

Cytogenetic and FISH Studies in Myelodysplasia, Acute Myeloid Leukemia, Chronic Lymphocytic Leukemia and Lymphoma

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Abstract

Conventional cytogenetic studies are widely used today to diagnose and manage patients with hematological malignancies. The application of fluorescence in situ hybridization (FISH) with chromosome-specific DNA probes helps to further define molecular subclasses and cytogenetic risk categories for patients with these disorders. Moreover, FISH permits analysis of proliferating (metaphase cells) and non-proliferating (interphase nuclei) cells, and is useful in establishing the percentage of neoplastic cells before and after therapy (minimal residual disease). For patients with myelodysplasia or acute myeloid leukemia, these chromosome techniques are important for accurate diagnosis and classification of disease and to help decide treatment and monitor response to therapy. Conventional cytogenetic studies have been problematic in chronic lymphocytic leukemia because the neoplastic cells divide infrequently. However, interphase FISH studies now permit detection of chromosome anomalies with prognostic significance in chronic lymphocytic leukemia. The World Health Organization recognizes that genetic anomalies are one of the most reliable criteria for classification of malignant lymphomas. New methods to extract individual nuclei from paraffin-embedded tissue are now available which permit the use of interphase FISH to detect important chromosome anomalies in lymphoma.

1. Introduction

Conventional cytogenetic studies are widely used today by physicians to diagnose and manage patients with hematological malignancies [1]. The application of fluorescence in situ hybridization (FISH) using chromosome-specific DNA probes further helps to define molecular subclasses and cytogenetic risk categories for patients with these disorders [1]. Moreover, FISH permits analysis of proliferating (metaphase cells) and non-proliferating (interphase nuclei) cells, and is useful in establishing the percentage of neoplastic cells before and after therapy (minimal residual disease) [2].

The myelodysplastic syndromes (MDS) are a clinically heterogeneous group of hematological disorders. Cytogenetic analyses detect an abnormal clone in many patients with MDS, especially patients with more aggressive forms of MDS such as t-MBS (therapy-related MDS) and RAEB (refractory anemia with excess blasts).

Recent interphase-FISH studies in patients with primary MDS and t-MDS have been exciting. Nevertheless, many patients with MDS appear to have a normal karyotype and owe their disease to one or more sub-microscopic gene mutations or other biological causes.

Acute myeloid leukemia (AML) has been subclassified into cytogenetic risk categories that have important implications for diagnosis, prognosis and treatment. Thus, it is important to perform conventional cytogenetic studies and/or FISH to properly treat patients and to monitor response to therapy.

Although the primary chromosome anomaly of B-cell chronic Lymphocytic leukemia (B-CLL) is unknown, aberrations of chromosomes 6, 11, 12, 13 and 17 are common in B-CLL. Each of these anomalies has been associated with a different prognosis [3]. Conventional cytogenetic studies are challenging in B-CLL because neoplastic cells divide infrequently. Today, interphase-FISH studies can be used to detect abnormal clones in

cludes using conventional chromosome studies to detect genetic aberrations. Today, various interphase-FISH tests are available to detect common chromosome anomalies in Lymphoma. In addition, new techniques have been developed to isolate individual nuclei from paraffin-embedded tissue that make routine interphase-FISH studies practical [6]. These FISH assays have considerable utility in clinical practice, in some cases for disease diagnosis and classification, and in other cases for prognosis.

This report deals with FISH and cytogenetic studies of MDS, AML, B-CLL, and lymphoma. The FISH probes and chromosome anomalies referred to in this report are summarized in Table 1. A more complete list of chromosome anomalies and associated gene rearrangements among patients with various hematological malignancies is shown in Table 2. The reader may wish to see other references that provide more detail about these disorders [1,5].

1.1. Genetic Testing Strategies

For some hematological malignancies, structural chromosome anomalies result in fusion of an oncogene and a promoter gene, which in turn produces a specific mRNA, which then produces a protein product with malignant potential. Most of these chromosome anomalies

can be detected by cytogenetic studies. At the DNA level, the fusion of genes associated with these chromosome anomalies can be detected by FISH, Southern blot, and/or PCR. At the RNA level, the messenger RNA transcript of these genes can be detected by RT-PCR. At the protein level, the chimera gene product can be detected by Western blot. Each of these methods can be used to quantify tumor burden, but they are associated with varying degrees of accuracy, cost, and turn around time. Since laboratories and personnel at different institutions vary, genetic testing strategies will not be identical among institutions.

