

Fluorescence In Situ Hybridization: Uses and Limitations

Alessandro Gozzetti and Michelle M. Le Beau

The development of molecular hybridization techniques such as fluorescence in situ hybridization (FISH) has had a major impact on efforts to detect and characterize the genetic changes that give rise to human tumors. With probes designed to identify specific chromosomes and chromosomal regions, FISH is used routinely by cytogenetics and pathology laboratories to identify recurring chromosomal abnormalities associated with hematologic malignant diseases. In many cases FISH analysis provides increased sensitivity, in that cytogenetic abnormalities have been found in samples that appeared to be normal by morphologic and conventional cytogenetic examination. The combination of cytogenetic, FISH, and molecular analyses provides a powerful approach for diagnosing and subclassifying malignant diseases into clinically and biologically relevant subgroups, in selecting appropriate therapy, and in monitoring the efficacy of therapeutic regimens.

Semin Hematol 37:320-333. Copyright © 2000 by W.B. Saunders Company.

RECURRING CHROMOSOMAL abnormalities are associated with distinct subtypes of leukemia or lymphoma with unique morphologic, immunophenotypic, and clinical features such as response to therapy.^{26,36,41} Thus cytogenetic analysis of an individual's malignant cells plays a major role in the diagnosis and subclassification of a hematologic neoplasm. Examples of the clinical-cytogenetic subtypes of acute myeloid leukemia (AML) include the t(15;17) in acute promyelocytic leukemia (AML-M3), and the t(8;21) in acute myeloblastic leukemia with maturation (AML-M2).^{26,36} A number of recurring abnormalities have also been recognized in solid tumors. The *Catalog of Chromosome Aberrations in Cancer* provides a registry of the abnormal karyotypes identified in tumors; the latest edition describes over 100,000 abnormalities in more than 30,000 neoplasms.⁴¹ The Cancer Chromosome Aberration Project integrates cytogenetic and genomic databases relevant to cancer (<http://www.ncbi.nlm.nih.gov/CCAP>). Molecular analysis has revealed that recurring chromosomal abnormalities result in the altered function of oncogenes; thus cytogenetic aberrations represent

genetic mutations that are involved in the pathogenesis and progression of human tumors.^{26,36,39}

The interpretation of chromosomal banding patterns for cytogenetics requires highly skilled personnel, and it is labor-intensive. Although automated karyotyping systems are available, the need to analyze many metaphase cells (20 to 30) remains time-consuming. Moreover, the analysis of human tumors is often technically difficult due to the presence of multiple abnormal cell lines and complex rearrangements. These factors have led investigators to seek alternative methods for identifying chromosomal abnormalities. One such method developed in the 1980s, fluorescence in situ hybridization (FISH), represented a major advance in chromosomal analysis.^{33,34,48} The high sensitivity and specificity of FISH and the speed with which the assays can be performed have made FISH a powerful technique with numerous applications (Table 1), and it has gained general acceptance as a clinical laboratory tool. In many cases, FISH has clarified the nature of chromosomal abnormalities, which were ambiguous with banding analysis alone. Here we present a review of FISH and outline how this technique can be applied to diagnosis, which in turn influences the treatment of hematologic malignant diseases.

From the Section of Hematology/Oncology, Department of Medicine, and The Cancer Research Center, University of Chicago, Chicago, IL.

Address reprint requests to Michelle M. Le Beau, PhD, Professor, Section of Hematology/Oncology, The University of Chicago, 5841 S Maryland Ave, MC2115, Chicago, IL 60637.

Copyright © 2000 by W.B. Saunders Company

0037-1963/00/3704-0002\$10.00/0

doi:10.1053/sbem.2000.16443

Fluorescence In Situ Hybridization Techniques

FISH techniques have been reviewed elsewhere³⁷ and are summarized here. FISH is based on the same principle as Southern blot analysis, namely, the ability

Table 1. Applications of Fluorescence in Situ Hybridization

- Detection of numerical and structural chromosomal abnormalities
- Identification of marker chromosomes (rearranged chromosomes of uncertain origin)
- Monitoring the effects of therapy
- Detection of early relapse or minimal residual disease
- Identification of the origin of bone marrow cells following stem cell transplantation
- Identification of the lineage(s) of neoplastic cells
- Examination of the karyotypic pattern of interphase cells, including nondividing or terminally differentiated cells
- Detection of gene deletions and gene amplification

of single-stranded DNA to anneal to complementary DNA. In the case of FISH, the target is the nuclear DNA of interphase cells or the DNA of metaphase chromosomes affixed to a microscope slide. FISH can also be performed using bone marrow or peripheral blood smears, or fixed and sectioned tissue. The test probe is labeled through incorporation of a chemically modified reporter nucleotide covalently bound to biotin or digoxigenin, or directly conjugated to fluorochromes. After the test probe anneals to its complementary sequences, the probe is detected by the attachment of a fluorochrome to molecules such as streptavidin or antidigoxigenin antibodies, which have a high affinity for the reporter nucleotide. A brilliantly colored signal at the hybridization site is visualized by fluorescence microscopy.

Probes for FISH

A variety of probes are used to detect chromosomal abnormalities by FISH; a partial list of those commercially available for cancer diagnostics is given in Table 2. A number of these probes are directly conjugated to fluorochromes, which has the advantage of eliminating the secondary detection step; directly labeled probes also provide strong signal with low background. FISH probes can be divided into three groups: (1) probes that identify *a specific chromosome structure*; (2) probes that hybridize to *multiple chromosomal sequences*; and (3) probes that hybridize to *unique DNA sequences*. An example of the first type, probes that hybridize to a specific chromosome structure, are α - and β -satellite sequences, which flank the centromeres of human chromosomes. In most instances, these sequences are distinct, such that an α -satellite probe derived from one chromosome will hybridize to that chromosome only. At present,

centromere-specific probes are available for chromosomes 1 to 4, 6 to 12, 15 to 18, and 20, as well as the X and Y chromosomes (marketed by Vysis, Downers Grove, IL, <http://www.vysis.com>, and Cytocell, Banbury, England, <http://www.cytocell.co.uk>). Satellite DNA probes hybridize to multiple copies of the repeat unit present at the centromeres, which results in a very bright fluorescent signal in metaphase and interphase cells. In a diploid cell, two hybridization signals are observed, representing the two chromosome homologs. Hybridization of centromere-specific probes is particularly suitable for the detection of monosomy, trisomy, and other aneuploidies in both leukemias and solid tumors (Fig 1A).^{28,49} For example, a centromere-specific probe for chromosome 8 can be used to detect trisomy 8 by FISH in newly diagnosed patients with a myelodysplastic syndrome (MDS) or AML, and to monitor the response to therapy.²⁸ FISH has also been used to detect minimal residual disease (MRD) with increased precision, since hundreds of cells (including nondividing cells) can be examined in a short time.²⁸

The second type, chromosome painting probes, utilize cloned DNA libraries derived from whole, flow-sorted human chromosomes.^{11,47} Hybridization results in the fluorescent staining or "painting" of the entire chromosome. Chromosome painting probes are available for each of the human chromosomes, and are particularly useful in characterizing marker chromosomes (Vysis, Cytocell, and Cambio, marketed by Vysis) (Fig 1B). Although they are suitable for use in metaphase cells, painting probes are not helpful in the analysis of interphase cells because the signal domains are so large and diffuse. An example of the power of FISH is that of the t(12;21)(p12;q22) in acute lymphoblastic leukemia (ALL), which was first detected through the use of FISH with chromosome painting probes.⁵² Due to the similar size and staining pattern of the chromosomal bands involved in this rearrangement, it is not visible in standard banding analysis.

