FIP1L1-PDGFR $\alpha$  · *ASS* gene · Imatinib mesylate (Gleevec) · Postpolycythemic myelofibrosis · Essential thrombocythemia · Primary myelofibrosis · *HMGA2* gene · Chronic neutrophilic leukemia · Normal karyotype · Chronic eosinophilic leukemia/idiopathic hypereosinophilic syndrome · Myeloid and lymphoid neoplasms with PDGFR $\alpha$  rearrangements · Polycythemia vera · Hypereosinophilia · *CHIC2* gene · Myelodysplastic syndrome · Chromosome 5q deletion · MDS associated with isolated del(5q) · Loss of the Y chromosome · MDS-FISH panel · Monosomy 5/del(5q) · Monosomy 7/del(7q) · Chromosome 11q deletion · Chromosome 13q deletion · Acute myeloid leukemia (AML) · Therapy-related- or t-AML · AML with t(8;21)(q22;q22) – RUNX1/RUNX1T1 · AML (promyelocytic) with t(15;17)(q22;q12) – PML/RAR $\alpha$  · ZBTB16/RAR $\alpha$  · NPM1/RAR $\alpha$  · NUMA1/RAR $\alpha$  · RAR $\alpha$  gene · All-trans-retinoic acid (ATRA) · AML with t(9;11)(p22;q23) – MLLT3/MLL · *MLL* (myeloid lymphoid lineage or mixed lineage leukemia)

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gene · t(9;11)(p21;q23) · t(11;19)(q23;p13.1) · t(11;19)(q23;p13.3) · MLL gene break-apart probe · AML with inv(16)(p13q22) or t(16;16)(p13;q22) – CBFβ/MYH · acute myelomonocytic leukemia (AMML) · Core binding factor beta subunit (CBFβ) · AML with t(6;9)(p23;q34) – DEK/NUP214 · Multilineage dysplasia · FLT3-ITD · AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2) – RPN1/EVI1 · AML (megakaryoblastic) with t(1;22)(p13;q13) – RBM15-MKL1 · Acute myeloid leukemia with myelodysplasia-related changes · Therapy-related myeloid neoplasms · Alkylating agent · Topoisomerase II inhibitor therapy · Acute myeloid leukemia · not otherwise specified · Acute myeloid leukemia with minimal differentiation · Acute myeloid leukemia without maturation · Acute myeloid leukemia with maturation · Acute myelomonocytic leukemia · Acute monoblastic and monocytic leukemia · Acute erythroid leukemia · Acute megakaryoblastic leukemia · Acute basophilic leukemia · Acute panmyelosis with myelofibrosis · B-lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities · Acute lymphoblastic leukemia (ALL) · Childhood ALL · B-lymphoblastic leukemia/lymphoma with hyperdiploidy · Hyperdiploid ALL · Hypodiploid ALL · B-lymphoblastic leukemia/lymphoma with hypodiploidy · B-lymphoblastic leukemia/lymphoma with t(12;21)(p13;q22) · TEL/AML1 (ETV6/RUNX1) · TEL (ETV6) gene · AML1 (CBFA2 or RUNX1) gene · B-lymphoblastic leukemia/lymphoma with t(9;22)(q34;q11.2) · BCR/ABL · B-lymphoblastic leukemia/lymphoma with t(v;11q23) · MLL rearranged · B-lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.3) · ETA/PBX1 (TCF3/PBX1) · B-lymphoblastic leukemia/lymphoma with t(5;14)(q31;q32) · IL3/IGH · Children's Oncology Group (COG) · T-lymphoblastic leukemia/lymphoma · T-cell receptor (TCR) genes · chronic lymphocytic leukemia/small lymphocytic lymphoma · MYB gene · ATM gene D13S319 locus · LAMP1 gene · p53 gene · CLL FISH panel · Plasmacell myeloma · IgH gene rearrangements · Monoclonal gammopathy of undetermined significance · plasma cell leukemia · Monosomy 13/del(13q), t(11;14)(q13;q32), t(4;14)(p16.3;q32), t(14;16)(q32;q23) · FGFR3/IgH · MAF/IgH · C-MYC gene · IgH/CCND1 · CCND1 gene · Plasma cell myeloma FISH panel · Non-Hodgkin lymphoma · API2-MALT1 · MALT lymphoma · Burkitt lymphoma · t(8;14)(q24;q32) · Mantle cell lymphoma v t(11;14)(q13;q32) · Diffuse large B-cell lymphoma · Complex karyotype · Follicular lymphoma · t(14;18)(q32;q21) · BCL2 gene · IgH gene · BCL6 gene · t(2;8)(p12;q24), t(8;22)(q24;q11) · Splenic marginal zone lymphoma · Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue · Anaplastic large-cell lymphoma · t(2;5)(p23;q35) · Anaplastic lymphoma kinase (ALK) gene

## Cytogenetic Analysis in the Diagnosis of Hematolymphoid Disorders

Non-random chromosomal abnormalities are a common feature of many hematolymphoid disorders and are a key component of their pathogenesis. As such,

routine chromosome analysis is critical in the laboratory workup of most known or suspected myelodysplastic/myeloproliferative disorders, leukemias, and lymphomas. Such studies can provide (1) diagnostic confirmation; (2) information useful for classification, staging, and prognostication; (3) information to guide appropriate choice of therapy; and (4) evidence of remission or relapse. In lymph node evaluation, cytogenetics can differentiate a reactive process from a malignant condition.

With the continued evolution of genetic laboratory methodologies, highly sensitive techniques have become commonplace in the laboratory workup of hematolymphoid disorders, including fluorescence in situ hybridization (FISH) and polymerase chain reaction (PCR). These technologies do not, however, provide the genome-wide coverage afforded by classical cytogenetics. Array comparative genomic hybridization (array CGH) promises the opportunity to study these malignancies in a genome-wide fashion and at a level of resolution not previously achievable by conventional cytogenetics. Using cytogenetic and molecular data, along with morphology and immunophenotype, hematolymphoid neoplasms can now be classified into clinically relevant categories that greatly improve tumor classification.

While earlier disease classification schemes included primarily clinical features, morphology, and immunophenotype, the recent advances in cytogenetic and molecular genetic analysis have greatly refined this process. Earlier attempts to classify myeloid and lymphoid neoplasms into meaningful subgroups resulted in the French–American–British (FAB) scheme proposed in 1976, which was based primarily on tissue morphology. Later revisions of the FAB system took into account immunocytochemical reactions of neoplastic cells; however, bone marrow morphology continued to be the backbone of classification. The “Revised European–American Classification of Lymphoid Neoplasms” (REAL) in 1994 extended the basis for classifying lymphoid neoplasms to include morphologic, immunologic, and genetic features; clinical presentations and disease course; and postulated normal cellular counterpart [1]. In 1997, the World Health Organization (WHO) released its first edition of a classification scheme developed jointly by pathologists, hematologists, and oncologists for hematologic malignancies [2]. This classification recognized specific disease entities based on a combination of morphologic and cytogenetic features. More recent editions of the *WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues* have included information about immunophenotype and molecular abnormalities. The latest edition (4th edition) published in 2008 [3] categorizes hematolymphoid neoplasms based on clinical and biological features, morphology, immunophenotype, cytogenetic abnormalities, and molecular genetic mutations. While certain conditions have specific immunophenotypic, cytogenetic, and/or molecular features, some myeloid and many lymphoid disorders demonstrate chromosomal abnormalities that may be observed in a number of entities. Nevertheless, these “non-specific” abnormalities can still provide important prognostic information that may guide choice of treatment. In addition, the classification of hematolymphoid neoplasms based on the multiple criteria described above has led to elucidation of involved genes and pathways, which has been critical for the development of “molecularly targeted” therapeutics.

## Classical Cytogenetic Analysis of Bone Marrow and Leukemic Blood

### *Historical Perspectives*

Despite the original description of chromosomes by Professor Walther Flemming in 1876 [4], it would be some 80 years later before the correct number of human chromosomes in a cell (46) was elucidated by Tjio and Levan [5]. Since then, the field of cytogenetics has witnessed several eras that have ushered in new and exciting discoveries that have dramatically improved the diagnostic capabilities of cytogenetics laboratories and made it an important specialty of medical genetics. The first cytogenetic abnormality associated with a specific type of malignancy was described by Nowell and Hungerford in 1960 [6]. The marker chromosome, named the Philadelphia chromosome for the city where it was identified, was associated with chronic myelogenous leukemia (CML). Utilizing better banding techniques, Janet Rowley at the University of Chicago later identified this marker as a derivative chromosome 22 originating from a reciprocal translocation between chromosomes 9 and 22 [t(9;22)(q34;q11.2)] [7]. Burkitt lymphoma was the first lymphoid neoplasm in which a characteristic chromosomal abnormality [t(8;14)(q24;q32)] was identified [8].

The “banding era” of the late 1960s and 1970s resulted in improved visualization of the human chromosome complement through the formation of unique banding patterns for each chromosome. The development of quinacrine banding, Giemsa banding, C-banding using barium hydroxide and reverse banding using acridine orange permitted delineation of individual chromosomes which improved the capability of cytogenetics laboratories to more accurately define numerical and structural chromosomal abnormalities.

The introduction of in situ hybridization methodologies in cytogenetics utilized DNA probes labeled with biotin and detected by sequential hybridizations with streptavidin–horseradish peroxidase and diaminobenzidine followed by visualization using standard bright-field microscopy. A slight modification of this enzymatic ISH procedure, known as chromogenic in situ hybridization (CISH), utilized fluorescently labeled DNA probes. This technique, known as fluorescence in situ hybridization (FISH), initially used single-fluorophore DNA probes and applied them to standard chromosome preparations for chromosome enumeration. As more single-copy FISH probes became commercially available, the diagnostic utility of FISH in the clinical cytogenetics laboratory increased. This technique became even more powerful as multicolor FISH probes became commonplace, permitting the identification of characteristic hematolymphoid chromosomal rearrangements such as the *BCR/ABL* fusion gene associated with the t(9;22)(q34;q11.2) in CML and the *PML/RARA* fusion gene associated with the t(15;17)(q22;q12) in acute promyelocytic leukemia. Additionally, because FISH did not require dividing cells, chromosomal abnormalities could be identified in non-dividing cells, including those in paraffin sections where tissue architecture is retained. As time went

on, more sophisticated molecular cytogenetic techniques were developed. These included comparative genomic hybridization (CGH) [9], spectral karyotyping [10], and Fiber FISH [11]. While these techniques extended the diagnostic capabilities of FISH, their technical complexity precluded their routine implementation in many cytogenetics laboratories.

With the approach of the 21st century, clinical cytogenetics found itself at yet another crossroads, defined by a powerful new diagnostic assay that truly blurs the line of demarcation between molecular genetic and cytogenetic analysis. Chromosome microarray analysis, or microarray CGH, is akin to a multiplex FISH experiment utilizing thousands of individual DNA probes arrayed to a glass slide. The “microarray era” has witnessed a substantial improvement in the diagnostic capability for identifying small (less than 5 Mb) unbalanced constitutional chromosomal rearrangements and has found great utility in the workup of children with developmental delay, mental retardation, autism/autism spectrum disorder, and multiple congenital anomalies [12, 13]. Recent literature has also demonstrated that this technology (using SNP arrays) will have a significant impact on the cytogenetic workup of hematolymphoid disorders, permitting detection of molecular mechanisms of tumorigenesis such as copy-number neutral loss of heterozygosity that cannot be identified using other cytogenetic methodologies [14, 15].

### *Specimen Collection and Storage*

Bone marrow is the tissue of choice for chromosome analysis in most hematological disorders including myeloproliferative neoplasms, myelodysplastic syndrome, chronic lymphocytic leukemia, and acute leukemias. Collection of 1–2 ml of bone marrow aspirate is adequate in most cases; however, a smaller sample may be acceptable if the marrow is hypercellular. If a bone marrow aspirate cannot be obtained, a bone core biopsy can be processed; however, the success rate for obtaining cytogenetic data on such a specimen is lower than that for a marrow aspirate. In patients with a white blood cell count greater than 10,000 billion/l and at least 10% circulating blast cells, a peripheral blood specimen cultured without phytohemagglutinin (PHA) can be studied. PHA will stimulate division of nonmalignant cells which can potentially interfere with the analysis of spontaneously dividing neoplastic cells. For lymphoma, sampling an involved lymph node is the method of choice. Cytogenetic analysis of bone marrow in lymphoid malignancies will yield positive results only if the bone marrow is involved as well; however, lymphoid-associated chromosomal abnormalities can sometimes be identified in bone marrow specimens without any overt morphological evidence of lymphoma involvement.