Treatment protocols for hematological disorders require testing procedures that can quantify tumor burden before and after therapy to assess response to therapy. This can be done using various genetic tests including quantitative cytogenetic studies, FISH, and RT-PCR. No single genetic testing procedure fulfills all the needs of clinical care for patients with acute leukemia. It is important to use combinations of testing methods that are both accurate and cost-effective for each clinical situation. Testing strategies need to be adjusted over time as

Table 1.

Chromosome Anomalies and FISH Probes in MAS, AML, B-CLL.

Disorder	Chromosome anomaly	FISH probes	Sensitivity	FISH strategy ¹
AML, MDS	del(5)(q13q33)	CEN5, EGR1	>7%	ND
AML, MDS	del(7)(q22q34)	CEN7, D7S46	>7%	ND
AML, MDS	t(11;var)(q23;var)	CEN11, MLL	>3%	BAP
MDS	del(13)(q12q14)	D13S319, D13S327	>7%	ND
AML, MDS	+8	CEN8	>4%	CEN
MDS	del(20)(q11q13)	D20S108	>10%	ND
AML	t(8;21)(q22;q22)	ETO, AML1	>1%	D
AML	t(15;17)(q22;q21)	PML, RAR α	>3%	ES
AML	inv(16)(q13q22)	MYH11, CBF β	>3%	BAP
B-CLL	del(6)(q13q23)	CEN6, c-MYB	>7%	ND
B-CLL	del(11)(q13q23)	CEN11, ATM	>7%	ND
B-CLL	+12	CEN12, MDM2	>4%	ND
B-CLL	del(13)(q12q14)	D13S319, D13S327	>7%	ND
B-CLL	del(17)(p11)	CEN17, p53	>7%	ND
Mantle cell lymphoma/leukemia	t(11;14)(q13;q32)	CCND1, IgH	>1%	D
Burkitt lymphoma	t(8;14)(q24;q32)	c-MYC, IgH	>1%	D
Anaplastic large cell lymphoma	t(2;var)(q23;var)	ALK	>3%	BAP
MALT lymphoma	t(11;18)(q21;q21)	API2, MALT1	>1%	D
MALT lymphoma	+3, +7, +12, +18	CEN 3, 7, 12, 18	>4%	CEN
Follicular lymphoma	t(14;18)(q32;q21)	IgH, BCL2	>1%	D
Diffuse large B-cell lymphoma	t(3;var)(q27;var)	BCL6	>3%	BAP

¹Strategies: ND-FISH, numeric and/or deletions; D-FISH, double fusion for translocations; BAP-FISH, breakpoint probes for translocations; ES-FISH, extra signals for translocations; CEN-FISH, numeric anomalies. See text for more information.

Table 2.