The third type of probe, unique DNA sequence probes, are usually genomic clones, which vary in size depending on the nature of the cloning vector. Probes in this group are particularly useful for detecting structural rearrangements. One early limitation of FISH was the inability to distinguish more than one target sequence at a time, which was overcome by the introduction of hybridization protocols to allow for the detection of several target sequences simulta-

Table 2. FISH Probes to Detect Chromosomal Abnormalities

Disease	Abnormality	Probe	Format*	Vendor†
AML-M2	t(8;21)	RUNX1/ETO	2-color dual fusion	Vysis
AML-M4Eo	inv(16)/t(16;16)	CBFB	2-color break-apart	Vysis
AML-M3	t(15;17)	PML/RARA	2-color single fusion	Ventana
AML	t(11q23)	MLL	2-color single fusion	Vysis
			Single color	Ventana
AML/MDS	-5/del(5q)	EGR1/5p	2-color deletion	Vysis
AML/MDS	-7/del(7q)	D7S522/CEP7	2-color deletion	Vysis
AML/MDS	del(20q)	D20S108	Single color	Vysis
AML/MDS	+8	CEP8	Single color	Vysis, Cytocell
CML	t(9;22)	BCR/ABL	2-color single fusion	Vysis
		BCR/ABL ES	2-color extra signal	Vysis
		MBCR/ABL	2-color single fusion	Ventana
		BCR/ABL	2-color dual fusion	Ventana
	+8	CEP8	Single color	Vysis
				Cytocell
	i(17q)	HER2/CEP17	2-color (17q/centromere)	Vysis
				Ventana
ALL	t(12;21)	TEL/AML1	2-color extra signal	Vysis
ALL	t(11q23)	MLL	2-color break-apart	Vysis
ALL	t(9;22)	BCR/ABL ES	2-color extra signal	Vysis
		MBCR/ABL	2-color single fusion	Ventana
		mBCR/ABL	2-color single fusion	Ventana
CLL	+12	CEP12	Single color	Vysis
	del(13q)	D13S25/D13S319	Single color	Vysis
NHL-ALCL	t(2;5)	ALK	2-color break-apart	Vysis
MCL	t(11;14)	CCND1/IGH	2-color dual fusion	Vysis
Miscellaneous				
Bone marrow transplants		CEPX/CEPY	Single color or 2 color	Vysis
Subtelomeric-each chromosomal arm			Single color	Vysis
			2 color	Cytocell

Abbreviations: ALCL, anaplastic large cell lymphoma; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CLL, chronic lymphocytic leukemia; CML, chronic myelogenous leukemia; MCL, Mantle cell lymphoma; MDS, myelodysplastic syndrome; NHL, non-Hodgkin's lymphoma.

*In 2-color break-apart probes, DNA sequences from the 5' and 3' regions of a single gene are labeled and detected with red and green fluorochromes. In the germline configuration, a yellow fusion signal is observed, whereas separate red and green signals are observed when the sequences are separated as a result of a translocation. With 2-color fusion probes, DNA sequences flanking the breakpoints of the involved genes are brought together to form either 1 (single-fusion probes) or 2 (dual-fusion probes) yellow fusion signal(s). With the 2-color extra-signal (ES) probes, DNA sequences flanking the breakpoint on the partner chromosomes are brought together to form a fusion yellow signal; however, part of the DNA sequences recognized by one of the probes may remain at the original site, giving rise to an extra signal in a single color.

†Vysis, Downers Grove, IL; Ventana, Tucson, AZ; Cytocell, Banbury, England.

neously. Using multicolor FISH, recurring translocations can be identified in interphase or metaphase cells by means of genomic probes that are derived from the breakpoints.^{42,56} For example, a locus-specific probe for the *BCR* gene at 22q11.2 detected with a green fluorochrome and a locus-specific probe for the *ABL* gene at 9q34 detected with a red fluorochrome will appear as a bright yellow spot (the combination of green and red fluorochromes) in

leukemia cells characterized by the *BCR/ABL* fusion gene, resulting from t(9;22)(q34;q11.2) (Fig 1C and D). The same strategy is being used in clinical laboratories to detect the fusion genes produced by other recurring translocations (Table 2).

One class of unique sequence probes currently available are the "subtelomeric" probes. The ends of mammalian chromosomes consist of tandem repeats of a simple six nucleotide unit (TTAGGG), which act

to protect the telomeres during the process of DNA replication and mitosis. Adjacent to the telomeres (100 to 300 kb from the end of each chromosome) is unique chromosome-specific DNA. Typically, the telomeric bands of most chromosomes appear as pale or unstained bands by G-banding methods. Translocations involving the exchange of telomeric material, one pale band for another, are difficult to discern. Hybridization of these subtelomeric probes to metaphase cells has resulted in the identification of cryptic translocations of telomeric bands, which were undetectable by G-banding.¹ The power of this method was first demonstrated for patients with unexplained mental retardation or congenital abnormalities,⁵⁰ but telomeric rearrangements probably also have been overlooked in neoplastic diseases. This possibility is important since pale subtelomeric bands are G-C-rich with a high gene content, and these regions may contain genes that are involved in tumorigenesis. A complete set of subtelomeric probes is available through Vysis; for humans, this set includes 41 probes, rather than 48, since the subtelomeric sequences of the p arms of the five acrocentric chromosomes (13 to 15, 21, and 22), and the X and Y chromosomes (shared pseudoautosomal region) are not included.