Immediate heparinization of a newly obtained bone marrow aspirate is critical, as clotting can make it difficult to process the specimen and may, in extreme cases, render the sample useless for cytogenetic study. Processing a clotted bone marrow specimen involves mechanical disaggregation of the clot and overnight treatment with 0.1 ml of heparin (stock solution 1,000 U/ml). In our experience, this procedure has proved successful in obtaining enough cells for tissue culture in most cases, with

more limited success in older bone marrow specimens. The newly obtained bone marrow aspirate should be transported in a sterile container containing preservative-free sodium heparin, tissue culture medium such as RPMI 1640 supplemented with heparin, or Hank's balanced salt solution (HBSS) containing heparin. A small sample of lymph node should be placed in sterile tissue culture medium or HBSS and transported to the cytogenetics lab as soon as possible. Every attempt should be made to transport the specimen to the lab without delay so that cultures can be initiated. If any delay in transport is expected, the specimens should be placed in sterile tissue culture medium to maintain cell viability. Samples can be stored at 4°C overnight and for no longer than 3 days. An important concern is the overgrowth of normal cells in specimens that have been subjected to delay prior to culture initiation. Thus, with longer delays comes an increased chance of a false-negative result. Specimens with high white blood cell counts and acute lymphoblastic leukemia specimens are particularly vulnerable and should be processed without delay.

### ***Specimen Processing and Tissue Culture***

Successful tissue culture of bone marrow specimens requires an optimal cell density, which for a bone marrow culture is approximately  $10^6$  cells. Extremely low and extremely high cell densities can compromise tissue culture outcomes. To determine the proper dilution of the original bone marrow suspension to ensure optimal cell density, two common methods are utilized. A hemocytometer can be used to perform a cell count on the original specimen, with the results used to determine the proper dilution of the original sample to  $10^6$  cells/ml per culture. The second method is cruder and utilizes the patient's white blood cell count to determine the number of drops of bone marrow suspension to add to 10 ml of tissue culture medium.

An experienced cancer cytogenetics laboratory will more often than not identify one or more chromosomal abnormalities, either by conventional analysis or FISH, in a bone marrow specimen with abnormal morphology. One exception to this rule are the chronic myeloproliferative neoplasms such as polycythemia vera, which are often characterized by molecular genetic changes such as the *JAK2 V617F* mutation. Success in obtaining positive cytogenetic results is highly dependent on choosing the appropriate culture conditions for the bone marrow, leukemic blood, and lymph node specimen. Providing clinical information and a suspected diagnosis (if possible) can aid greatly in determining the type and number of cultures to be established. Table 2.1 provides an overview of various culture regimens.

While a short-term culture of 24–48 h is initiated in most studies along with additional cultures as described above (given an adequate specimen volume), a direct method is also used in some laboratories. In this method, cells are treated with Colcemid for 1 h followed by incubation in a warm hypotonic solution (0.075 M KCl) for 15 min and fixation with 3:1 methanol:glacial acetic acid. The direct method often yields suitable metaphase cells for analysis and can provide a result within 24 h; however, short-term (24 h) cultures have two major advantages over direct preparations. First, the metaphase quality obtained with the direct preparation is not as good as that obtained in a short-term culture. Second, in some cases, clonal

**Table 2.1** Culture conditions used for hematolymphoid disorders

Clinical findings/suspected diagnosis	Types of cultures to be initiated
Myelodysplastic syndrome and myeloproliferative neoplasms	ST + CM
Acute myeloid leukemia	ST + CM
Anemia	
Bicytopenia	
Chronic myelogenous leukemia <sup>a</sup>	
Eosinophilia	
Pancytopenia	
Thrombocytosis	
Leukopenia or neutropenia	ST + CM (adult) ST + GCT (child)
B-cell lymphocytic leukemia	ST + GCT + LPS + PWM (if adequate specimen volume)
B-cell lymphoma	
Chronic lymphocytic leukemia <sup>b</sup>	
Hairy cell leukemia	
Lymphadenopathy	
Lymphocytosis	
Lymphoma	
Lymphoproliferative disorders	
Mantle cell lymphoma	
Monoclonal gammopathy	
Non-Hodgkin lymphoma	
Plasmacytoma	
Plasma cell leukemia	
Plasma cell myeloma	
T-cell leukemia/lymphoma	ST + GCT + PHA

ST – short-term culture (unstimulated 24-h culture)

CM – conditioned medium (48–72-h culture). Preparation: 1 ml supernatant from HTB-9 ladder carcinoma cell line culture (obtained from ATCC) added to 9 ml complete medium

GCT – giant cell tumor culture supplement (48–72-h culture). Preparation: 1 ml supernatant from TIB-223 human lung histiocytoma cell line culture (obtained from ATCC) added to 9 ml complete medium.

LPS – lipopolysaccharide (3–4-day culture)

PHA – phytohemagglutinin

PWM – pokeweed mitogen (3–4-day culture)

<sup>a</sup> For peripheral blood, if WBC <50.0, set up buffy coat; if WBC >50.0, set up whole blood

<sup>b</sup> If peripheral blood, LPS + PWM only; if post bone marrow transplant, ST + GCT

rearrangements are detectable only in cultured preparations, such as the diagnostic t(15;17) in acute promyelocytic leukemia. If the specimen is extremely limited in quantity, it may be necessary to initiate only one culture, which is usually a short-term unstimulated culture.

The protocol for studying peripheral blood is similar; however, transport medium should not be added to the blood sample. Transport in a sodium heparin vacutainer (alternatively lithium heparin) is necessary. The culture conditions described above would also be appropriate for peripheral blood samples. Mitogens are added to stimulate the growth of B or T cells as clinically indicated (Table 2.1).



## ***Culture Harvesting, Slide Preparation, and Staining***

Specimen harvesting involves incubating the culture in a mitotic spindle inhibitor, such as Colcemid (0.05  $\mu\text{g/ml}$ ) to collect metaphases, then adding a hypotonic solution (0.075 M KCl), and incubating at 37°C for 10–15 min. This is followed by several fixation steps using chilled 3:1 methanol:glacial acetic acid. With each successive fixation, the cell pellet is being “cleaned up,” ensuring an optimal cell suspension with little or no background when slides are prepared. After 4–5 fixation steps, the cells are resuspended in fixative and can be stored at –20°C.

Preparing slides is as much an art as it is a science, and each lab will have slight variations on their technique. The overall goal is to prepare slides with well-spread chromosomes that can be recognized as individual “metaphase spreads.” Precleaning microscope slides with 95% ethanol can facilitate uniformity of chromosome spreading and enhance the quality of metaphase preparations. Optimal humidity (45–55%) and ambient temperature (70–75°F) are also important, necessitating in some cases the use of an environmentally controlled chamber for slide preparation.

In the metaphase stage of the cell cycle, chromosomes are condensed such that individual chromosome morphology can be recognized. Chromosome morphology is characterized by size, centromere position, and banding pattern. The bands observed in metaphase chromosomes are prepared by processing slides using various different methodologies and staining solutions, including quinacrine mustard and fluorescence microscopy (Q banding), Giemsa or an equivalent stain (G banding), hot alkali followed by staining with Giemsa or acridine orange (R banding), chromosome denaturation prior to Giemsa staining (C banding) to visualize heterochromatic DNA, and staining with silver nitrate (AgNOR banding) to visualize nucleolar organizing regions in the short arm of acrocentric chromosomes.

## ***Guidelines for Microscopic Analysis of Bone Marrow and Leukemic Blood***

In most cases, normal and neoplastic cells will coexist in the specimen. The goal, therefore, is to identify those neoplastic cells that potentially carry one or more chromosomal abnormalities. Care should be exercised when making any clinical predictions based on the proportion of normal to abnormal cells in a given specimen, as this can be influenced by cell culture conditions as well as sampling error. The microscopists performing the cytogenetic examination must be aware that in some conditions, particularly ALL, it is those metaphases with poorer morphology that may be representative of the neoplastic clone. Care should, therefore, always be taken to examine a variety of metaphase cells of differing quality. In addition, a case which is found to be cytogenetically normal may still harbor significant molecular abnormalities. Approximately 40–50% of AML cases demonstrate a normal karyotype but possess one or more acquired mutational changes [14] or have



submicroscopic abnormalities detectable by higher resolution techniques such as array comparative genomic hybridization [16].

The typical oncology chromosome study requires the examination of 20 metaphase cells. A clonal abnormality as defined in ISCN 2009 [17] consists of two or more cells with the same chromosome gain or structural rearrangement, or three cells with the same chromosome loss. If a single-cell abnormality is identified, the process cannot be defined as clonal; however, if it is a characteristic abnormality associated with a specific hematolymphoid disorder or is observed in a patient that demonstrated it as part of an abnormal clone in a previous study, an extended workup is indicated. Sometimes, an apparently balanced rearrangement not known to be associated with any hematolymphoid disorder will be observed. This observation necessitates the examination of a PHA-stimulated peripheral blood culture to determine if the abnormality is constitutional in nature.

### *The Karyotype and Cytogenetic Nomenclature*

The karyotype is a pictorial representation of the 46 chromosomes present in each cell (Fig. 2.1). They are classified by their size and centromere position into seven groups. Within each of these groups, individual chromosome homologues are paired with each other based on their similar banding pattern generated by G, Q, or R banding.



**Fig. 2.1** A normal G-banded bone marrow karyotype

The development and refinement of a specific and descriptive way to describe karyotype abnormalities has been integral in the growth of cytogenetics over the last 30 years. The original attempt to achieve standardization of chromosome nomenclature was the document *A Proposed Standard System of Nomenclature of Human Mitotic Chromosomes* presented at the Denver Conference in 1960, while the newest revision of *An International System of Human Cytogenetic Nomenclature* (2009) has just been published [17]. Each revision in between has addressed the enhanced methodologies for studying chromosomes that were developed since the previous release. Techniques such as high-resolution banding, FISH, and most recently array CGH have resulted in a refinement of chromosome morphology, necessitating an expansion of chromosome nomenclature in each revision.

The reader is referred to ISCN 2009 [17] for an in depth description of human chromosome nomenclature. Only a few basic features will be presented here. Chromosomal abnormalities are of two types, numerical and structural. The normal modal chromosome number is 46, designated as a diploid cell. If more or less than 46 chromosomes are present, the cell is referred to as being aneuploid. If more than 46 chromosomes are present, the cell is hyperdiploid; if less than 45 chromosomes, the cell is hypodiploid. Gain of a chromosome is referred to as trisomy, while loss of a chromosome is referred to as monosomy. These are described in the karyotype designation by a “+” or “-,” respectively.

Structural abnormalities are designated by the type of abnormality present and the breakpoints involved. The breakpoints will lie either within a chromosome band or at the junction between two chromosome bands. A chromosome band is a portion of a chromosome clearly distinguishable from adjacent segments which may be lighter or darker depending on the banding technique. There are specific “landmark” bands that help to distinguish one chromosome from another. Each band is, at successively higher levels of resolution, further divided into subbands. The bands and subbands are numbered outward from the centromere.

Descriptions of structural rearrangements commonly observed in neoplastic disorders are provided in Table 2.2. Table 2.3 lists several examples of common chromosomal abnormalities and their description using ISCN 2009 nomenclature.