154 Chromosome Anomalies in Malignant Hematologic Disorders.

Anomaly	Associated disorders	Genes	Anomaly	Associated disorders	Genes
Translocations			Inversions		
t(1;3)(p36;q21)	AML-M1, AML-M4, MDS		inv(3)(q21q26)	AML-M4, AML-M6, CML Ph+, MDS	Ribophorin I, EVII
der(1;7)(q10;q10)	AML, CMD, MDS, t-MDS		inv(14)(q11q32)	T-CLD, T-CLL, T-NHL, T-PLL	TCRad, IgH
t(1;11)(p32;q23)	ALL, AML	TAL1, MLL	inv(16)(p13q22)	AML-M4Eo	MYH11, CBFb
t(1;11)(q21;q23)	AML-M4, AML-M5	AF1r, MLL	Deletions		
t(1;14)(p32;q11)	T-ALL	TAL1, TCRad	del(1)(p32p36)	CLD, NHL	
t(1;17)(p36;q21)	AML-M3	? , RARa	del(1)(p22)	AML, ALL, MDS, NHL	
t(1;19)(q23;p13)	pre-B-ALL	PBX1, E2A	del(1)(q21)	NHL	
t(1;22)(p13;q13)	AML-M7		del(1)(q32)	ALL, NHL	
t(2;3)(p12;q27)	DLCL, FL	Igk, BCL6	del(1)(q42)	NHL	
t(2;5)(p23;q35)	ALCL	ALK, NPM	del(2)(p23)	AML	
t(2;8)(p12;q24)	ALL-L3, BL, NHL	Igk, c-MYC	del(2)(p21)	CLD, NHL	
t(2;18)(p12;q21)	NHL		del(2)(q31)	AML, CLD	
t(2;11)(p21;q23)	MDS		del(3)(p21)	ALL, MDS	
t(2;14)(p13;q32)	B-CLL		del(3)(q21)	ALL, AML, NHL	
t(3;3)(q21;q26)	AML, MDS	Pibophorin I, EVII	del(4)(p14)	NHL	
ins(3;3)(q26;q21q26)	AML, MDS		del(4)(q21)	NHL	
t(3;5)(q21;q31)	AML-M6	MLF1, NPM	del(5)(p13)	NHL	
t(3;5)(q25;q34)	AML		del(5)(q13q33)	AML, MDS, MPD, 5q-syn	
t(3;14)(q27;q32)	DLCL, FL	BCL6, IgH	del(6)(p21)	ALL, AML	
t(3;21)(q26;q22)	AML, CML Ph+, MDS	EAP, AML1	del(6)(q13-15q23)	ALL, CLL, FL, MCL, SLL, T-NHL	
t(3;22)(q27;q11)	DLCL, FL	EAP, AML2	del(6)(q21)	ALL, AML, CLL, NHL	
t(4;11)(q21;q23)	ALL, AML	AF4, MLL	del(7)(p13)	AML, CLD, NHL	
t(4;14)(p16.3;q32.3)	MM	FGFR3, IgH	del(7)(q11)	ALL, AML, MDS	
t(5;12)(q33;p13)	CMML, MDS, MPD	PDGFRB, TEL	del(7)(q22q34)	AML, CLD, CMD, MDS, NHL	
t(5;14)(q31;q32)	ALL	IL3, IgH	del(7)(q32)	AML, CLL, MDS, NHL	
t(5;17)(p23;q11-12)	AML-M3	NPM, RARa	del(8)(p21)	ALL, CLD, NHL	
t(6;9)(p23;q34)	AML-M1, AML-M2, AML-M4, MDS	DEK, CAN	del(8)(q22)	AML, NHL	
t(6;11)(q27;q23)	AML-M4, AML-M5	AF6, MLL	del(9)(p13)	ALL, NHL	
t(6;12)(q15;p13)	CLD		del(9)(p21)	ALL, AML, CLD, NHL	
t(6;14)(p25.3;q32)	MM	IRF4, IgH	del(9)(q11q13-q22)	AML, MDS	
t(7;11)(p15;p15)	AML, AML-M2	HOXA9, NUP98	del(10)(p13)	NHL, CLD	
t(8;13)(p11;q12)	MPD	FGFR1, ZNF198	del(10)(p12)	AML	
t(8;14)(q11;q32)	ALL		del(10)(q22)	NHL	
t(8;14)(q24;q32)	ALL-L3, BL, MM, NHL	c-MYC, IgH	del(10)(q24)	ALL, CLD, NHL	
t(8;14)(q24;q11)	T-ALL	c-MYC, TCRad	del(11)(p11)	CLD, NHL	
t(8;16)(p11;p13)	AML-M4, AML-M5		del(11)(q13q14-q23)	AML, CLD, CLL, MDS, NHL	
t(8;21)(q22;q22)	AML-M2, AML-M4, MDS	ETO, AML1	del(11)(q23)	AML, ALL, CLD, CLL, MDS, NHL	
t(8;22)(q24;q11)	ALL-L3, BL	c-MYC, Ig1	del(12)(p12)	AML, AML-M2, AML-M4, MDS, NHL	
t(9;11)(p22;q23)	ALL, AML-M5, MDS, t-MDS	AF9, MLL	del(12)(p11p12-p13)	AML, MDS	
dic(9;12)(p13;p11)	ALL	PAX5, IgH	del(13)(q12-q22)	AML, AMM, CLD, CLL, MM, MDS, NHL	RB1, D13S25
t(9;14)(p12.13;q32)	B-NHL, LPL	ABL, BCR	del(13)(q12-q14)	AML, AMM, CLD, MDS, NHL	