FISH Strategies for the Detection of Recurring Translocations

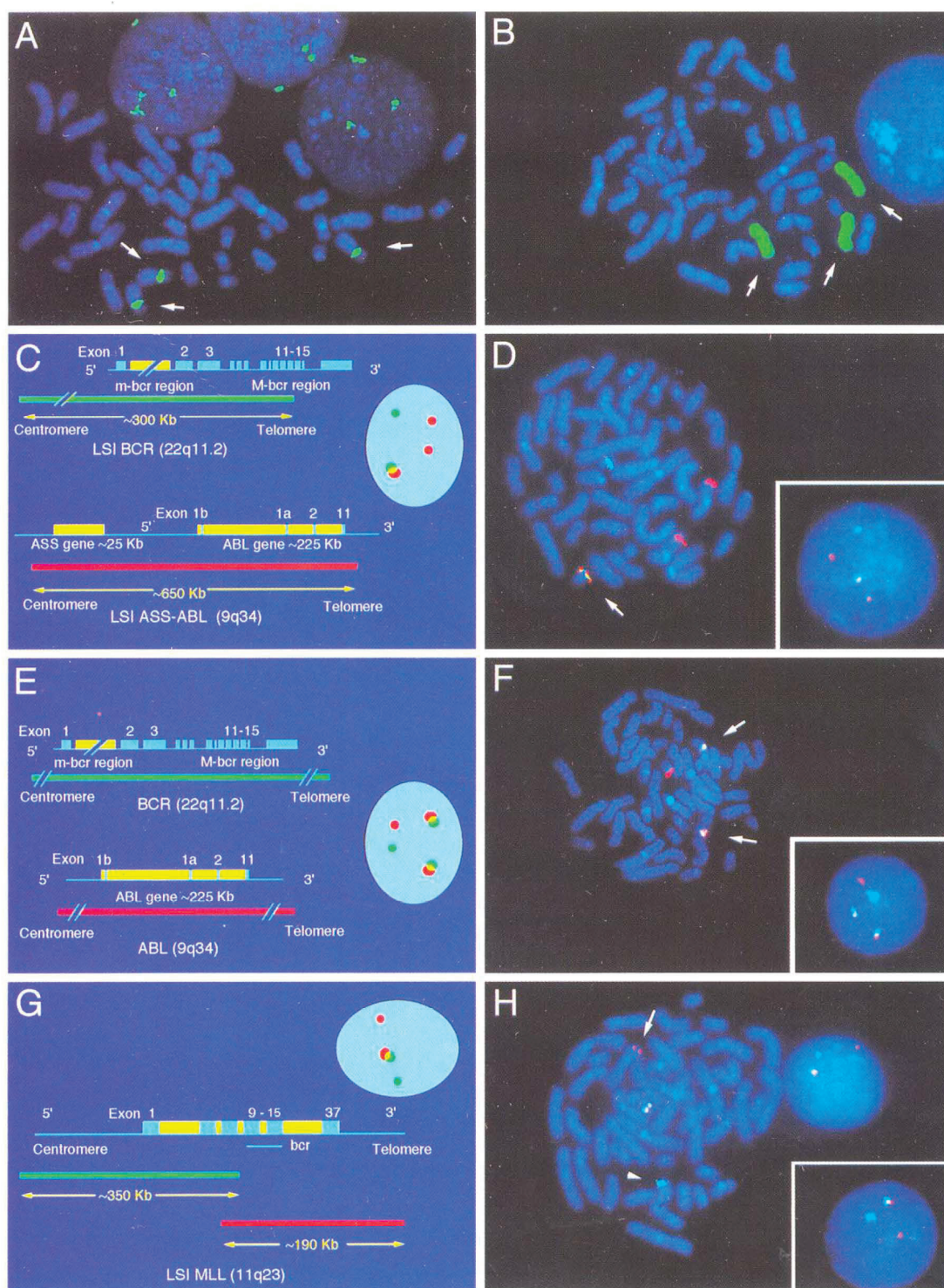
A variety of strategies have been developed for the detection of recurring translocations in interphase cells that take advantage of the ability to detect hybridized probes using multiple fluorochromes. In the following discussion, we describe the various probes available to detect the t(9;22), and emphasize the variable sensitivity. Thus the selection of a particular probe configuration may vary depending on the application. In the conventional method, a *single fusion signal* is detected by the hybridization of dual-color locus-specific probes to their target sequences at the junction of the translocation breakpoints. In the example of t(9;22), the directly labeled probe contains the 5' portion of the *BCR* gene at 22q11.2 (Spectrum Green), and the 3' portion of the *ABL* gene (Spectrum Orange) at 9q34 (LSI bcr/abl dual-color probe; Vysis). In cells with the t(9;22), one green and one red signal are observed on the normal homologs, and a yellow fusion signal is seen on the Ph

chromosome [der(22)t(9;22)], as a result of the translocation of the *ABL* sequences to chromosome 22. Probes of this type have a relatively high number of false-positive fusion signals (2% to 6%) as a result of the close proximity and random juxtaposition of target chromosomes in interphase nuclei. The sensitivity of single-fusion FISH probes is 5% to 10% (mean number of fusion signals in normal samples plus 2 SD), limiting their use in the detection of MRD.¹³

The next generation of *BCR/ABL* FISH probes, *extra-signal (ES) probes*, was introduced recently (Vysis). With the two-color ES probes, DNA sequences flanking the breakpoints on the chromosomes are brought together to form a fusion yellow signal; however, part of the DNA sequences recognized by one of the probes (*ABL*) remains at the original site, giving rise to an extra signal in a single color (red) (Fig 1C and D). False-positive signals can be distinguished from genuine fusion signals resulting from t(9;22) by the absence of the extra red signal for the 5' *ABL* sequences. The application of these probes results in improved sensitivity, and they can detect Philadelphia chromosome (Ph)-positive cells in less than 1% of nuclei in a sample.⁵⁴

A third type of probe for detecting the t(9;22) is a *dual fusion signal BCR/ABL* probe (Ventana Medical Systems, Tucson, AZ <http://www.ventanamed.com>). In this case, the probes span the involved genes: cells with t(9;22) have one red and one green signal observed on the normal homologs, and yellow fusion signals observed on both der(22) and der(9) homologs (Fig 1E and F). There is a substantially lower rate of false-positive cells, and the cut-off value for a positive result is about 1%.¹⁴ The results of a recent study suggested that quantitative analysis of 6,000 nuclei could be used to detect MRD with a sensitivity of approximately 0.2%.¹⁴

A fourth type of probe is the *two-color break-apart probe*. An example is the *MLL* probe marketed by Vysis to detect the recurring translocations of 11q23. In two-color break-apart probes, DNA sequences from the 5' and 3' regions of a single gene are differentially labeled and detected with red and green fluorochromes. In the germline configuration, a yellow fusion signal is observed, whereas separate red and green signals are observed when the sequences are separated as a result of a translocation (Fig 1G and H). The sensitivity of this type of probe is exceedingly high (cut-off value of ~2%) with excellent specificity.



Applications of FISH

The applications of FISH in cancer diagnosis are summarized in Table 1 and have been described in depth in several recent reviews.^{4,32,37,51} In many cases, FISH analysis provides increased sensitivity, in that cytogenetic abnormalities have been detected in samples that appeared to be normal by morphologic and conventional cytogenetic analyses. FISH is most powerful when targeted toward those abnormalities that are known to be associated with a particular disease or to occur in a specific patient's tumor. For example, cytogenetic analysis is often performed at the time of diagnosis to identify the chromosomal abnormalities in an individual patient's malignant cells. Thereafter, FISH can be used to detect residual disease or early relapse, and to assess the efficacy of therapeutic regimens. Several clinical-cytogenetic subsets for which FISH is feasible in a clinical setting are described below; other examples are given in Table 2.

t(9;22)(q34;q11.2), *BCR/ABL*

Approximately 92% of patients with chronic myelogenous leukemia (CML) have the standard t(9;22); the remaining 7% to 8% patients have variant, three-way translocations involving chromosomes 9 and 22, and a third chromosome. In both standard and variant translocations, the *ABL* oncogene (9q34) is relocated adjacent to the *BCR* gene on chromosome 22 (q11.2). Most breaks in *BCR* cluster in a small, 5.8-kb region between exons b2 and b3, or between b3 and b4, known as the breakpoint cluster region (bcr). In contrast, the breaks on chromosome 9 occur over more than 200 kb, usually upstream (5') of exon II, and the *ABL* coding sequences are translocated to

chromosome 22 and fused in-frame with the *BCR* gene, to produce a chimeric protein (p210). In ALL, the incidence of the t(9;22) is 30% in adults and 5% in children. In about 30% of adult cases, the molecular rearrangement is identical to that observed in CML; in the remaining patients the breakpoint occurs upstream (5') of bcr but still within the *BCR* gene, giving rise to smaller fusion proteins (185 to 190 kd).