## **The Molecular Mechanisms Responsible for Chromosomal Rearrangements in Neoplasia**

Many cancers are associated with specific chromosomal abnormalities that disrupt normal cellular processes leading to malignant transformation. Much work has focused on the molecular mechanisms that lead to the visible chromosomal rearrangements observed in a variety of human constitutional chromosome disorders [18]. The mechanisms that underlie those pathogenetic rearrangements observed in various hematolymphoid disorders may not be dissimilar. A chromosome translocation appears to be initiated by a DNA double-strand break

**Table 2.2** Overview of structural chromosomal rearrangements observed in neoplasia

Type of structural chromosomal abnormality	Morphological change observed
Translocation	Exchange of two chromosomal segments distal to the designated breakpoints in two chromosomes; can be balanced or unbalanced
Derivative	A structurally rearranged chromosome generated from events involving two or more chromosomes or multiple events within a single chromosome
Inversion	180° inversion of chromosome segment between two designated breakpoints Pericentric – breakpoints are in p and q arms Paracentric – both breakpoints in same arm
Deletion	Loss of chromosome segment Terminal – Loss of segment distal to single breakpoint Interstitial – Loss of segment between two breakpoints
Isochromosome	Chromosome arms are identical with a single centromere
Isodicentric chromosome	A mirror-image chromosome with two centromeres
Ring	Chromosome with one breakpoint in each arm followed by reunion of two ends
Marker	Chromosome that appears to be mitotically stable but cannot be classified by conventional banding studies
Double minutes	Acentric chromosome fragments often in multiple copies
Homogenously staining region	Chromosome region that stains uniformly (Both are cytogenetic manifestations of gene amplification)
Add	Chromosome with “additional” material of unknown origin attached to the long or short arm

**Table 2.3** Common hematolymphoid chromosomal abnormalities and their description using ISCN nomenclature

<i>t(9;22)(q34;q11.2)</i>
t – denotes translocation
(9;22) – translocation between chromosomes 9 and 22
(q34;q11.2) – breakpoints are in the long arm of both chromosomes at bands 9q34 and 22q11.2
<i>der(22)t(9;22)(q34;q11.2)</i>
der(22) – denotes derivative chromosome 22 originating from the t(9;22)(q34;q11.2)
<i>inv(16)(p13q22)</i>
inv – denotes inversion
(16) – inversion involves chromosome 16
(p13q22) – the inverted segment lies between the breakpoints 16p13 (in the p arm) and 16q22 (in the q arm); the segment between the two breakpoints rotates 180°
<i>del(5)(q13q33)</i>
del – denotes deletion
(5) – deletion involves chromosome 5
(q13q33) – deleted segment is interstitial between bands 5q13 and 5q33 in the long arm
<i>i(17)(q10)</i>
i – denotes isochromosome
(17) – isochromosome involves chromosome 17
(q10) – chromosome arms are composed of two identical complete chromosome 17 long arms

followed by “misrepair.” Proposed mechanisms that result in recurrent, balanced translocations in hematolymphoid disorders include the following: (1) illegitimate V(D)J or switch recombination, (2) presence of repetitive sequences such as *ALU* sequences or LINE elements at “broken” ends, and (3) error-prone non-homologous end joining [19]. The joining of the two “broken” ends appears to be facilitated by the presence of specific DNA sequences at these breaks, including *ALU* sequences, translin-binding consensus sequences, and scaffold-associated regions [20]. In addition, the formation of recurrent translocations also requires a clustering within the nucleus of the two involved chromosomes. This has been demonstrated for the *BCR* and *ABL1* genes associated with chronic myelogenous leukemia and the *BCL6* and *MYC* genes that rearrange with *IGH* in B-cell disorders [19]. The mechanism(s) that facilitate this clustering within the nucleus are not known. Finally, the identification of recurrent hematolymphoid translocations including the t(9;22)(q34;q11.2), t(15;17)(q22;q12), and t(14;18)(q32;q21) rearrangements by the polymerase chain reaction (PCR) in apparently healthy individuals raises the possibility that the mechanisms required for malignant transformation may be far more involved than what is currently appreciated [21].

## **Molecular Cytogenetic (FISH) Analysis of Bone Marrow and Leukemic Blood**

### ***Basic Principles of Fluorescence In Situ Hybridization***

Conventional cytogenetic analysis of bone marrow or leukemic blood cultures permits a genome-wide assessment of chromosomal abnormalities; however, it is sometimes hampered by low mitotic index, poor chromosome morphology, considerable karyotypic complexity, and normal karyotypes. Fluorescence in situ hybridization (FISH) can overcome these problems by targeting specific nucleic acid sequences in a highly sensitive and rapid manner. The powerful diagnostic capabilities of FISH are rooted in its relative ease of use in the clinical laboratory, enhanced sensitivity over conventional banding studies, and ability to probe for one or more specific genomic regions of interest in either dividing or non-dividing cells, as well as in in situ tissue preparations permitting identification of cytogenetic changes in a specific cell lineage. By utilizing fluorescently labeled DNA probes to detect genetic aberrations that are generally beyond the resolution of conventional chromosome banding studies, FISH in a sense merges conventional cytogenetic analysis with molecular genetics.

FISH is based on the principle that a single-stranded DNA molecule will recognize and bind to its complementary sequence on a metaphase chromosome or in an interphase nucleus. The overall hybridization is similar to in situ hybridization using radioisotope-labeled probes. The major advantage of FISH, however, is the utilization of a DNA probe labeled with a fluorescent dye, which results in a

highly sensitive, simple, and rapid assay. Both the probe and the target DNA are treated with heated formamide solution to denature double-stranded DNA, followed by probe application to target DNA and incubation at 37°C. During the incubation process, annealing of the probe to the target sequence occurs through complementary base pairing. A fluorescence microscope equipped with appropriate filters is then used to detect the hybridized probe on the target material, appearing as bright-colored signals. Multiple probes labeled with different colored fluorescent tags can be applied simultaneously on the same target to detect one or more specific regions of the genome.

FISH analysis can be performed on either metaphase chromosomes derived from cultured cells or non-dividing cells, allowing identification of chromosomal aberrations irrespective of cell cycle stage. This latter technique, known as interphase FISH, is a powerful cytogenetic tool that can be applied to a wide variety of clinical specimens to enumerate chromosomes and identify chromosomal rearrangements. When viable specimens are not available, interphase FISH can be performed on a bone marrow or a blood smear, disaggregated cells from a paraffin block, touch preparation from a lymph node, or cytopsin cells fixed on a microscope slide. FISH can also be performed on a paraffin-embedded tissue section. While this technique has the advantage of maintaining tissue architecture, its inherent disadvantages include nuclear truncation artifact and overlapping cells that may make analysis difficult.

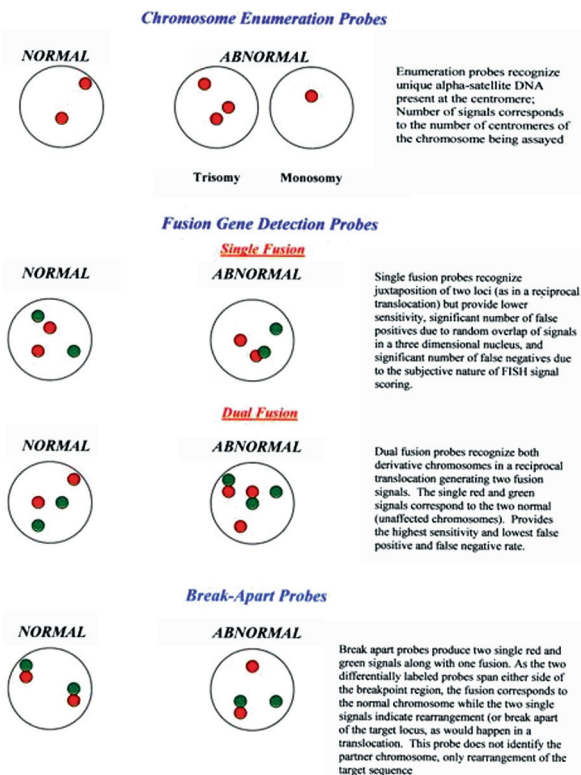
### ***Clinical Indications for FISH Testing in Hematolymphoid Disorders***

Common indications for FISH testing in hematolymphoid malignancies include the following: (1) confirmation of chromosomal abnormalities detected by conventional cytogenetics and establishment of FISH signal pattern for follow-up study, (2) detection of chromosomal abnormalities when clinical and morphologic findings are suggestive of a specific chromosomal abnormality [e.g., t(11;14) in mantle cell lymphoma], (3) characterization of genetic aberrations using a panel of disease-specific FISH probes for risk stratification and therapeutic management, such as in acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), and plasma cell myeloma (PCM), (4) detection of cryptic or masked translocations when chromosome analysis is inconclusive or yields a normal karyotype [such as the t(12;21) in ALL or t(4;14) in myeloma], (5) detection of lymphoma-associated translocations in paraffin-embedded tissue sections, (6) quantitation of minimal residual disease and detection of cytogenetic remission and relapse through analysis of a large number of both dividing and non-dividing cells, (7) monitoring cross-sex bone marrow transplantation patients for engraftment status (chimerism), and (8) rapid detection of *PML/RARA* gene fusion in acute promyelocytic leukemia, where quick diagnosis is required for prompt treatment.

## Types of FISH Probes Routinely Used in Hematological Disorders

There are primarily three types of probes used in clinical FISH testing: centromere enumeration probes (CEPs), locus-specific identifier (LSI) probes, and whole-chromosome paint (WCP) probes (Fig. 2.2).

The CEPs recognize a highly repetitive alpha-satellite DNA sequence located at the centromere of each chromosome. These probes are labeled in one color and give a large, bright signal, useful for chromosome enumeration in both interphase and metaphase cells. The LSI probes hybridize to single-copy DNA sequences in a specific chromosomal region or gene. These probes can identify fusion gene products generated from a reciprocal translocation, chromosome inversions, and gene deletion or amplification. On metaphase cells, the LSI probes give two small, discrete signals per chromosome. The gain of LSI signals within a nucleus is consistent with duplications or amplifications, while the loss of LSI signal indicates a deletion. The design of LSI probes targeting specific translocations has evolved considerably, minimizing the false-positive and false-negative rates. Dual-color, dual-fusion (DCDF) LSI probes are designed to span both sides of the breakpoints in two different chromosome regions/genes involved in a reciprocal translocation, resulting



**Fig. 2.2** Examples of FISH probe designs commonly used in hematolymphoid disorders and their resulting hybridization patterns (reproduced with permission from [58])

in remarkably improved specificity. To assess the rearrangement of a gene that may be associated with multiple translocation partners, a dual-color break-apart (DCBA) LSI probe has been designed. The DCBA probe is a combination of two differently labeled probes that bind to sequences that flank the 5'- and 3'-ends of the break-point within the involved chromosome region. The separation of the two colors is indicative of rearrangement. WCP probes are cocktails of unique sequence DNA probes derived from flow-sorted chromosomes, chromosome-specific libraries, or chromosome-microdissected regions that recognize specific sequences spanning the length of a chromosome. In normal metaphase preparations, this gives the effect that both chromosome homologues are "painted." WCP probes are useful to identify marker chromosomes and to detect cryptic translocations; however, their utility in interphase nuclei is limited. An overview of commercially available FISH probes useful to characterize hematolymphoid disorders is provided in Table 2.4.

**Table 2.4** Commercially available FISH probes used to characterize hematolymphoid disorders

Disease	Chromosomal abnormality	Gene(s) involved	FISH probe(s)
CML	t(9;22)(q34;q11.2)	<i>ABL</i> , <i>BCR</i>	<i>BCR/ABL</i> fusion
		<i>ASS</i>	<i>BCR/ABL</i> fusion + 9q34
AML	t(8;21)(q22;q22)	<i>RUNX1T1 (ETO)</i> , <i>RUNX1 (AML1)</i>	<i>RUNX1T1/RUNX1</i> fusion
	inv(16)(p13q22)/t(16;16)	<i>MYH11</i> , <i>CBFβ</i>	<i>CBFβ</i> or <i>MYH11</i> break apart
			<i>MYH11/CBFβ</i> fusion
	t(v;11)(v;q23), del 11q23	<i>MLL</i>	<i>MLL</i> break apart
	Monosomy 5/del 5q33-34	<i>CSF1R</i>	<i>CSF1R/5p</i>
	Monosomy 5/del 5q31	<i>EGR1</i>	<i>EGR1/5p</i>
	Monosomy 7/del 7q		D7S522/CEP7
	del 20q		D20S108
	Trisomy 8		CEP8
AML-M3 (APL)	t(15;17)(q22;q12)	<i>PML</i> , <i>RARA</i>	<i>PML/RARA</i> fusion,
	t(v;17)(v;q12)		<i>RARA</i> break apart
MDS	Monosomy 5/del 5q33-34	<i>CSF1R</i>	<i>CSF1R/5p</i>
	Monosomy 5/del 5q31	<i>EGR1</i>	<i>EGR1/5p</i>
	Monosomy 7/del 7q		D7S522/CEP7
	del 20q		D20S108
	Trisomy 8		CEP 8
	del(11)(q23)	<i>MLL</i>	<i>MLL</i> break apart
	del(13)(q14)	<i>RB1</i>	<i>RB1/13q14</i>
			D13S319/13q14
MPN	Trisomy 8		CEP 8
	Trisomy 9		CEP 9
	del(4)(q12q12)	<i>CHIC2</i>	<i>CHIC2/4qter</i>
		<i>PDGFRA/FIP1L1</i>	<i>PDGFRA/FIP1L1 fusion</i>
	del 20q		D20S108
B-ALL	Trisomy 4, 10, 17		CEP 4, 10, 17
	t(12;21)(p13;q22)	<i>ETV6 (TEL)</i> , <i>RUNX1 (AML1)</i>	<i>TEL/AML1 ES fusion</i>
	t(v;11)(v;q23)	<i>MLL</i>	<i>MLL</i> break apart
	t(9;22)(q34;q11.2)	<i>ABL</i> , <i>BCR</i>	<i>BCR/ABL</i> fusion