t(9;22)(q34;q11)	ALL, AML-M1, AML-M2, CML, MPD	AF10, MLL	del(14)(q24)	CLD, NHL	
t(10;11)(p13;q23)	AML-M4, AML-M5	HOX11, TCRad	del(15)(q21q22)	NHL	
t(10;14)(q24;q11)	T-ALL	Rhom2, TCRad	del(16)(q22)	AML, AML-M4Eo, NHL	
t(11;14)(p13;q11)	T-ALL, NHL	BCL1, IgH	del(17)(p11)	ALL, AML, CLD, MDS, NHL	P53
t(11;14)(q13;q32)	B-PLL, CLD, MM, MCL, MGUS, NHL	PLZF, RARa	del(17)(q23)	NHL	
t(11;17)(q23;q21)	AML-M3	MLL, AF17	del(18)(p11)	AML	
t(11;17)(q23;q21)	AML-M4, AML-M5	AP12, MLT	del(18)(q21)	AML, CLD, NHL	
t(11;18)(q21;q21)	MZL, NHL	MLL, EML	del(19)(p13)	NHL	
t(11;19)(q23;p13)	ALL, AML-M4, AML-M5, t-AML	TEL, AML1	del(20)(q11)	AML, MDS, MPD, PV	
t(12;21)(p13;q22)	ALL	IgH, c-MAF	del(20)(q11q13)	AML, CMD, MDS, PV	
t(14;16)(q32;q23)	MM	IgH, BCL2	del(22)(q11)	AML, ALL, CLD, MDS, NHL	
t(14;18)(q32;q21)	CLD, FL, DLCL, MM, NHL	IgH, BCL3			
t(14;19)(q32;q13)	CLD, CLL, NHL	IgH, BCR			
t(14;22)(q32;q11)	ALL	PML, RARa			
t(15;17)(q22;q21)	AML-M3, CML Ph+	MYH11, CBFb			
t(16;16)(p13;q22)	AML-M4Eo, MDS	MTG16, AML-M2			
t(16;21)(q24;q22)	AML-M2, AML-M4, Childhood AML	FUS, ERG			
t(16;21)(q24;q22)	AML-M1, AML-M2	HLF, E2A			
t(17;19)(q21-22;p13)	ALL	AFIP, MLL			
t(X;11)(q13;q23)	T-ALL				
der(Y)(Y:1)(q12;q21)	MDS				
Duplications			Numeric anomalies		
dup(1)(q12q32)	ALL, NHL		+3	ATL, MZL, SLVL	
dup(1)(q21q32)	ALL, CLD, NHL		+4	AML	
dup(1)(q25q44)	NHL		+6	AML, MDS	
dup(11)(q13q25)	NHL		+7	CLD, NHL	
dup(12)(q13q21)	NHL		+8	ALL, AML, CLD, MPD, MDS, PV	
			+9	AML, MDS, MPD, PV	
			+10	AML	
			+11	AML, MDS	
			+12	AML, CLL, CLD, NHL	
			+13	AML	
			+15	MM	
			+18	ALL, CLD	
			+19	AML, CLD	
			+21	AML, ALL, MDS, MPD	
			+22	AML	
			+X	NHL	
			-5	AML, MDS	
			-7	AML, MDS, MPD	
			-9	AML	
			-17	CLL	
			-18	CLD	
			-20	ALL	
			-21	AML	
			-Y	ALL, AML, CLD, MDS, MM, MPD, NHL, PV	
<p>ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia; AMM, agnogenic myeloid metaplasia; ALCL, anaplastic large cell lymphoma; ATL, adult T-cell lymphoma; BL, Burkitt's lymphoma; CLD, chronic lymphoproliferative disorder; CLL, chronic lymphocytic leukemia; CML Ph+, chronic myeloid leukemia with Philadelphia chromosome; CMML, chronic myelomonocytic leukemia; DLCL, diffuse large cell lymphoma; FL, follicular lymphoma; HSgdTL, hepatosplenic gamma delta T-cell lymphoma; LPL, lymphoplasmacytic lymphoma; MCL, mantle cell lymphoma; MDS, myelodysplastic syndrome; MM, multiple myeloma; MPD, myeloproliferative disorder; MGUS, monoclonal gammopathy of undetermined significance; MZL, marginal zone lymphoma; NHL, Non-Hodgkin's lymphoma; pre-B-ALL, precursor B-cell acute lymphocytic leukemia; PLL, polyclonal lymphocytic leukemia; PV, polycythemia vera; RARS, refractory anemia with ringed sideroblasts; SLVL, splenic lymphoma with villous lymphocytes; t-AML, therapy-related acute myeloid leukemia; t-MDS, therapy-related mye</p>					