As described above, several types of FISH probes are available to detect the t(9;22) in both metaphase and interphase cells.^{7,14,54} All three configurations of *BCR/ABL* FISH probes (single-fusion, single-fusion ES, and dual-fusion) can be used for both standard and variant translocations; however, the signal pattern of the dual-fusion probe is altered for variant translocations (one red—one green—two fusion signals becomes two red—two green—one fusion signal in variant translocations).^{14,54} FISH can be performed at diagnosis or to monitor the response to treatment.¹⁴ Of importance is that FISH can detect the *BCR/ABL* fusion in rare cases that are *BCR/ABL*-positive but normal by cytogenetic analysis.

t(12;21)(p12;q22), *ETV6/RUNX1*

The t(12;21) is one of the most common translocations in childhood acute leukemia, accounting for 25% of childhood precursor-B ALL, and defines a distinct group of patients characterized by age between 1 and 10 years, B-lineage immunophenotype (CD10⁺, CD19⁺), and favorable outcome.^{4,51} The translocation fuses the 5' helix-loop-helix domain of *ETV6* with the 3' DNA-binding and transactivation domain of *RUNX1*. The t(12;21) is not detected by

Figure 1. (A, B, D, F, H) Photomicrographs of metaphase and interphase cells following FISH; the cells are counterstained with 4,6-diamidino-2-phenylindole-dihydrochloride (DAPI). A detailed description of the signal configurations for each type of probe is provided in the text. (A) Hybridization of a directly labeled centromere-specific probe for chromosome 8 (CEP8 Spectrum Green; Vysis) to metaphase and interphase cells with trisomy 8 from a bone marrow aspirate of a patient with AML. The chromosome 8 homologs are identified with arrows. (B) Hybridization of a directly-labeled chromosome 8-specific painting probe (WCP8 Spectrum Green; Vysis) to a metaphase cell with trisomy 8 from a bone marrow aspirate of a patient with AML. The chromosome 8 homologs are identified with arrows. (C) Schematic diagram of the *BCR* and *ABL* loci, location of the *BCR/ABL* single-fusion ES probe (Vysis), and configuration of signals in interphase cells. (D) Hybridization of the *BCR/ABL* ES probe to metaphase and interphase cells with the t(9;22). The fusion signal on the Ph chromosome [der(22)] is identified with an arrow. (E) Schematic diagram of the *BCR* and *ABL* loci, approximate location of the *BCR* and *ABL* dual-fusion probe (Ventana Medical Systems), and configuration of signals in interphase cells. (F) Hybridization of the *BCR/ABL* dual fusion probe to metaphase and interphase cells with the t(9;22). The dual fusion signals on the der(9) and der(22) are identified with arrows. (G) Schematic diagram of the *MLL* gene, location of the *MLL* break-apart probe (Vysis), and configuration of signals in interphase cells. (H) Hybridization of the *MLL* break-apart probe to metaphase and interphase cells with the t(9;11). The der(9) is labeled with the 3' portion of the *MLL* gene (red signal, arrow), and the der(22) is labeled with the 5' portion of *MLL* (green signal, arrowhead). Panels C and D are modified from the product literature provided by Vysis.

cytogenetic analysis, due to the similarity in size and banding pattern of 12p and 21q, but the translocation can be found using FISH.

t(11q23)/*MLL* Rearrangements

Recurring translocations involving the *MLL* gene at 11q23 are of great interest in acute leukemia for the following reasons: (1) more than 30 different recurring rearrangements involve *MLL*; (2) the resulting translocations occur in both lymphoid and myeloid leukemias; and (3) they have a very unusual age distribution, occurring in approximately 75% of infant leukemias. Translocations of *MLL* result in the formation of a chimeric gene on the rearranged chromosome 11, der(11), consisting of the 5' region of *MLL* and the 3' region of the partner gene. These translocations have been associated with an intermediate to poor clinical outcome. FISH can detect the rearrangement, and a dual-color *MLL* break-apart probe is available commercially (Vysis). In the past, the use of commercially available *MLL* probes for FISH was limited because *MLL* sequences 3' of the breakpoint are deleted in about 25% of patients. As a consequence, only two signals are observed, rather than the pattern of three signals indicative of a translocation. This problem has been largely eliminated with the break-apart probe currently available, which contains a larger amount of 3' *MLL* sequences.

t(15;17)(q22;q11.2-12)/*PML RARA*

The t(15;17) is highly specific for AML-M3. The breakpoint on chromosome 17 occurs within the first intron of the alpha retinoic acid receptor gene (*RARA*), and the break on chromosome 15 occurs within the *PML* gene. Establishing the diagnosis of APL with the typical t(15;17) is important because this disease is sensitive to therapy with all-*trans* retinoic acid (ATRA), whereas other cases of AML and other M3-like disorders associated with variant translocations (~2%) do not respond to this treatment. A FISH probe for the t(15;17) is available, and it can be used in metaphase and interphase cells as a supplement to conventional cytogenetic analysis in uncertain cases, and to monitor the effects of ATRA, arsenic trioxide, or other therapies.

t(8;21)(q22;q22)/*RUNX1/ETO*

The t(8;21) is observed in 18% of all AML patients with an abnormal karyotype and in 30% of AML-M2

patients, and identifies a morphologically and clinically distinct subset. At the molecular level, the translocation results in the *RUNX1/ETO* chimeric protein. The *RUNX1* protein heterodimerizes with *CBFB* (see description of the inv(16) below) to form transcription factor essential for hematopoiesis. The *RUNX1/ETO* fusion protein disrupts the *RUNX1* pathway. AML-M2 with the t(8;21) has a favorable prognosis in adults, but the outcome in children is poor. A dual-fusion FISH probe has recently become available to detect this translocation (Table 2).

inv(16) or t(16;16)(p13;q22)/*CBFB/MYH11*

The inv(16), or related t(16;16), occurs in virtually all cases of AML-M4eo leukemia, and overall they account for 8% of all AML cases. A fusion protein containing the 5' region of *CBFB*, fused to the 3' portion of *MYH11* is produced. *CBFB/MYH11* disrupts the *RUNX1* transcription factor pathway required for normal hematopoiesis. The inv(16) is associated with a good response to chemotherapy, with an overall 5-year survival rate of 75%. FISH analysis has revealed that some rearrangements involving 16p13 or 16q22 were actually masked inv(16).⁵¹ A two-color break-apart *CBFB* probe is available to detect these rearrangements (Table 2).

t(2;5)(p23;q35)/*ALK/NPM*

The molecular consequence of the t(2;5) is the production of a unique fusion protein, resulting from the joining of the nucleophosmin (*NPM*) gene at 5q35 to a tyrosine protein kinase gene (*ALK*) at 2p23. This rearrangement is characteristic of anaplastic large cell lymphomas with a T or null phenotype, usually arising in young patients. The tumors express CD30 (Ki-1), which is also observed in Hodgkin's disease. The availability of a two-color break-apart *ALK* FISH probe (Vysis) is useful to distinguish between these diseases. *ALK* can also be deregulated by genes other than *NPM* in variant 2p23 rearrangements.

t(11;14)(q13;q32)/*CCND1/IGH*

The t(11;14) is observed in approximately 75% of mantle cell lymphomas (MCL), in 20% of polyclonal lymphocytic leukemias, and up to 20% of multiple myeloma cases. The consequence of the rearrangement is the activation of the cyclin D1 gene (*CCND1*) by the *IGH* gene. *CCND1* is located 100 to 130 kb away from the breakpoint at 11q13, which represents

an obstacle for the detection of rearrangements by molecular analysis. Recently, a two-color fusion FISH probe for MCL, which spans the 350-kb breakpoint region at 11q13, and the JH and VH regions of *IGH*, was introduced (Vysis). FISH analysis using a similar strategy reported the detection of t(11;14) in 100% of histologically confirmed MCL cases.³⁸