**Table 2.4** (continued)

Disease	Chromosomal abnormality	Gene(s) involved	FISH probe(s)
T-ALL	t(1;19), t(17;19)		<i>E2A TC3F/PBX1</i> fusion
	t(5;14)(q35;q32)	<i>TLX3 (HOX11L2)</i>	<i>TLX3</i> break apart
	del(9)(p21)	<i>p16</i>	<i>p16/D9Z3</i>
	7q35 rearrangement	<i>TCRbeta</i>	<i>TCRbeta</i> break apart
	7p14-15 rearrangement	<i>TCRgamma</i>	<i>TCRgamma</i> break apart
	14q11.2 rearrangement	<i>TCRalpha/delta</i>	<i>TCRalpha/delta</i> break apart
CLL	del(11)(q22.3)	<i>ATM</i>	<i>ATM</i>
	Trisomy 12		CEP 12
	del (13)(q14.3)	<i>Micro-RNA genes (miR-16-1, miR-15a)</i>	<i>D13S319/13q14</i>
	del(17)(p13)	<i>TP53</i>	<i>TP53</i>
PCM	del(6)(q23)	<i>MYB</i>	<i>MYB</i>
	Monosomy 13/del(13)(q14)		<i>D13S319/LAMP1</i>
	Trisomy 5,9,15,19		<i>RB1/LAMP1</i>
	del(17)(p13)	<i>TP53</i>	CEP 5, 9, 15, 19
	t(11;14)(q13;q32)	<i>CCND1, IGH</i>	<i>TP53</i>
	t(4;14)(p16.3;q32)	<i>FGFR3, IGH</i>	<i>CCND1/IGH</i> fusion
NHL	t(14;16)(q32;q23)	<i>IGH, MAF</i>	<i>FGFR3/IGH</i> fusion
	t(V;8)(V;q24)	<i>MYC</i>	<i>MAF/IGH</i> fusion
	t(V;14)(V;q32)	<i>IGH</i>	<i>MYC</i> break apart
	t(11;14)(q13;q32)	<i>CCND1, IGH</i>	<i>IGH</i> break apart
	t(14;18)(q32;q21)	<i>IGH, BCL2</i>	<i>CCND1/IGH</i> fusion
	t(8;14)(q24;q32)	<i>MYC, IGH</i>	<i>BCL2/IGH</i> fusion
MCL	t(8;14)(q24;q32)	<i>MYC, IGH</i>	<i>IGH/MYC, CEP8</i>
	t(2;8)(p12;q24)	<i>MYC, IGK</i>	<i>MYC</i> break apart
	t(8;22)(q24;q11.1)	<i>MYC, IGL</i>	
DLBCL	t(3;14)(q27;q32), t(2;3)(p12;q27), t(3;22)(q27;q11.2)	<i>BCL6, IGH</i> <i>IGK, BCL6</i> <i>BCL6, IGL</i>	<i>BCL6</i> break apart
	t(11;18)(q21;q21), t(14;18)(q32;q21)	<i>API2, MALT</i> <i>IGH, MALT</i>	<i>API2/MALT1</i> fusion
	t(2;5)(p23;q35), t(V;5)(V;q35)	<i>ALK, NPM</i>	<i>MALT1</i> break apart
ALCL			<i>ALK</i> break apart

CML, Chronic myelogenous leukemia; AML, acute myelogenous leukemia; APL, acute promyelocytic leukemia; MDS, myelodysplastic syndrome; MPD, myeloproliferative disorder; ALL, acute lymphoid leukemia (B or T cell); CLL, chronic lymphocytic leukemia; PCM, plasma cell myeloma; NHL, non-Hodgkin lymphoma; MCL, mantle cell lymphoma; FL, follicular lymphoma; BL, Burkitt lymphoma; DLCL, diffuse large-cell lymphoma; MALT, extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue; ALCL, anaplastic large-cell lymphoma; BMT, bone marrow transplantation

## Advantages and Disadvantages of FISH

FISH analysis has both advantages and disadvantages over conventional cytogenetic analysis. FISH can (1) be performed on metaphase cells or interphase nuclei

(non-dividing cells) and on fresh or fixed tissue samples, (2) target genetic aberrations that pinpoint candidate genes involved in leukemogenesis, (3) simultaneously assess chromosomal aberrations, cellular phenotype, and tissue morphology utilizing paraffin-embedded tissue sections (paraffin FISH), (4) provide in a rapid fashion highly specific, sensitive, and reproducible results that are interpreted objectively, (5) simultaneously assess multiple genomic targets, (6) provide superior resolution (interphase FISH  $\geq 20$  kb, metaphase FISH  $\geq 100$  kb) compared with standard karyotyping ( $>10$  Mb), and (7) detect specific cryptic chromosomal abnormalities. Limitations of FISH include the following: (1) its inability to provide a genome-wide assessment of chromosomes; (2) the necessity for clinical information or a differential diagnosis to guide the appropriate choice of probes to be used, and (3) the requirement for a high-quality fluorescence microscope with multiple filters, a CCD camera that can detect low-level light emission, and sophisticated imaging software.

## Diagnostic and Prognostic Cytogenetic Markers in Myeloid Disorders

### *Myeloproliferative Neoplasms (MPN)*

Most MPNs are not characterized by a unique cytogenetic abnormality but instead demonstrate molecular mutations in genes that code for cytoplasmic or receptor protein tyrosine kinases. As such, these mutations such as the *JAK2 V617F* and *FIP1L1-PDGFR* fusion gene do not affect differentiation but instead convey a proliferative advantage [22]. Those cytogenetic abnormalities that are identified are found in a variety of myeloid neoplasms, precluding their use as a marker to subclassify the disease process. Despite the relatively low frequency of karyotypic abnormalities at diagnosis in these disorders, cytogenetic analysis is still important. It can distinguish a clonal process from a reactive myeloproliferation, it can exclude chronic myelogenous leukemia characterized by the Philadelphia (Ph) chromosome, and it can be used throughout the course of the disease to identify cytogenetic progression associated with disease progression and an increased risk of leukemic transformation. In addition, identification of a complex karyotype in the diagnostic bone marrow is associated with a poorer prognosis.

*Chronic myelogenous leukemia, BCR/ABL1 positive.* CML was the first hematological disorder to be associated with a specific chromosomal abnormality, the t(9;22)(q34;q11.2) which generates the Philadelphia chromosome (truncated chromosome 22) (Fig. 2.3).

The molecular consequence of this translocation is fusion of the 3' segment of the Abelson (*ABL1*) proto-oncogene on chromosome 9q34 to the 5' segment of the *BCR* gene on chromosome 22q11.2, producing a chimeric 210-kDa *BCR/ABL* fusion gene product that has constitutive tyrosine kinase activity. At diagnosis, over 90% of CML patients will demonstrate the t(9;22)(q34;q11.2) by conventional cytogenetic



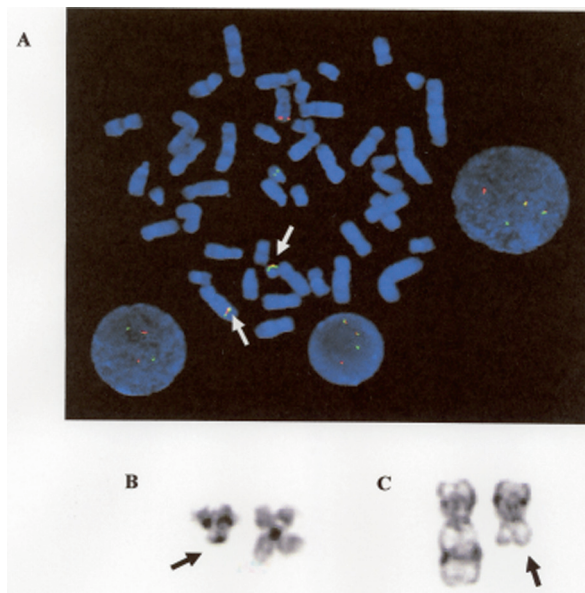
**Fig. 2.3** An abnormal female karyotype demonstrating the  $t(9;22)(q34;q11.2)$  which generates the Philadelphia chromosome [der(22) chromosome]

analysis. The remaining cases present either a submicroscopic rearrangement or a variant  $t(v;9;22)$  translocation. In these cases, FISH analysis can readily detect the *BCR/ABL1* fusion, and failure to do so would suggest that another MPN, such as chronic neutrophilic leukemia, should be considered. The dual-color, dual-fusion FISH (D-FISH) assay utilizing the *BCR/ABL1* probe (Fig. 2.4a) not only will detect translocations occurring at the typical major breakpoint cluster region (M-BCR) that generates the p210 product but will also identify a breakpoint in the micro breakpoint cluster region ( $\mu$ -BCR) which produces a larger fusion protein (p230) rarely observed in CML, as well as a breakpoint in the minor breakpoint cluster region (m-BCR) producing the shorter fusion product (p190) most often observed in Ph+ ALL.

Deletion of DNA sequences proximal to the 9q34 breakpoint, which includes the *ASS* gene, has been observed in approximately 10–30% of CML patients at diagnosis. These deletions have been associated in some studies with a shortened chronic phase and decreased overall survival; however, other studies have reported no significant difference in those patients with a der(9) deletion with regard to response rate or overall survival [23].

Effective treatments for CML including imatinib mesylate (Gleevec),  $\alpha$ -interferon, and allogeneic stem cell transplantation result in a decrease in the percentage of Ph+ neoplastic cells. *BCR/ABL1* FISH can accurately quantify cytogenetic response to therapy, determine remission status, and identify relapse. With

**Fig. 2.4** Common cytogenetic abnormalities in MPNs. (a) A *BCR/ABL1* fusion in CML is demonstrated utilizing the dual-color, dual-fusion FISH assay. The *BCR/ABL1*-positive nuclei (with two fusion signals) are at 3 and 6 o'clock. The nucleus in the *bottom left corner* is negative for *BCR/ABL1* fusion (two red, two green signals). The metaphase cell in the center is also positive for fusion [arrows identify the der(9) and der(22) chromosomes]. Deletion of chromosomes 20q (b) and 11q (c) is a relatively common abnormality in MPNs



successful treatment, the D-FISH assay can monitor regression of the clone down to 1%. Much has been written about the use of *BCR/ABL1* FISH analysis of peripheral blood specimens. This is a common practice for routinely monitoring CML patients, as it can be performed at regular intervals without the need for an invasive bone marrow aspiration, even for patients in complete cytogenetic remission. Some studies have suggested a similar performance of the *BCR/ABL1* quantitative FISH assay in peripheral blood versus bone marrow for detection of minimal residual disease; however, other studies have suggested that measuring *BCR/ABL1* positivity in peripheral blood may underestimate the tumor burden [23]. Nevertheless, it is generally acknowledged that FISH analysis of peripheral blood utilizing D-FISH is adequate for CML disease monitoring.

Of the three diagnostic modalities (karyotyping, FISH, and RT-PCR), only conventional cytogenetics provides a genome-wide assessment that permits identification of clonal evolution including acquisition of abnormalities such as trisomy 8, isochromosome 17q, trisomy 19, and an additional copy of the der(22) chromosome. These abnormalities herald the onset of accelerated phase or blast phase CML which would necessitate modifications of the treatment plan. Thus, neither RT-PCR nor *BCR/ABL1* FISH negate the importance of bone marrow cytogenetic analysis as an important management tool in CML.

*Polycythemia vera.* The most common cytogenetic markers identified in PV in decreasing frequency are del(20q) (Fig. 2.4b), +8, +9, 9p rearrangement, gains of 1q, and del(13q). These abnormalities are observed in 15–25% of cases. Trisomy 8 may be the sole change or may be found in combination with trisomy 9. A clone with

trisomies 8 and 9 may persist for several decades without clonal evolution or transformation to acute leukemia. As PV evolves to postpolycythemic myelofibrosis or acute leukemia, additional cytogenetic abnormalities are acquired [23]. This cytogenetic evolution is apparent when comparing follow-up bone marrow biopsies with the baseline karyotype performed on the diagnostic bone marrow specimen. The identification of unfavorable prognostic markers [any aberration other than del(13q) or del(20q)] appears to be the strongest predictor of a poor prognosis in secondary myelofibrosis [24].

*Essential thrombocythemia.* Less than 10% of ET cases demonstrate cytogenetic abnormalities, and none are specific for this disorder. Like other MPNs, deletions of chromosomes 5q, 13q, and 20q, along with +8, +9, and gains of 1q are commonly observed. One important prognostic cytogenetic marker in ET is the presence of abnormalities involving chromosomes 7 and 17, which appear to be associated with a higher risk of leukemic transformation. Since none of these abnormalities are specific for ET, the greatest benefit of cytogenetic testing in this disorder is to exclude the presence of the Ph chromosome as a cause of thrombocytosis [22].

*Primary myelofibrosis.* Chromosomal abnormalities are found in 40–50% of cases and are found in greater numbers with disease progression. The presence of either del(13)(q12–22) or der(6)t(1;6)(q21–23;p21.3) is strongly suggestive but not diagnostic for PMF. Non-random abnormalities are similar to those found in PV, including trisomy for chromosomes 8, 9, and 21 as well as del(13q) and del(20q) chromosomes. As the disease progresses, structural abnormalities become more common, including gain of 1q, chromosome 7q abnormalities, and del(17p). The chromosome 7q abnormalities along with chromosome 5q deletions may be therapy-related changes related to cytotoxic therapy used to treat the myeloproliferative process. The *HMGA2* (high-mobility group protein A2) is disrupted by a recurrent breakpoint at chromosome band 12q14 in some cases [3, 22].