Chronic Lymphocytic Leukemia

As a result of the low proliferative index, only 50% of chronic lymphocytic leukemia (CLL) cases are adequate for cytogenetic analysis; of these, about half have clonal abnormalities.^{26,30,45} The application of FISH to CLL has substantially enhanced our ability to detect chromosomal aberrations; the incidence of a number of recurring abnormalities has been established (see below), providing new insights into the pathogenesis of this disease.

del(13q)/t(13q14). Abnormalities of 13q14 are the most common aberrations in CLL, identified in 50% of cases examined by FISH but in only 10% to 20% studied by cytogenetic analysis.²⁴ The putative tumor-suppressor gene at 13q14 is unknown. Early studies suggested that the retinoblastoma gene (*RBI*) was deleted in about 30% of cases, but homozygous deletions or absence of *RBI* mRNA are rarely observed. More recently the relevant gene has been localized distal to *RBI*, in the interval containing the D13S25/D13S19 loci. The smallest overlapping deleted segment of 300 kb has been identified and cloned; however, mutations have not yet been identified in candidate genes within this interval.^{17,31}

Previous studies based on cytogenetic analysis found that abnormalities of 13q were associated with early-stage disease and a better survival; the results obtained by FISH are conflicting. In two FISH studies, loss of *RBI* (24/54, 44%) or the D13S25/D13S19 loci (28/54, 48%) correlated with longer survival,²⁷ and early-stage disease (104/300, 34%) (the latter finding was not statistically significant).²⁴ In contrast, in another recent examination, deletions of D13S25/D13S19 were found in 37% of 78 CLL patients; those who were Rai stage 0 to II with deletion of 13q14 had a more aggressive disease.⁵⁵

Trisomy 12. FISH analysis using a centromere probe for chromosome 12 identified trisomy in 12% to 54% of the cases (mean, 20%).^{2,21,30} In several large cytogenetic series, trisomy 12 was associated with atypical morphology, advanced stage, and shorter survival. Little is known about the genomic conse-

quences of this alteration, although a gene dosage effect has been proposed. A small region at 12q13-15 containing the murine double minute-2 gene (*MDM2*) has been implicated, and overexpression of *MDM2* was identified in patients with aggressive disease.¹⁵

del(11q22-23). A deletion of the long arm of chromosome 11 has been identified in 13% to 19% of CLL cases examined by FISH analysis, as compared to 7% by cytogenetic analysis. *del(11q)* is associated with advanced disease stage, disease progression, and shorter survival, particularly in patients less than 55 years old.¹⁸ The ataxia-telangiectasia gene (*ATM*) is a tumor-suppressor gene, and absence of the ATM protein correlates with poor outcome. Some B-CLL patients with loss of heterozygosity for alleles on 11q have mutations in the remaining *ATM* allele.¹⁷

Other abnormalities. Deletion of 17p13 with loss of *TP53*, observed by FISH in approximately 10% of patients with CLL, is associated with a poor outcome and drug resistance.¹⁶ Deletions of 6q have been reported in about 10% of B-CLL cases; most involve 6q21-23, but the relevant gene is unknown. Abnormalities of 14q32 occur in about 8% of cases; most common is the t(11;14), and the presence of this translocation may be indicative of MCL. Another recurring translocation is the t(14;19)(q32;q13) in approximately 1% of CLL cases, which results in deregulation of the *BCL3* gene.^{17,40}

Multiple Myeloma

Chromosomal abnormalities are detected by cytogenetic analysis in 30% to 50% of multiple myeloma (MM) patients.^{9,23,26} The overall frequency of chromosomal abnormalities correlates with the stage of the disease: stage I, 20%; stage III, 60%; patients with an extramedullary mass, 80%, most likely reflecting increased proliferative activity with disease progression. By multivariate analysis, patients undergoing autologous bone marrow transplantation who have abnormalities of chromosomes 13 and 11 represent an unfavorable prognostic group.^{6,57}

The use of FISH and spectral karyotyping to examine MM has had a dramatic effect on our understanding of the chromosomal pattern of this disease. First, aneuploidy has been demonstrated in up to 90% of patients with MM²⁰; trisomy 6, 9, and 17 are associated with improved survival, and trisomy 8 has been correlated with disease progression.²³

Moreover, translocations were identified in virtually all patients.³ Of note is that aneuploidy for chromosomes 3, 7, 9, or 11 was also detected in monoclonal gammopathy of undetermined significance (MGUS), a disorder that evolves into MM in about 25% of cases.⁵⁸ Furthermore, a recent study of 158 patients with MM showed that, in 20 patients with a previous history of MGUS, the incidence of monosomy 13 detected by FISH was 71% versus 31% in MM patients without previous MGUS, suggesting the existence of two distinct diseases.⁵

t(14q32). The *IGH* locus is involved in 20% to 40% of abnormal MM cases by cytogenetic analysis.²⁵ In two recent studies, metaphase and interphase FISH analysis revealed *IGH* rearrangements in 75% of MM patients and in 100% of human myeloma cell lines.^{3,44} More than 20 translocation partners have been identified, confirming that *IGH* is the most frequently rearranged gene in this disease. In contrast to other B-cell neoplasms, where the rearrangement involves the J region of *IGH*, translocations in MM usually involve the switch region as a consequence of an illegitimate switch recombination.⁸ A FISH probe that spans the entire *IGH* locus, which holds promise for detecting virtually all *IGH* translocations, is currently under development (Vysis).

The fibroblast growth factor receptor 3 gene (*FGFR3*) at 4p16 is involved in 15% to 33% of myeloma cases. Of importance is that this translocation is usually not detected by cytogenetic analysis because it involves two pale telomeric bands.¹⁰ The consequence of the t(4;14) is overexpression of FGF3, as well as the production of a hybrid mRNA IgH-MMSET (multiple myeloma set domain), resulting in increased proliferation and decreased apoptosis of myeloma cells. A second common translocation is the t(6;14)(p25;q32); the partner gene at 6p25, *MUM1/IRF4*, is a member of the interferon regulatory factor family. The t(11;14)(q13;q32), characteristic of MCL, is observed in approximately 20% of cases analyzed by FISH; this translocation results in overexpression of *CCND1*, and a poor prognosis.⁵³ Because the breakpoint in *IGH* involves the switch region, the commercially available *CCND1/IGH* probe developed for MCL is not applicable for the detection of the translocation in myeloma (a reconfigured probe useful in myeloma is expected to be available soon). The *MAF* gene, which encodes a member of the large family of basic leucine-zipper transcription factors, is overexpressed in 25% of MM cell lines, as a result of

the t(14;16)(q32;q23). Finally, rearrangements of *MYC* at 8q24 occur in 10% of myeloma cases, and involvement of *BCL2* at 18q21 has been demonstrated in rare cases.

del(13q14). A del(13q) is identified in 15% to 20% of MM cases by cytogenetic analysis, and has been correlated with poor outcome.^{6,25} A recent study using FISH detected 13q14 abnormalities in up to 40% of the cases and confirmed that loss of 13q is an independent adverse prognostic factor.⁵⁹ Although the *RBI* gene is deleted in about half of the patients, no correlation has been found between *RBI* gene deletion and protein levels.²⁹ The minimal deleted region, apparently the same as that involved in other B-cell neoplasms, spans the region containing the D13S319/D13S25 markers.¹²

del(17p13). Monoallelic deletions of *TP53* have been detected by FISH in up to 50% of newly diagnosed patients. Loss of *TP53* is correlated with aggressive disease and short survival after treatment with conventional chemotherapy.¹⁹

Sensitivity and Specificity of FISH

Several studies have examined the normal range of values and determined the specificity and sensitivity of interphase FISH analysis. The reproducibility, sensitivity, and specificity have been established for a centromere-specific probe for chromosome 8 (Spectrum Orange Cep8, Vysis).²⁸ The distribution of signals in bone marrow samples from healthy individuals was used to establish the cut-off point of 2.65%, or the maximum number of cells with three signals allowed before a sample is categorized as positive (mean $\pm$ 2 SD). Of 216 samples examined by cytogenetic analysis, 61 (28.2%) had trisomy 8, 142 (65.7%) were negative for trisomy 8, and 13 (6.1%) were ambiguous (one metaphase cells with trisomy 8 detected in 20 or 30 cells). Of the 61 cytogenetically positive cases, 55 were correctly identified as positive for trisomy 8 by FISH, resulting in an overall sensitivity of 91%, equivalent to the percentage of abnormal cases that are appropriately classified. Of the 142 cytogenetically negative cases, 140 were correctly scored as negative for trisomy 8 by FISH, resulting in an overall specificity of 99%, equal to the percentage of normal cases that are correctly classified. These findings emphasize that, prior to use on clinical specimens, investigators must establish the perfor-

mance of the assay within their laboratory, using appropriate tissue samples.

Reverse transcription polymerase chain reaction is used to detect many fusion genes, and it is the method of choice for detecting MRD. The sensitivity varies from 10^{-4} to 10^{-6} . In contrast, the sensitivity of FISH to detect the t(9;22) was determined to be 10^{-3} , with a cut-off value of 0.079%.¹⁴

Advantages and Limitations of FISH

FISH has greatly expanded the capabilities of cytogenetics and pathology laboratories (Table 3). FISH is highly sensitive and specific, and it can be performed rapidly. In contrast, cytogenetic analysis is very labor-intensive, and results are not available for 1 to 3 weeks in all but exceptional cases. Material for FISH can be

processed in 4 to 24 hours, and the analysis of 1,000 to 2,000 cells accomplished in 15 to 45 minutes. FISH enables information on the cytogenetic pattern of tumor cells to be obtained in a time-frame sufficient for data to be considered in treatment decisions. The complementary use of cytogenetic analysis and FISH provides a remarkably powerful tool for cancer cytogeneticists. For example, the ability to target FISH studies toward specific cytogenetic abnormalities can provide data that are essential for the clinical management of a cancer patient, such as the use of a chromosome 8-specific centromere probe to detect MRD following treatment in an AML patient who had trisomy 8 on cytogenetic analysis at the time of diagnosis.

The limitations of FISH are given in Table 3. Most notable is that the detection of abnormalities is restricted by the probes currently available (Table 2). At present, there are centromere-specific probes for all of the human chromosomes; thus, a gain of a chromosome can be detected with high sensitivity. Abnormalities such as recurring translocations and deletions are best identified with probes that are derived from the bands containing the breakpoints or from probes within the deleted segment. In the past, relatively few translocations could be detected by FISH; however, this situation has dramatically improved over the past few years as probes have (or soon will) become available for the most commonly recurring translocations, particularly those with diagnostic and prognostic significance.

Other limitations of FISH are technical. First, the method has been demonstrated to be highly sensitive for the detection of trisomy but less so for chromosome loss.⁴⁹ The false monosomy rate (presence of only one signal in a diploid cell) can vary from 3% to 9% of all nucleated cells in preparations from bone marrow or peripheral blood; therefore, the ability of FISH to detect monosomy in a small percentage of the cell population, as in MRD, is limited. Second, processing of bone marrow and peripheral blood cells from patients with hematologic malignant diseases is relatively simple, but the preparation of other tissues, such as paraffin-embedded tissues or frozen sections from lymph node biopsies, is much more difficult. Artifacts created by crushing and sectioning of tissue, and technical factors related to penetration of the probe into the cell nucleus, can lead to misleading results. In these tissues, the percentage of cells showing false monosomy may be very high (>50%). Autofluo-

Table 3. Advantages and Limitations of FISH

Advantages

- Rapid technique, and large numbers of cells can be scored in a short period.
- Efficiency of hybridization and detection is high.
- Sensitivity and specificity is high.
- Cytogenetic data can be obtained from nondividing or terminally differentiated cells.
- Cytogenetic data can be obtained from poor samples that contain too few cells for routine cytogenetic analysis (post-treatment samples or hypocellular samples).
- Technique permits direct correlation of cytogenetic and morphologic features, enabling pathologists to differentiate malignant from benign conditions in equivocal cases.
- Method has been adapted for automated systems (useful for some probes, such as centromere-specific probes).

Limitations

- Restricted to those abnormalities that can be detected with currently available probes.
- Only one or a few abnormalities can be assessed simultaneously.
- Cytogenetic data can be obtained only for the target chromosomes; thus, FISH is not a good screening tool for cytogenetically heterogeneous diseases, eg, AML or ALL.
- Due to failure to detect signal, FISH is highly sensitive for trisomy but less sensitive for detecting chromosome loss or deletion.
- Processing of bone marrow or peripheral blood samples is relatively simple; however, preparation of paraffin-embedded tissues or frozen sections from solid tumors or lymph node biopsies is substantially more difficult.
- Requires fluorescence microscopy and an image analysis system.

rescence of tissue or precipitation of the probe or fluorochrome may lead to an incorrect interpretation of additional copies of the target chromosome. Many of these problems have been partially eliminated by improvements in hybridization techniques; in other cases, evaluation of the efficiency of a particular hybridization can be facilitated by cohybridization of a control probe.

A third major problem with FISH is that data can be obtained only for the target chromosomes. In contrast, conventional cytogenetic analysis can detect the presence of virtually all chromosomal abnormalities, including multiple lesions, by a single test. Multicolor FISH improves this situation by allowing the simultaneous visualization of two or more DNA regions; spectral karyotyping (SKY), multiplex FISH, and cross-species color banding (Rx-FISH) also alleviate this difficulty (see chapter by Schröck and Padilla-Nash).

FISH requires an imaging system, including a charge-coupled device camera, computer, and image analysis software. Together with the expense of a fluorescence microscope, the start-up costs can range from \$80,000 to \$120,000. Cumbersome early software has been replaced by improved current versions. Probes and reagents represent an additional expense (with the necessary positive and negative controls, the costs are about \$80 to \$100 per case).

Further limitations are that each laboratory must establish criteria for acceptable performance (sensitivity and specificity) and cut-off values for each probe, and validate each batch of probe. The Laboratory Practice Committee of the American College of Medical Genetics has established the "Standards and Guidelines for Clinical Genetics Laboratories" (second edition 1999; available through www.faseb.org/genetics/acmg). These establish procedures for probe validation, assay validation, and analytical sensitivity. With the exception of several probes marketed by Vysis, most of the FISH probes available commercially have not yet received US Food and Drug Administration approval for diagnostic use. As a result, some laboratories continue to use both cytogenetic and FISH methods to test the same clinical sample, rather than FISH alone.

Because of these limitations, FISH does not yet circumvent the need for a complete cytogenetic analysis of banded chromosomes in the initial diagnos-

tic specimen. At present, FISH is mostly used in conjunction with cytogenetic analysis.