*Chronic neutrophilic leukemia.* Most patients with CNL demonstrate a normal karyotype; however, +8, +9, +21, del(11q) (Fig. 2.4c), del(12p), and del(20q) have been reported as clonal aberrations. As the disease progresses, clonal cytogenetic abnormalities may emerge [3].

*Chronic eosinophilic leukemia/idiopathic hypereosinophilic syndrome.* CEL/HES belongs to the WHO subgroup of *Myeloid and Lymphoid Neoplasms with PDGFRA Rearrangement*. These disorders are characterized by a persistent unexplained hypereosinophilia and rearrangement of the *PDGFRA* gene. The most common rearrangement of *PDGFRA* involves formation of a hybrid fusion tyrosine kinase between the 5'-portion of the *FIP1L1* gene and the 3'-portion of the *PDGFRA* gene through a cryptic 800-kb interstitial deletion within chromosome band 4q12 [25, 26]. This event can be identified in 40–60% of CEL patients and can be readily demonstrated by FISH utilizing a probe for the *CHIC2* gene, which lies between the *FIP1L1* and *PDGFRA* genes and is deleted when the fusion event occurs. Recently, a FISH probe which recognizes the *PDGFRA/FIP1L1* fusion gene has become available. A subset of patients with CES have benefited from treatment with imatinib mesylate, which appears to target *FIP1L1/PDGFRA* [25].

## ***Myelodysplastic Syndromes (MDS)***

Bone marrow cytogenetic analysis is a standard practice in the evaluation of a patient with suspected MDS and is considered an independent predictor of clinical outcome, overall survival, and progression to acute leukemia. The extent and nature of cytogenetic abnormalities is one of the three parameters in the International Prognostic Scoring System (IPSS), along with degree of peripheral cytopenia and bone marrow blast cell percentage that separates patients into one of the four prognostic groups (good, intermediate-1, intermediate-2, and poor) with regard to both survival and AML evolution [27]. As the disease becomes more severe, the frequency of cytogenetic abnormalities increases. Cytogenetic analysis can also distinguish a monoclonal proliferation from a reactive process in a morphologically unremarkable bone marrow and can, through serial cytogenetic studies, identify clonal evolution which accompanies progression of disease.

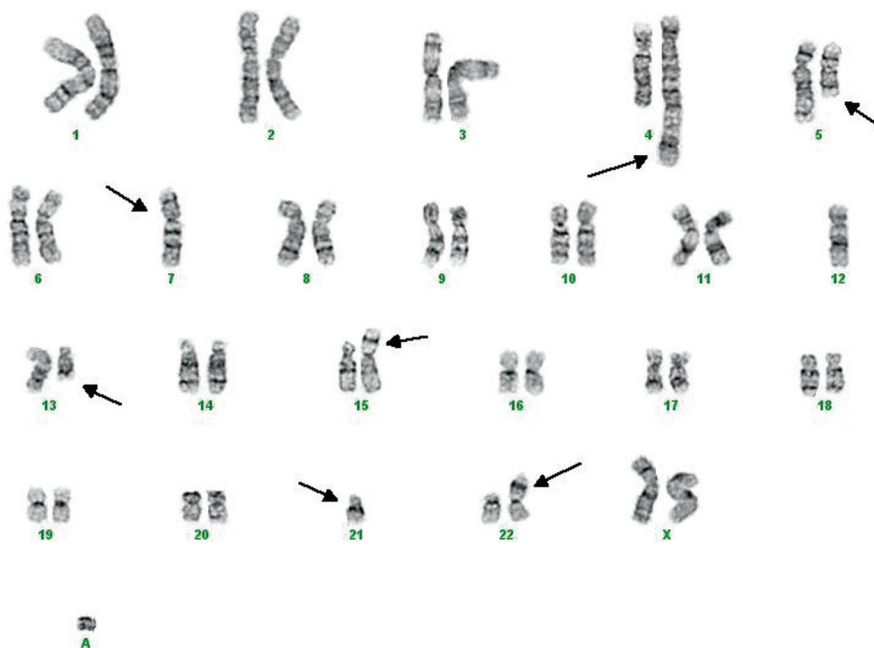
Conventional cytogenetic analysis can identify chromosomal abnormalities in 40–70% of de novo MDS cases and in almost 95% of t-MDS at diagnosis [28], with none specific for a particular MDS subtype except for the chromosome 5q deletion [WHO classification: *MDS associated with isolated del(5q)*]. Recurrent chromosome changes in MDS include loss of chromosome 5 or 7, deletions of chromosome 5q or 7q, trisomy 8, and chromosome 20q deletion. Loss of the Y chromosome is also relatively common in MDS, but this may be an age-related artifact in many patients. The identification of trisomy 8 and/or del(20q) in the absence of morphological evidence does not provide a definitive diagnosis of MDS. Close clinical and laboratory follow-up of such patients is necessary to identify emerging evidence of myelodysplasia [3]. Less frequently, structural rearrangements involving chromosomes 3q; deletion of chromosomes 11q, 13q, and 17p; and trisomies 9 and 21 are observed. Many of these chromosomal changes are also observed in AML, a finding indicative of the pathobiologic similarity between the two diseases. Complex karyotypes are often associated with advanced disease and a greater likelihood of leukemic transformation (Fig. 2.5).

The primary utility of FISH analysis in MDS is based on the finding that 15–20% of MDS patients demonstrate a normal karyotype, yet possess one or more clonal abnormalities of prognostic and/or therapeutic significance when analyzed by FISH [28, 29]. These patients will often demonstrate an increase in bone marrow blasts, an increase in rate of leukemic transformation, and a poorer prognosis [29]. Based on this and other studies, most advocate the use of an MDS-FISH panel on the diagnostic specimen. The MDS-FISH panel utilized in many laboratories includes probes to detect monosomy 5/del(5q), monosomy 7/del(7q), trisomy 8, chromosome 20q deletion, chromosome 11q deletion, and chromosome 13q deletion [28].

## ***Acute Myeloid Leukemia***

Acute myeloid leukemia (AML) is characterized by excessive accumulation of myeloid blasts ( $\geq 20\%$ ) in bone marrow, peripheral blood, and other tissues. AML can be de novo or can occur following exposure to cytotoxic agents including





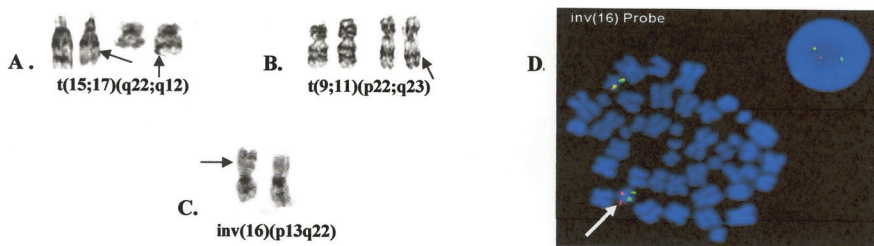
**Fig. 2.5** An abnormal cytogenetic clone presenting this complex karyotype was identified in an 83-year-old female with pancytopenia. Her history is positive for right breast infiltrating ductal carcinoma in 2002 with chemotherapy and radiation therapy. Bone marrow examination revealed refractory anemia with excess blasts, type 1. Abnormalities are identified by *arrows* and include the myeloid markers *del(5q)* and monosomy 7 which are consistent with secondary (therapy-related) myelodysplasia

chemotherapy and radiotherapy (therapy-related- or t-AML). Some 10–15% of AML cases are related to such previous cytotoxic exposure. The identification of specific cytogenetic abnormalities is diagnostic for specific AML subtypes and can be powerful predictors of prognosis and response to therapy. Overall, cytogenetic abnormalities are identified in approximately 55% of adults at diagnosis, with a range of 50–80% [28]; however, only a subset of these chromosome changes are associated with clinical, morphological, and immunophenotypic specificity for a particular AML subtype.

In the current WHO classification scheme, the following AMLs are characterized by a recurrent cytogenetic abnormality associated with a specific molecular rearrangement (Fig. 2.6a–d):

- *AML with  $t(8;21)(q22;q22)$  – *RUNX1/RUNX1T1**: Identified in 5–12% cases of AML with maturation [FAB classification: AML-M2] and in 40–50% of karyotypically abnormal cases of AML with maturation.
- *AML (promyelocytic) with  $t(15;17)(q22;q12)$  – *PML/RAR $\alpha$**  (Fig. 2.6a): Acute promyelocytic leukemia (APL) [FAB classification: AML-M3], a disease





**Fig. 2.6** (a–c) Common rearrangements identified in acute myeloid leukemia (arrows point to derivative chromosomes). (d) FISH detection of chromosome 16 inversion using a break-apart probe for CBF $\beta$  in an interphase nucleus and metaphase spread (arrow indicates break-apart signal)

primarily observed in young adults, is characterized by the presence of abnormal hypergranular promyelocytes. All AML cases with the  $t(15;17)(q22;q12)$  are diagnosed as APL; however, not all cases of APL will present the classic  $t(15;17)(q22;q12)$  due to the presence of (1) a complex karyotype involving both chromosomes 15 and 17 with additional cytogenetic changes, (2) a submicroscopic event leading to insertion of the retinoic acid receptor alpha ( $RAR\alpha$ ) gene into the promyelocytic leukemia ( $PML$ ) gene, or (3) a variant translocation such as  $t(11;17)(q23;q12)$  with  $ZBTB16/RAR\alpha$  fusion,  $t(5;17)(q35;q12)$  with  $NPM1/RAR\alpha$  fusion, or  $t(11;17)(q13;q12)$  with  $NUMA1/RAR\alpha$  fusion. The  $t(15;17)$  and variant translocations all have in common disruption of the  $RAR\alpha$  gene, with the typical  $t(15;17)$  giving rise to the  $PML/RAR\alpha$  gene fusion product which causes a block in differentiation at the promyelocyte stage [3]. The identification of the  $t(15;17)$  and the genes involved in this rearrangement has led to a successful treatment for APL utilizing all-*trans*-retinoic acid (ATRA), which acts as a differentiating agent [28]. Identification of variant translocations is important, as some APL variants such as  $t(11;17)(q23;q12)$  are resistant to this drug.

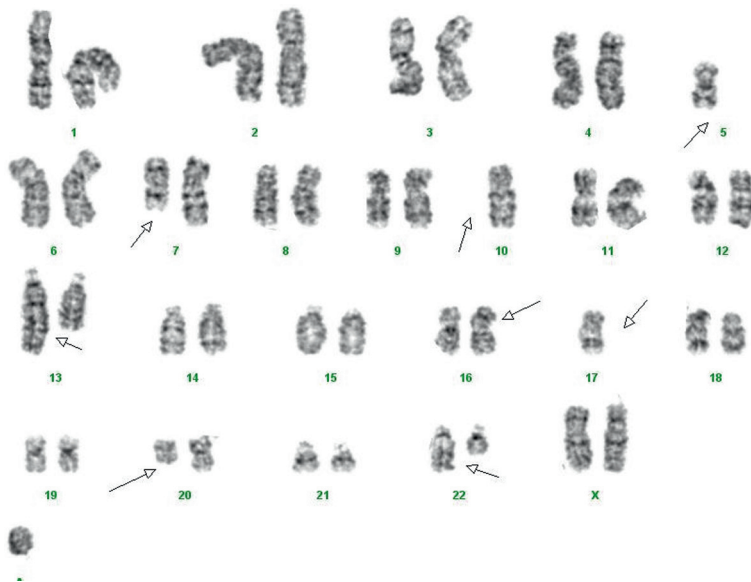
- **AML with  $t(9;11)(p22;q23)$  –  $MLLT3/MLL$**  (Fig. 2.6b): Acute myeloid leukemia with chromosome 11q23 abnormalities generally presents with monocytic features and involves disruption of the  $MLL$  (myeloid lymphoid lineage or mixed lineage leukemia) gene. Abnormalities of 11q23 are identified in 5–6% of AML cases occurring at any age; however, it is more common in childhood AML. The two AML subgroups that demonstrate 11q23 rearrangement most often are AML in infants and therapy-related AML (following topoisomerase II therapy). The most common translocations in childhood AML include  $t(9;11)(p21;q23)$  and  $t(11;19)(q23;p13.1)$  or  $t(11;19)(q23;p13.3)$ . The  $MLL$  gene is very promiscuous, as it is known to be involved in 73 recurrent translocations and partner with 54 partner genes in all acute leukemias [3, 30]. Because of this, the most effective method to detect  $MLL$  gene rearrangement is to utilize an  $MLL$  gene break-apart probe that can detect involvement of  $MLL$  regardless of which partner chromosome band/gene is involved [3].