Recent Developments in FISH

Despite its high sensitivity and specificity, FISH has not yet had the full impact on clinical diagnostics that many geneticists predicted 10 years ago. Ultimately, FISH has been limited by the availability of commercially available, specific probes with high performance in a clinical setting. This situation has changed dramatically in the past few years, as probes to detect the most frequent abnormalities in myeloid disorders are now available (Table 2). The development of probes for recurring abnormalities in lymphoid malignancies has lagged behind that in the myeloid disorders; however, probes for the t(11;14) in MCL have been introduced recently, and those for the t(14;18) and t(8;14) are under development and are expected to be available by the end of 2000. Another important probe that will be available soon spans the *IGH* locus. Such a probe will enable the detection of any *IGH* translocation in interphase cells and should be extremely valuable in distinguishing a reactive from a malignant process in lymph node or tissue biopsies.

The development of collections of probes (a myeloid panel, a lymphoid panel, a multiple myeloma panel, etc) is under consideration by several commercial providers and holds the promise of reducing the cost of applying multiple FISH probes, as well as increasing the speed with which multiple assays are performed.²² The ready availability of such panels as well as probes for clinically relevant abnormalities would make feasible rapid FISH frontline test for detecting chromosomal abnormalities at diagnosis. Other recent developments, such as the recognition that dual-fusion signal probes and two-color break-apart probes provide increased sensitivity, will improve FISH, as current configurations are converted to more sensitive schemas in the near future. Other promising advances include peptide nucleic acid probes (DNA analogs in which the deoxyribose has been replaced by 2-aminoethyl glycine), which have very rapid hybridization kinetics and stable formation of duplexes with complementary DNA,³⁵ and padlock probes.⁴³

Some of the most recent innovations lend themselves well to automation, as in hybridization chambers. Several commercially available imaging systems

offer flexibility in image acquisition and analysis, including the enumeration of fluorescent signals in interphase nuclei. Microscope and imaging companies have turned their attention to the development of automated metaphase finding systems adapted for fluorescence; such systems would eliminate a time-consuming step in the FISH analysis of metaphase cells.

Finally, SKY and Rx-FISH now provides the power to analyze the entire genome in a single hybridization experiment. Together, the combination of cytogenetic, FISH, and SKY analyses incorporates the screening potential of cytogenetics with the accuracy of molecular genetics techniques, which will lead to better understanding of neoplastic diseases, more accurate diagnosis, stratification of patients into genetic subgroups, and eventual improvements in treatment and outcome.

Acknowledgment

We thank Ventana Medical Systems for providing the dual fusion signal BCR/ABL probe, and John Proffitt (Vysis, Inc) for providing information on FISH probes.

References

1. A complete set of human telomeric probes and their clinical application. National Institutes of Health and Institute of Molecular Medicine collaboration. *Nat Genet* 14:86-89, 1996
2. Anastasi J, Le Beau MM, Vardiman J, et al: Detection of trisomy 12 in chronic lymphocytic leukemia by fluorescence in situ hybridization to interphase cells: A simple and sensitive method. *Blood* 79:1796-1801, 1992
3. Avet-Loiseau H, Brigaudeau C, Morineau N, et al: High incidence of cryptic translocations involving the Ig heavy chain gene in multiple myeloma, as shown by fluorescence in situ hybridization. *Genes Chromosomes Cancer* 24:9-15, 1999
4. Avet-Loiseau H: Fish analysis at diagnosis in acute lymphoblastic leukemia. *Leuk Lymphoma* 33:441-449, 1999
5. Avet-Loiseau H, Li J-Y, Morineau N, et al: Monosomy 13 is associated with the transition of monoclonal gammopathy of undetermined significance to multiple myeloma. *Blood* 94:2583-2589, 1999
6. Barlogie B, Jagannath S, Desikan KR, et al: Total therapy with tandem transplants for newly diagnosed multiple myeloma. *Blood* 93:55-65, 1999
7. Bentz M, Cabot G, Moos M, et al: Detection of chimeric *BCR-ABL* genes on bone marrow samples and blood smears in chronic myeloid and acute lymphoblastic leukemia by in situ hybridization. *Blood* 83:1922-1928, 1994
8. Bergsagel PL, Chesi M, Nardini E, et al: Promiscuous translocations into immunoglobulin heavy chain switch regions in multiple myeloma. *Proc Natl Acad Sci USA* 93:13931-13936, 1996
9. Calasanz MJ, Cigudosa JC, Odero MD, et al: Cytogenetic analysis of 280 patients with multiple myeloma and related disorders: Primary breakpoints and clinical correlations. *Genes Chromosomes Cancer* 18:84-93, 1997
10. Chesi M, Nardini E, Brents LA, et al: Frequent translocation t(4;14)(p16.3;q32.3) in multiple myeloma is associated with increased expression and activating mutations of fibroblast growth factor receptor 3. *Nat Genet* 16:260-264, 1997
11. Cremer T, Lichter P, Borden J, et al: Detection of chromosome aberrations in metaphase and interphase tumor cells by in situ hybridization using chromosome specific library probes. *Hum Genet* 80:235-246, 1988
12. Dao D, Sawyer J, Epstein J, et al: Deletion of the retinoblastoma gene in multiple myeloma. *Leukemia* 8:1280-1284, 1994
13. Dewald GW, Schad CR, Christenson ER, et al: The application of fluorescent in situ hybridization to detect *Mbcr/abl* fusion in variant Ph chromosomes in CML and ALL. *Cancer Genet Cytogenet* 71:7-14, 1993
14. Dewald GW, Wyatt WA, Juneau AL, et al: Highly sensitive fluorescence in situ hybridization method to detect double BCR/ABL fusion and monitor response to therapy in chronic myeloid leukemia. *Blood* 91:3357-3365, 1998
15. Dierlamm J, Michaux L, Criel A, et al: Genetic abnormalities in chronic lymphocytic leukemia and their clinical and prognostic implications. *Cancer Genet Cytogenet* 94:27-35, 1997
16. Döhner H, Fischer K, Bentz M, et al: p53 gene deletion predicts for poor survival and non-response to therapy with purine analogs in chronic B-cell leukemias. *Blood* 85:1580-1589, 1995
17. Döhner H, Stilgenbauer S, Döhner K, et al: Chromosome aberrations in B-cell chronic lymphocytic leukemia: Reassessment based on molecular cytogenetic analysis. *J Mol Med* 77:266-281, 1999
18. Döhner H, Stilgenbauer S, James M, et al: 11q deletions identify a new subset of B-cell chronic lymphocytic leukemia characterized by extensive nodal involvement and inferior prognosis. *Blood* 89:2516-2522, 1997
19. Drach J, Ackermann J, Fritz E, et al: Presence of a p53 gene deletion in patients with multiple myeloma predicts for short survival after conventional-dose chemotherapy. *Blood* 92:802-809, 1998
20. Drach J, Schuster J, Nowotny H, et al: Multiple myeloma: high incidence of chromosomal aneuploidy as detected by interphase fluorescence in situ hybridization. *Cancer Res* 55:3854-3859, 1995
21. Escudier SM, Pereira-Leahy JM, Drach JW, et al: Fluorescent in situ hybridization and cytogenetic studies of trisomy 12 in chronic lymphocytic leukemia. *Blood* 81:2702-2707, 1993

22. Fischer K, Scholl C, Salat J, et al: Design and validation of DNA probe sets for a comprehensive interphase cytogenetic analysis of acute myeloid leukemia. *Blood* 88:3962-3971, 1996
23. Fonseca R, Coignet LJ, Dewald GW: Cytogenetic abnormalities in multiple myeloma. *Hematol Oncol Clin North Am* 13:1169-1180, 1999
24. Garcia-Marco JA, Price CM, Catovsky D: Interphase cytogenetics in chronic lymphocytic leukemia. *Cancer Genet Cytogenet* 94:52-58, 1997
25. Hallek M, Bergsagel PL, Anderson KC: Multiple myeloma: Increasing evidence for a multistep transformation process. *Blood* 91:3-21, 1998
26. Heim S, Mitelman F: *Cancer Cytogenetics* (ed 2). New York, NY, Wiley-Liss, 1995
27. Hogan WJ, Tefferi A, Borell TJ, et al: Prognostic relevance of monosomy at the 13q14 locus detected by fluorescence in situ hybridization in B-cell chronic lymphocytic leukemia. *Cancer Genet Cytogenet* 110:77-81, 1999
28. Jenkins RB, Le Beau MM, Kraker WJ, et al: Interphase fluorescence in situ hybridization: A sensitive method for trisomy 8 detection in bone marrow specimens. *Blood* 79:3307-3315, 1992
29. Juge-Morineau N, Mellerin MP, Francois S, et al: High incidence of deletions but infrequent inactivation of the retinoblastoma gene in human myeloma cells. *Br J Haematol* 91:664-667, 1995
30. Juliusson G, Merup M: Cytogenetics in chronic lymphocytic leukemia. *Semin Oncol* 25:19-26, 1998
31. Kalachikov S, Migliazza A, Cayanis E, et al: Cloning and gene mapping of the chromosome 13q14 region deleted in chronic lymphocytic leukemia. *Genomics* 42:369-377, 1997
32. Kearney L: The impact of the new FISH technologies on the cytogenetics of haematological malignancies. *Br J Haematol* 104:648-658, 1999
33. Landegent JE, Jansen in de Wal N, Baan RA, et al: 2-Acetylaminofluorene-modified probes for the indirect hybridocytochemical detection of specific nucleic acid sequences. *Exp Cell Res* 153:61-72, 1984
34. Langer PR, Waldrop AA, Ward DC: Enzymatic synthesis of biotin labeled polynucleotides: Novel nucleic acid affinity probes. *Proc Natl Acad Sci USA* 78:6633-6637, 1981
35. Lansdorp PM, Verwoerd NP, van der Rijke V, et al: Heterogeneity in telomere length of human chromosomes. *Hum Mol Genet* 5:685-691, 1996
36. Le Beau MM: Molecular biology of cancer: Cytogenetics, in De Vita VT, Hellman S, Rosenberg SA (eds): *Cancer: Principles and Practice of Oncology* (ed 5). Philadelphia, PA, Lippincott, 1997, pp 103-119
37. Le Beau MM: Fluorescence in situ hybridization in cancer diagnosis, in De Vita VT, Hellman S, Rosenberg SA (eds): *Important Advances in Oncology*. Philadelphia, PA, Lippincott, 1993, pp 29-45
38. Li JY, Gaillard F, Moreau A, et al: Detection of translocation t(11;14)(q13;q32) in mantle cell lymphoma by fluorescence in situ hybridization. *Am J Pathol* 154:1449-1452, 1999
39. Look AT: Oncogenic transcription factors in the human acute leukemias. *Science* 278:1059-1063, 1997
40. McKeithan TW, Takimoto GS, Ohno H, et al: BCL3 rearrangements and t(14;19) in chronic lymphocytic leukemia and other B-cell malignancies: A molecular and cytogenetic study. *Genes Chromosomes Cancer* 20:64-72, 1997
41. Mitelman F: *Catalog of Chromosome Aberrations in Cancer*. New York, NY, Wiley-Liss, 1994
42. Nederlof PM, van der Flier S, Wiegant J, et al: Multiple fluorescence in situ hybridization. *Cytometry* 11:126-131, 1990
43. Nilsson M, Krejci K, Koch J, et al: Padlock probes reveal single nucleotide differences, parent of origin and in situ distribution of centromeric sequences in human chromosomes 13 and 21. *Nat Genet* 16:252-254, 1997
44. Nishida K, Tamura A, Nakazawa N, et al: The Ig heavy chain gene is frequently involved in chromosomal translocations in multiple myeloma and plasma cell leukemia as detected by in situ hybridization. *Blood* 90:526-534, 1997
45. Panayiotidis P, Kotsi P: Genetics of small lymphocyte disorders. *Semin Hematol* 36:171-177, 1999
46. Perez-Simon JA, Garcia-Sanz R, Tabernero MD, et al: Prognostic value of numerical chromosome aberrations in multiple myeloma: A FISH analysis of 15 different chromosomes. *Blood* 91:3366-3371, 1998
47. Pinkel D, Landegent J, Collins C, et al: Fluorescence in situ hybridization with human chromosome specific libraries: Detection of trisomy 21 and translocation of chromosome 4. *Proc Natl Acad Sci USA* 85:9138-9142, 1988
48. Pinkel D, Straume T, Gray JW: Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. *Proc Natl Acad Sci USA* 83:2934-2938, 1986
49. Poddighe PJ, Moesker O, Smeets D, et al: Interphase cytogenetics of hematological cancer: comparison of classical karyotyping and in situ hybridization using a panel of eleven chromosome specific DNA probes. *Cancer Res* 51:1959-1967, 1991
50. Precht KS, Lese CM, Spiro RP, et al: Two 22q telomere deletions serendipitously detected by FISH. *J Med Genet* 35:939-942, 1998
51. Raimondi SC: Fluorescence in situ hybridization: Molecular probes for diagnosis of pediatric neoplastic diseases. *Cancer Invest* 18:135-147, 2000
52. Romana SP, Poirel H, Le Coniat M, et al: High frequency of t(12;21) in childhood B-lineage acute lymphoblastic leukemia. *Blood* 86:4263-4269, 1995
53. Ronchetti D, Finelli P, Richelda R, et al: Molecular analysis of 11q13 breakpoints in multiple myeloma. *Blood* 93:1330-1337, 1999
54. Sinclair PB, Green AR, Grace C, et al: Improved sensitivity of BCR-ABL detection: A triple probe three-color fluorescence in situ hybridization system. *Blood* 90:1395-1402, 1997
55. Starostik P, O'Brien S, Chung CY, et al: The prognostic significance of 13q14 deletions in chronic lymphocytic leukemia. *Leuk Res* 23:795-801, 1999
56. Tkachuk DC, Westbrook CA, Andreef M, et al: Detection of

- bcr-abl* fusion in chronic myelogenous leukemia by in situ hybridization. *Science* 250:559-562, 1990
57. Tricot G, Sawyer J, Jagannath S, et al: Poor prognosis in multiple myeloma is associated only with partial or complete deletions of chromosome 13 or abnormalities involving 11q and not with other karyotype abnormalities. *Blood* 86:4250-4256, 1995
58. Zandecki M, Lai JL, Genevieve F, et al: Several subclones may be identified within plasma cells of patients with monoclonal gammopathy of undetermined significance, both at diagnosis and during the indolent course of the condition. *Blood* 90:3682-3690, 1997
59. Zojer N, Konisberg R, Ackermann J, et al: Deletion of 13q14 remains an independent adverse prognostic variable in multiple myeloma despite its frequent detection by fluorescence in situ hybridization. *Blood* 95:1925-1930, 2000