- *AML with inv(16)(p13q22) or t(16;16)(p13;q22) – CBF $\beta$ /MYH11* (Fig. 2.6c): Acute myelomonocytic leukemia (AMML) [FAB classification: AML-M4eo] accounts for approximately 10% of all AML cases and is characterized by an increase in myeloid and monocytic cell lines with a characteristically abnormal eosinophil component in bone marrow. The genetic basis for AML-M4eo is the fusion of the core binding factor beta subunit (*CBF $\beta$* ) gene at chromosome 16q22 to the smooth muscle myosin heavy chain gene (*MYH11*) at chromosome 16p13 through either the *inv(16)* or the *t(16;16)*.
- *AML with t(6;9)(p23;q34) – DEK/NUP214*: AML with or without monocytic features that is often associated with basophilia and multilineage dysplasia. The *t(6;9)* is the sole abnormality in most cases, although it can sometimes be part of a complex karyotype. The concurrent identification of the *FLT3-ITD* mutation occurs in 69% of pediatric cases and 78% of adult cases [3].
- *AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2) – RPN1/EVII*: AML with increased atypical bone marrow megakaryocytes and associated multilineage dysplasia. Patients may present de novo or have a prior MDS phase [3].
- *AML (megakaryoblastic) with t(1;22)(p13;q13) – RBM15/MKL1*: A rare AML (<1% of cases) that demonstrates small and large megakaryoblasts. This disease is de novo in most cases and almost exclusively seen in infants and young children [3].

Of these AMLs, the rearrangements considered to be favorable with regard to response to chemotherapy, high remission rate, and long-term survival include *t(8;21)(q22;q22)*, *inv(16)(p13q22)* or *t(16;16)(p13;q22)*, and *t(15;17)(q22;q12)* treated with all-*trans*-retinoic acid. The *(9;11)(p22;q23)* is associated with an intermediate prognosis, while the *t(6;9)(p23;q34)*, *inv(3)(q21q26.2)*, and *t(3;3)(q21;q26.2)* are associated with a poor prognostic outcome [3].

In addition to the cytogenetically characterized myeloid leukemias described above, several additional AML groups are recognized. *Acute myeloid leukemia with myelodysplasia-related changes* often present with severe pancytopenia. More often observed in the elderly, this disease is characterized by chromosomal abnormalities similar to those found in MDS. These include monosomy 5/*del(5q)* and monosomy 7/*del(7q)*, often as part of a complex karyotype [3].

The latency period for development of *therapy-related myeloid neoplasms* varies with the type of chemotherapeutic agents used. Therapy-related AML (t-AML) associated with alkylating agent chemotherapy or radiation therapy is often preceded by MDS and can develop after a period of 2–7 years, while topoisomerase II inhibitor therapy-associated AML develops after a shorter latency period and is not associated with a preceding myelodysplastic phase [31]. Alkylating agent t-AML is characterized by deletions involving chromosomes 5 and 7, often as part of a complex karyotype (Fig. 2.7). Topoisomerase-associated t-AML is associated with disruptions of the *MLL* gene at 11q23, often through a balanced translocation. While in general the outcome of t-AML/t-MDS is poor, certain cytogenetic results including *inv(16)*, *t(8;21)*, and *t(15;17)* are associated with a better prognosis (comparable to de novo AML with favorable cytogenetics).



**Fig. 2.7** This complex karyotype was identified in a patient who had undergone multiple rounds of chemotherapy and radiation for persistent follicular lymphoma over many years. The concurrent bone marrow examination demonstrated marked erythroid hyperplasia and megakaryocytic hyperplasia along with erythroid and megakaryocytic dyspoiesis and ringed sideroblasts. t-MDS was favored, given her therapy history. This karyotype confirms t-MDS with abnormalities of chromosomes 5 and 7. A little over 1 month later, this patient presented with acute myeloid leukemia (t-AML), not surprising given the complexity of this karyotype

Those AMLs subtyped in the WHO classification as *acute myeloid leukemia, not otherwise specified* include acute myeloid leukemia with minimal differentiation, acute myeloid leukemia without maturation, acute myeloid leukemia with maturation, acute myelomonocytic leukemia, acute monoblastic and monocytic leukemia, acute erythroid leukemia, acute megakaryoblastic leukemia, acute basophilic leukemia, and acute panmyelosis with myelofibrosis. These diseases are not associated with a specific cytogenetic abnormality but instead demonstrate abnormalities that are best classified as “myeloid cytogenetic markers.” These include monosomy 5/del(5q), monosomy 7/del(7q), +8, del(11q), del(20q). Complex karyotypes are often observed as well [3].

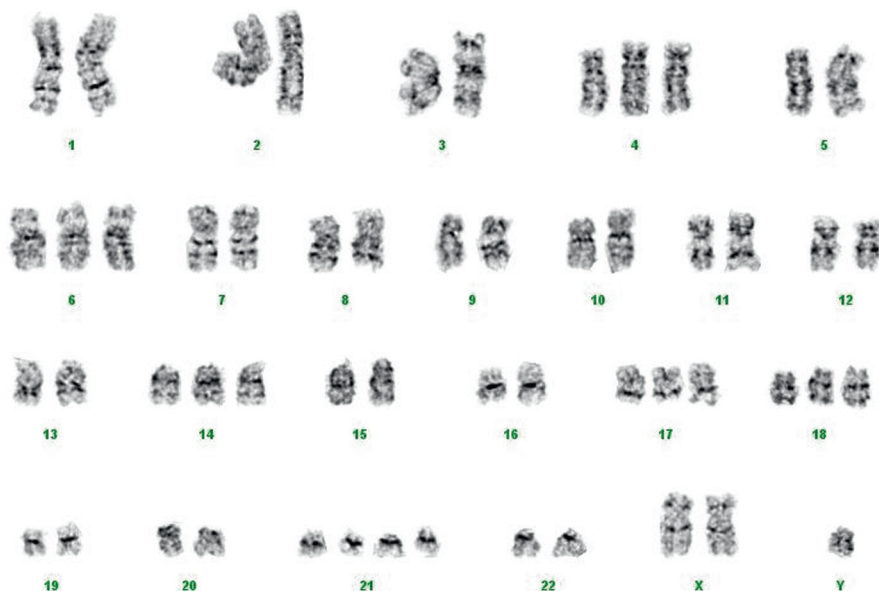
## Diagnostic and Prognostic Cytogenetic Markers in Lymphoid Disorders

### *B-Lymphoblastic Leukemia/Lymphoma with Recurrent Genetic Abnormalities*

This new WHO classification defines a group of diseases characterized by recurrent numerical and structural chromosomal abnormalities. In childhood ALL, the identification of recurrent chromosomal aberrations as prognostic markers has had a major

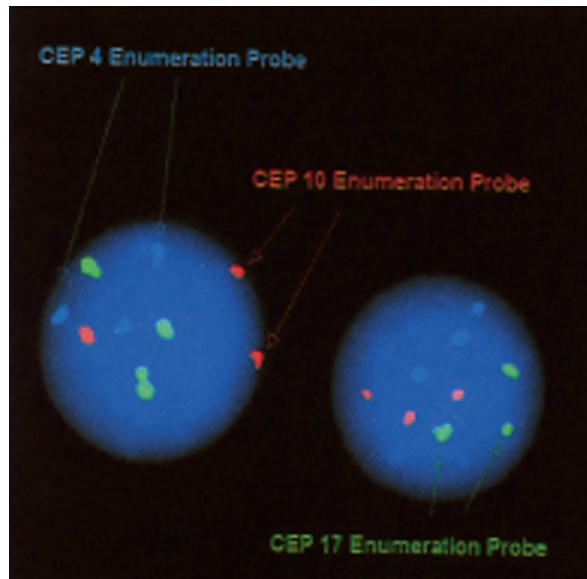
impact on efforts to cure this disease, as they have permitted effective stratification of patients into appropriate treatment regimens. Approximately 80% of ALL cases demonstrate clonal chromosomal abnormalities, while the remaining cases either present a normal karyotype or cannot be analyzed due to a variety of factors such as poor chromosome morphology and the apoptotic tendency of ALL blasts in culture. For this reason, FISH has become an important tool for the assessment of genetic aberrations in ALL [32].

High hyperdiploidy, defined as  $>50$  chromosomes per karyotype, occurs in approximately 25% of ALL cases (*B-lymphoblastic leukemia/lymphoma with hyperdiploidy*) and constitutes a distinct subset characterized by a favorable prognosis (Fig. 2.8). The gains are non-random, with chromosomes 4, 6, 10, 14, 17, 18, 21, and X accounting for close to 80% [32]. More specifically, hyperdiploid ALL with simultaneous trisomy of chromosomes 4, 10, and 17 has the least treatment failure and the greatest clinical outcome [33]. Enumeration of chromosomes 4, 10, and 17 by FISH (triple trisomy FISH) can identify these numerical changes (Fig. 2.9), providing important prognostic information when chromosome analysis is unsuccessful or when a normal karyotype is identified by banding studies. In contrast, hypodiploid ALL (*B-lymphoblastic leukemia/lymphoma with hypodiploidy*) defines a subgroup characterized by  $<45$  chromosomes per karyotype. This is observed in both adults and children; however, near-haploid ALL (with 23–29 chromosomes) is identified almost exclusively in children. Hypodiploid ALL is associated with a poor prognosis [3]. Care should be exercised when a high



**Fig. 2.8** Pediatric bone marrow with precursor B-cell ALL demonstrating a hyperdiploid karyotype. Note trisomy for chromosomes 4 and 17 (see text)

**Fig. 2.9** Pediatric B-cell ALL demonstrating trisomy of chromosomes 4, 10, and 17 by FISH



hyperdiploid/near-triploid karyotype is identified to examine the pattern of chromosome gain, as duplication of a near-haploid/hypodiploid karyotype can appear as a hyperdiploid karyotype, yet will be associated with the poor prognosis characteristic of hypodiploid ALL.

In the non-hyperdiploid ALL subgroup, four major translocations have been observed. *B-lymphoblastic leukemia/lymphoma with t(12;21)(p13;q22); TEL/AML1 (ETV6/RUNX1)* is recognized in up to 30% of childhood B-precursor ALL; however, this translocation is rare or absent in infants and in adults with ALL. The t(12;21) translocation fuses the *TEL (ETV6)* and *AML1 (CBFA2 or RUNX1)* genes, normally localized to 12p13 and 21q22, respectively. Many studies have demonstrated that ALL patients with *TEL/AML1* fusion do extremely well. This translocation cannot be detected by conventional cytogenetics due to its cryptic nature, therefore necessitating the use of a *TEL/AML1* fusion FISH probe for detection [3].

*B-lymphoblastic leukemia/lymphoma with t(9;22)(q34;q11.2); BCR/ABL1* is observed in approximately 5% of children but up to 25% of adults with ALL. The resulting *BCR/ABL1* hybrid gene product is a 190-kDa protein that, as in CML, possesses dysregulated tyrosine kinase activity and is responsible for leukemic transformation. Ph+ALL is one of the most difficult childhood leukemias to treat and is generally associated with a poor prognosis [3]. Any of the *BCR/ABL1* FISH probe formats utilized in CML will also detect the fusion gene associated with breakpoints within the minor breakpoint region in ALL.

*B-lymphoblastic leukemia/lymphoma with t(v;11q23); MLL rearranged* constitutes a subgroup characterized by translocations of chromosome band 11q23

causing rearrangements of the *MLL* gene. This is seen in 80% of infant leukemia and in secondary leukemia that arises in patients treated with topoisomerase II inhibitors. Leukemic cells containing 11q23/*MLL* rearrangement are usually non-hyperdiploid, have an early pre-B-cell immunophenotype, and coexpress myeloid antigens except for CD10. Generally, ALL that involves *MLL* gene rearrangement is a clinically aggressive disease with a poor prognosis. Greater than 50 translocation partners with 11q23 have been described in ALL, suggesting that disruption or destabilization of *MLL* function underlies leukemogenesis in these cases. As these leukemias have been observed in very young infants, it has been theorized that *MLL* gene rearrangement may occur in utero. The most common translocations are t(4;11) followed by t(11;19) and t(9;11). As many different variant t(v;11q23) translocations exist, the most sensitive method for detecting *MLL* gene rearrangement is to utilize an *MLL* gene rearrangement probe [3, 30].

*B-lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.3); E2A/PBX1 (TCF3/PBX1)* is seen in approximately 5% of adult and childhood ALLs and encodes the fusion protein *E2A/PBX1*. This was previously thought to represent a poor prognostic marker, but intensification of therapy in pediatric patients has overcome its effects on outcome. This translocation can be detected utilizing an *E2A* gene break-apart FISH probe [3].

*B-lymphoblastic leukemia/lymphoma with t(5;14)(q31;q32); IL3/IGH* is a rare entity accounting for less than 1% of all cases of ALL. Seen in both children and adults, the prognostic significance of this translocation is not firmly established. Conventional cytogenetic analysis can usually identify this abnormality [3].

FISH has become an invaluable tool for identifying the major genetic aberrations in ALL and for risk-stratifying patients with this disease. In one large study, FISH screening using probes for *TEL/AML1*, *BCR/ABL1*, and *MLL* gene rearrangements along with selected centromeric probes increased the success rate to 91% and the detection rate of genetic aberrations to 89% [34]. Many clinical trials, including those established by the Children's Oncology Group (COG), require all newly diagnosed ALL cases to undergo both conventional cytogenetic testing and molecular cytogenetic characterization for risk stratification utilizing a panel to identify *TEL/AML1* and *BCR/ABL1* fusion; *MLL* rearrangement; and chromosomes 4, 10, and 17 triple trisomy.

## ***T-Lymphoblastic Leukemia/Lymphoma***

*T-lymphoblastic leukemia/lymphoma* is a malignancy of lymphoblasts committed to the T-cell lineage. T-cell acute lymphoblastic leukemia (T-ALL) comprises about 15% of all childhood ALL cases. It is more commonly found in older than younger children and more often in males than females. In adults, T-ALL comprises about 25% of all ALL cases. T-cell lymphoblastic lymphoma (T-LBL) is found in all age groups and makes up approximately 90–95% of all lymphoblastic lymphomas. Both T-ALL and T-LBL demonstrate clonal rearrangements of the T-cell receptor (*TCR*) genes including the alpha/delta *TCR* loci at 14q11.2, the beta locus at 7q35, and



the gamma locus at 7p14-15. These genes rearrange with various partner genes including *MYC*, *HOX11* (*TLX1*), *HOX11L2* (*TLX3*), and *TAL1* and lead to dysregulation of the partner gene when it juxtaposes next to one of the *TCR* gene promoter regions. While an abnormal karyotype is identified in 50–70% of cases, FISH break-apart probes to detect rearrangement of the TCR alpha/delta, TCR beta, and TCR gamma loci (Table 2.4) are available [3].

### ***Chronic Lymphocytic Leukemia/Small Lymphocytic Leukemia***

Low proliferation activity of leukemic B cells in culture and overgrowth of normal cells preclude the routine detection of chromosomal abnormalities by conventional cytogenetic analysis in chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL). For this reason, FISH utilizing a panel of DNA probes that interrogate the *MYB* gene at 6q23, *ATM* gene at 11q22.3, chromosome 12 alpha-satellite region, D13S319 locus at 13q14.3, *LAMP1* gene at 13q34, and *p53* gene at 17p13.1 is a useful adjunct to conventional analysis in the workup of a newly diagnosed CLL/SLL case. G banding reveals a clonal chromosomal abnormality in approximately 40% of CLL/SLL cases (with trisomy 12 being the most frequent), while FISH identifies genetic aberrations in over 80% of CLL/SLL cases. With FISH, the most common single chromosomal abnormality is a deletion of 13q14 found in 55–65% of cases followed by trisomy 12 in 15–25%, deletion of 11q22/*ATM* in 11–18%, deletion of 17p13/*p53* in 7–8%, and deletion of 6q in 5–6% [35–37]. Identification of these abnormalities is of prognostic value, with isolated 13q deletion or a normal karyotype predicting a better prognosis, while del(6q), del(11q), and del(17p) characterize a group with a poorer prognosis. Trisomy 12 carries a high risk of disease progression, but unlike patients with del(17p) and del(11q), patients with trisomy 12 respond to therapy with better survival [35]. Follow-up FISH studies are also clinically useful, as demonstrated by one study which showed that 27% of CLL patients acquired new chromosomal aberrations during the course of their disease, and in one-third of these patients, the newly detected abnormalities changed their disease status from low risk to high risk [38].

### ***Plasma Cell Myeloma***

Identification of recurrent chromosomal abnormalities by conventional analysis in plasma cell myeloma (PCM) has been hindered by patchy bone marrow infiltration, low mitotic index of malignant plasma cells in vitro, the poor quality of metaphase chromosomes, and the cryptic nature of some *IgH* gene rearrangements observed in this disease. An abnormal karyotype is found in 30–40% of cases, more often in advanced stages than in newly diagnosed patients. Highly complex karyotypes are also common, mostly in later stage disease [39–42].

Three distinct cytogenetic groups are recognized: (1) a hyperdiploid group with 47 or more chromosomes observed in 30–50% of cases with a lower frequency



of *IgH/14q32* rearrangement and monosomy 13/del(13q), (2) a hypodiploid group accounting for 20–35% of cases with a higher frequency of *IgH/14q32* rearrangement and monosomy 13/del(13q), and (3) a pseudodiploid group in 20–35% of cases characterized by *IgH/14q32* rearrangement [41]. Clinically, this cytogenetic classification is valuable, since hyperdiploid PCM patients seem to have a better outcome than do non-hyperdiploid patients.

FISH analysis has demonstrated that chromosomal aberrations can be found in the majority of PCM cases, despite the relatively high incidence of a normal karyotype identified by conventional banding studies. In a Mayo Clinic study, Dewald et al. [42] identified one or more abnormalities by FISH in 86% of newly diagnosed PCM cases using a panel of FISH probes to detect t(4;14), t(11;14), t(14;16), 17p13 deletion, and monosomy 13/del(13q).

Identification of monosomy 13/del(13q) by FISH is the most common abnormality detected in PCM. FISH studies have revealed that monosomy 13/del(13q) occurs in all stages of plasma cell neoplasms including monoclonal gammopathy of undetermined significance (MGUS), PCM, and plasma cell leukemia (PCL); however, the net effect of monosomy 13/del(13q) on prognosis is stronger when monosomy 13/del(13q) is detected by karyotype than when it is observed by FISH [42]. This is because the observation of abnormal metaphases indicates a larger tumor burden with a highly proliferative malignant plasma cell component. While monosomy 13/del(13q) has been associated with shorter survival and lower response rates to treatment, some recent studies have suggested that it may not, as the sole abnormality, be as important a prognostic marker. Instead, its close association with other poor prognostic markers such as t(4;14), t(14;16), and *p53* gene deletion may contribute to the perception that monosomy 13/del(13q) predicts an adverse outcome. At present, the prognostic significance of chromosome 13 abnormalities in PCM is not completely clear [41].

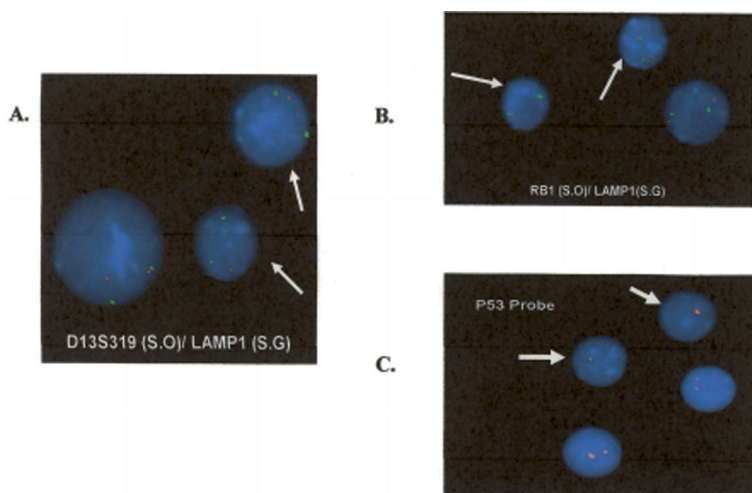
The *IgH/14q32* translocation is detected in more than 50% of PCM cases and is strongly associated with the non-hyperdiploid group [43]. This rearrangement is mediated mostly by errors in immunoglobulin class switch recombination [44] and is believed to be an early, possibly pathogenic event in many cases [45]. Three major specific *IgH* translocations t(11;14)(q13;q32), t(4;14)(p16.3;q32), and t(14;16)(q32;q23) are identified in PCM. The t(4;14) and t(14;16) are cryptic translocations found in less than 15 and 5% of patients, respectively. They can only be detected accurately utilizing the *FGFR3/IgH* and *MAF/IgH* FISH dual-fusion FISH probes. Both t(4;14) and t(14;16) are associated with hypodiploidy, an adverse disease outcome with shorter survival, and aggressive clinical features. Less common translocations involving *IgH* have also been described involving partner genes such as *IRF4*, *IRTA1/IRTA2*, and *C-MYC* [41]. Secondary *IgH* translocations that dysregulate the *C-MYC* proto-oncogene are found in 5% of PCM cases [40]. Those types of translocations are considered late progression events and are likely to have a negative impact on overall prognosis.

The t(11;14) is the most common translocation observed in PCM, being seen in 15–20% of cases. While it can be detected easily by G banding, the *IgH/CCND1* FISH probe is useful to examine metaphase cells with a complex karyotype or poor

morphology, and to assess interphase nuclei in cases that yield normal metaphases. The t(11;14) results in upregulation of *CCND1* and is associated with a favorable prognosis. Other cyclin genes such as *CCND2* and *CCND3* have also been found to be upregulated in both MGUS and PCM. It appears that almost all cases of MGUS and PCM tumors upregulate at least one cyclin gene, sometimes as a consequence of an *IgH* translocation [45]. Some have proposed that a classification system for PCM based on the type of cyclin gene expressed together with the karyotypic profile may generate useful biological and clinical subgroups.

Deletion of 17p13/*p53* gene using a locus-specific *p53* gene FISH probe is detected in 9–30% of PCM cases. This abnormality is identified more often in non-hyperdiploid PCM (26%) than in the hyperdiploid group (1%) [40]. Deletion of 17p13 is associated with a poor prognosis in PCM.

It is clear that identification of cytogenetic abnormalities by both conventional karyotyping and FISH studies provides important diagnostic and prognostic information in PCM. The FISH panel utilized by most cytogenetics laboratories in newly diagnosed PCM cases includes enumeration probes for chromosomes 3, 9, and 15 to screen for ploidy (gain of chromosomes 3, 9, and 15 is found in >90% of hyperdiploid cases) as well as probes to detect monosomy 13/13q deletion (RB1/LAMP1), *p53* gene deletion, and common *IgH* translocations (Fig. 2.10). This methodology yields significant prognostic information for risk assessment and treatment stratification in patients with PCM. In order to increase the sensitivity of FISH in PCM, some labs are now employing techniques that enrich



**Fig. 2.10** Plasma cell myeloma FISH panel demonstrates the following: (a, b) loss of the D13S319 and RB1 loci (one orange signal) with retention of the LAMP1 locus at 13q34 (two green signals) [this differentiates a chromosome 13q deletion from monosomy 13] and (c) loss of the *p53* gene (one orange signal indicated by arrows)

for the plasma cell component in either whole-nuclei preparations or paraffin-embedded tissue sections. Originally described by Ahmann and colleagues [46], simultaneous FISH and cytoplasmic immunoglobulin staining permits analysis of only cells that express a plasma cell phenotype. Other techniques including May-Grunwald Giemsa (MGG) staining and FISH (target FISH or T-FISH) [47], FICTION (fluorescence immunophenotyping and interphase cytogenetics as a tool for the investigation of neoplasms) [48], and SNP microarrays combined with FISH [49] have been used to enrich for the malignant component in FISH analysis of plasma cell disorders.

## ***Non-Hodgkin Lymphoma***

The majority of non-Hodgkin lymphomas (NHLs) demonstrate clonal chromosomal abnormalities. The primary aberrations are commonly translocations that cause relocation of oncogenes to the vicinity of highly active promoter/enhancer elements of immunoglobulin or T-cell receptor genes in B-cell or T-cell lymphoma, respectively, resulting in gene deregulation [50]. Unlike most of the translocations in acute and chronic leukemias that result in a hybrid fusion gene with altered activity, the translocations in B-cell lymphoma mostly result in juxtaposition (not fusion) of the oncogene to an immunoglobulin gene regulatory sequence. One exception is the *API2-MALT1* fusion gene generated by the t(11;18)(q21;q21) in MALT lymphoma. In some B-lineage lymphomas such as Burkitt lymphoma [t(8;14)(q24;q32) and its variants] or mantle cell lymphoma [t(11;14)(q13;q32)], one or a few specific *IgH* rearrangements are identified in the majority of cases and are thus considered pathognomonic for the disease; however, in other B-cell neoplasms such as diffuse large B-cell lymphoma, *IgH* rearrangements are detectable in a smaller number of cases and are translocated with a wide variety of partner genes. In general, few translocations identified in B-cell neoplasms are characteristic of a specific lymphoma subtype [41].

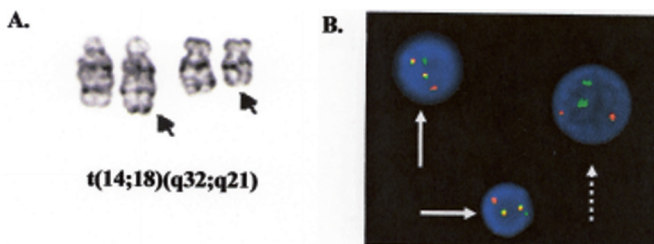
Cytogenetic and molecular studies have provided evidence that the process of oncogenesis in many lymphomas follows a multistep process similar to that originally described for colorectal cancer. It appears that the primary genetic event of a tumor clone initiates the lymphoid malignancy. These genetic alterations thus serve as diagnostic markers for the malignancy; however, additional changes would appear to be necessary for sustained lymphomagenesis. One line of evidence to support this notion is the molecular identification in apparently healthy individuals of genetic alterations such as the t(14;18) or the t(11;14). Whether these individuals are at higher risk for subsequent development of malignancy is not clear, but it seems that one or more additional genetic alterations are necessary for the development of frank malignancy. These secondary genetic changes are often identified along with the defined primary change in the diagnostic specimen. These, and further genetic changes, result in increasing complexity of the karyotype and are associated with transformation of an indolent lymphoma to one with more aggressive biological behavior. Thus, identification of complex karyotypes in the

diagnostic lymphoma specimen, or cytogenetic evolution with increasing karyotypic complexity, is associated with a poorer prognosis [41].

Conventional cytogenetic analysis is not always possible in lymphomas due to the lack of fresh tissue and small biopsy specimens. FISH can be used to establish the diagnosis in viable and fixed tissue and to assess the involvement of bone marrow by lymphoid tumor. As unfixed tissue may not be available, FISH on paraffin-embedded tissue sections can be an invaluable technique to identify genetic aberrations in lymphoid malignancies, as can FISH analysis of touch imprint specimens [51]. Studies have shown that the sensitivity of FISH for detecting lymphoma-associated chromosome translocations is higher and more specific than PCR owing, in part, to the large genomic region over which some of the translocation breakpoints are spread. This can preclude their detection by molecular methods in a highly sensitive fashion. In mantle cell lymphoma, for instance, FISH was found to be superior to PCR with a 95–100% detection rate of *IgH/CCND1* gene fusion as compared with a detection rate of 35–40% by PCR [52].

**Follicular lymphoma.** The most frequent translocation in B-cell NHL, t(14;18)(q32;q21), juxtaposes the *BCL2* proto-oncogene at 18q21 next to the *IgH* gene locus at 14q32 (Fig. 2.11). This translocation is identified in 80–90% of follicular lymphoma (FL) cases and to a lesser extent in diffuse large B-cell lymphoma (20–30%). The translocated *BCL2* gene encodes an aberrant protein that inhibits apoptosis. Only in 10% of cases is the t(14;18) the sole abnormality. A number of non-random secondary changes are documented, the most common of which is an additional copy of the derivative chromosome 18 originating from the t(14;18) [der(18)t(14;18)(q32;q21)]. Low-grade FL can progress to high-grade FL or transform to diffuse large B-cell lymphoma (DLBCL) through acquisition of additional cytogenetic changes, an event associated with a poorer prognosis [41].

Another recurrent primary abnormality in FL is rearrangement of the *BCL6* gene at band 3q27. This rearrangement occurs through a variety of chromosomal abnormalities involving various partner genes. In fact, *BCL6* rearrangement appears to be an extremely common event in a variety of B-cell disorders, in particular DLBCL. Rearrangements of *BCL6* result in dysregulation through its interaction



**Fig. 2.11** (a) A patient with follicular lymphoma demonstrates the t(14;18) by conventional cytogenetic analysis. (b) FISH analysis utilizing a dual-color, dual-fusion probe reveals one red, one green, and two fusion signals (solid arrows) indicating fusion of *IGH* and *BCL2* genes. The hatched arrow indicates a nucleus with a normal signal pattern

with gene regulatory sequences of the partner gene in the translocation. High-grade FL with *BCL6* rearrangement but without the t(14;18) often progresses to *DLBCL* as well [41].

**Diffuse large B-cell lymphoma.** No chromosomal abnormality is specific for diffuse large B-cell lymphoma. Many of the chromosomal abnormalities observed in other B-cell lymphomas can be observed in this disease as well. These abnormalities include *BCL6* gene disruption (20–40% of cases); translocations of 14q32 involving the *IgH* locus (20–40%); gain of chromosomes X, 3, 7, 12, and 18; and loss of chromosomes Y, 6, 13, 15, and 17. There is at present conflicting evidence regarding the prognostic significance of either *BCL6* rearrangement or t(14;18), the most commonly observed translocation of 14q32 being observed in DLBCL. However, like in other lymphomas, del(17p) involving the *p53* gene as well as karyotypic complexity indicates disease progression and a poorer prognosis [41]. Of interest is the finding that t(14;18) can occur concurrently with chromosome 8q24/*MYC* gene translocation in a number of B-cell neoplasms, including DLBCL. These neoplasms are of high grade and are associated with a poorer prognosis [53].

**Burkitt lymphoma.** The Burkitt lymphoma (BL)-associated translocations include t(8;14)(q24;q32), t(2;8)(p12;q24), and t(8;22)(q24;q11). The t(8;14) is observed in 75–85% of all BL patients (Fig. 2.12), while the remaining 15–25% of patients present one of the variant translocations, with the t(8;22) seen twice as frequently as



**Fig. 2.12** This karyotype from a patient with Burkitt lymphoma demonstrates the t(8;14)(q24;q32) (solid arrows). An add (19q) chromosome is also present (open arrow)

the t(2;8). These translocations juxtapose the *C-MYC* proto-oncogene at 8q24 next to the promoter for the Ig heavy chain gene at 14q32, Ig kappa locus at 2p12, or Ig lambda locus at 22q11. This repositioning of the *MYC* gene disrupts its regulation and results in its constitutive overexpression leading to malignant transformation. Activation of *MYC* takes place on the der(14) in the t(8;14) and on the der(8) in the t(2;8) and t(8;22). Molecular analysis of the breakpoints in sporadic, endemic, and immunodeficiency-associated BL demonstrates different clustering on the der(8) and the der(14), suggesting that different pathogenetic mechanisms may generate the t(8;14) in different disease settings. A characteristic feature of BL is that one of the three characteristic translocations is generally part of a relatively simple karyotype, with karyotypic complexity indicating disease progression. Among secondary chromosomal abnormalities, the most common is structural rearrangement of chromosome 1, especially the long arm, as well as trisomy 7 and trisomy 12 [41].

*Mantle cell lymphoma.* The t(11;14)(q13;q32) is present in virtually all cases of mantle cell lymphoma (MCL). In 20% of cases, it is part of a more complex karyotype, sometimes associated with loss of the der(11) chromosome. Chromosome numbers are generally in the diploid or the hyperdiploid range, except in the blastic variants where polyploidy is often observed. The t(11;14) involves a breakpoint within the *BCL1* gene locus at 11q13 that results in relocation of the *CCND1* gene (which is positioned downstream from *BCL1*) next to the promoter for the *IgH* gene. This results in the overexpression of *CCND1*. Identification of the t(11;14) is important as it can differentiate MCL from other low-grade lymphomas, especially if immunophenotyping is inconclusive [41].

*Splenic marginal zone lymphoma.* Up to 40% of splenic marginal zone lymphomas present a del(7q) chromosome. The t(11;14)(q13;q32) has also been reported; however, it is unclear whether these cases may have been MCL [41].

*Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT type).* Three recurrent translocations are observed in MALT lymphomas. These include t(11;18)(q21;q21), t(14;18)(q32;q21), and t(1;14)(p22;q32). Trisomies 3 and 18 are observed in translocation-negative MALT lymphomas. Like other lymphomas, as MALT lymphomas progress, they acquire additional secondary chromosomal changes including *MYC* gene translocations (8q24), del(17p) with loss of *p53* and del(9p) with loss of the *CDKN2A* locus. The presence of the t(11;18) and possibly the t(1;14) is associated with a low probability of cure by antibiotic therapy that targets *Helicobacter pylori*, the infectious agent responsible for the development of gastric MALT lymphoma [41].

*Anaplastic large-cell lymphoma.* The t(2;5)(p23;q35), which fuses the nucleophosmin (*NPM*) gene at 5q35 with the anaplastic lymphoma kinase (*ALK*) gene at 2p23, is the most common translocation observed in anaplastic large-cell lymphoma (ALCL). Tumors with this translocation are generally of high grade and express the CD30 (Ki-1) antigen. The t(2;5) leads to the formation of a chimeric fusion protein with constitutive tyrosine kinase activity. Other translocations which fuse *ALK* to other partner genes have been identified in ALCL as well [41].



## ***Hodgkin Lymphoma***

Chromosome analysis in classical Hodgkin lymphoma (HL) often reveals normal karyotypes due to the abundance of nonmalignant cells in the lesion; however, cytogenetic studies by classical and FISH methods combined with CD-30 immunofluorescence staining have revealed highly complex karyotypes with cytogenetic instability, triploid/tetraploid metaphases, and multiple aneuploidies in the neoplastic Reed–Sternberg cells. In nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL), abnormalities involving the *BCL6* gene at 3q27 are identified in up to 50% of cases, not surprising given that NLPHL shares many features with DLBCL, and may in fact be a non-Hodgkin lymphoma rather than a HL [41].

## **Array-Based Genomic Profiling of Hematolymphoid Disorders**

The newest generation of hematolymphoid molecular analysis is based on the simultaneous examination of thousands of small genomic segments utilizing arrays containing either oligonucleotides (60-mers) or single-nucleotide polymorphisms (SNPs). SNP analysis appears to be better suited for studying neoplasia as it can detect gene-dosage changes at a higher level of resolution than can oligos and can also detect copy-number neutral loss of heterozygosity (acquired uniparental disomy). From a few hundred thousand to over one million individual loci can be interrogated in a single assay depending on the type of SNP array used. Two technologies currently available involve the spotting of individual SNPs onto gene chips (Affymetrix SNP Array) or adsorbed on microbeads (Illumina Infinium HD BeadChip). Some have referred to this technology as “molecular allelokaryotyping” [54, 55]. One significant disadvantage of array-based studies is that present platforms cannot detect balanced chromosomal rearrangements, a common feature of many hematolymphoid disorders.

SNP arrays appear to provide concordant results when compared with FISH analysis using disease-specific panels; however, SNP analysis may not be as sensitive as FISH for detecting low-level mosaicism. Sargent et al. [56] studied 100 CLL samples utilizing both a typical CLL FISH panel and a 44 K oligonucleotide array and demonstrated a high degree of concordance between FISH and array CGH, although low-level mosaicism (<25% of nuclei positive for a chromosomal abnormality) was often not detected by array CGH.

Studies utilizing SNP array technology to genomically profile hematolymphoid neoplasms are becoming more numerous in the literature. These studies have revealed clinically significant information previously unattainable by classical cytogenetic and FISH analysis. Lehmann et al. performed SNP chip analysis on 56 patients with early stage untreated CLL and identified not only abnormalities that were detected by simultaneous FISH analysis but also additional abnormalities including deletions of chromosomes 5q, 6q, and Xp. Whole-chromosome 13 uniparental disomy (UPD) was also identified and appears to be a common finding in early stage CLL [54]. Kawamata et al. performed SNP chip analysis on 14



ALL samples at diagnosis, remission, and relapse. All cases demonstrated genomic abnormalities at relapse, with 10 samples acquiring additional changes not observed in the diagnostic specimen. These changes included deletion of the *INK4A/ARF* and *NF2* genes. Also at relapse, uniparental disomy of chromosomes originally presenting in the diagnostic specimen as trisomy was identified, along with UPD of chromosome region 16p12.3-pter. Interestingly, this SNP chip study also revealed disappearance of deletions at relapse, possibly indicating that some of the clones identified at relapse were present but not identified at initial diagnosis [55]. SNP chip analysis of AML/MDS samples reported by Akagi et al. [57] identified genomic abnormalities including uniparental disomy in 49% of samples previously found to have a normal karyotype. These and other studies clearly demonstrate the power of SNP array-based genetic analysis; however, for the foreseeable future, a combination of conventional cytogenetics, FISH, and SNP array analysis will likely be the best approach to studying hematolymphoid disorders.

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<http://www.springer.com/978-1-60761-261-2>

Hematopathology

Genomic Mechanisms of Neoplastic Diseases

Crisan, D. (Ed.)

2011, XII, 360 p., Hardcover

ISBN: 978-1-60761-261-2

A product of Humana Press