methodologies improve or change. The effort to develop enhanced genetic testing methods should be part of a continuous quality improvement program. Moreover, the availability and experience with different genetic tests varies among institutions. Thus, different institutions may employ varying genetic testing strategies for hematological malignancies.

1.2. Conventional Cytogenetic Studies versus FISH Studies

Conventional chromosome studies are the standard method to study proliferating cells in hematological malignancies. Generally cytogenetic studies are based on analysis of up to 25 metaphases and can be used to detect most chromosome anomalies and determine the percentage of proliferating neoplastic cells. To accurately quantify proliferating disease, it is important to randomly select metaphases because bias can influence the percentage of normal and abnormal metaphases [7]. Conventional chromosome studies are particularly valuable when a specific hematological diagnosis or classification is not apparent by morphologic criteria. Cytogenetics studies are important screening methods to detect chromosome anomalies, but the technique is limited by the need for mitotic neoplastic cells and require 2 to 3 days to perform.

FISH is rapidly becoming part of clinical practice in the work up of patients with hematological malignancies. Probes for FISH are classified by where they hybridize. Some hybridize to specific centromeres [8,9] and others to specific gene loci [2,10-12]. Some probes hybridize to unique DNA sequences over the length of each chromosome producing a paint-like pattern for each chromosome [13]. Still, other probes hybridize to chromosome specific telomere regions [14]. DNA probes can be used individually or in combinations and can be labeled with different colored fluorors.

The advantages of FISH are significant. Many DNA probes can be purchased, but "home brew" probes are easy to make. Commercial probes are usually preferred to avoid the rigorous quality control needed with making "home brew" probes. FISH can be used to study both non-proliferating and proliferating cells. Most FISH tests cost approximately half of a complete chromosome study. FISH can be performed overnight and so is particularly useful in urgent medical conditions. Using FISH, it is easy to analyze large numbers of cells and the results are reproducible.

FISH can be performed on various types of specimens. Fixed cell pellets left over from cytogenetic studies on bone marrow or blood are very suitable for FISH [2,10,15]. FISH also works well with bone marrow or peripheral blood smears, touch preparations and paraffin-embedded tissues [6,16,17]. FISH is particularly useful to detect genetic anomalies when results of cytogenetic studies are inconclusive. For example, in the absence of metaphases, or when the results are normal because of cryptic or masked translocations.

A good FISH strategy to detect aneuploidy and dele-

tions uses a probe of one color for the centromere and a probe of another color for an interstitial site on the same chromosome [1]. For purposes of this paper, this method is referred to as ND-FISH to indicate detection of numeric and/or deletion chromosome anomalies. The most common colors of fluorors are red and green. This FISH strategy produces two red and two green signals in interphase cells. False positive signal patterns occur when signals overlap, any probe fails to hybridize, or when cross-hybridization happens. It is important to establish the normal range and gain experience with FISH in untreated patients before using this method in clinical practice. This method can detect neoplastic clones in which >3% cells are trisomy, >5% cells are monosomy or >7% cells have deletions.

Several FISH strategies can detect fusion of loci associated with chromosome translocations and inversions. S-FISH produces a single fusion signal [18]. ES-FISH produces a fusion signal and an extra signal. D-FISH produces double or two fusion signals [2]. BAP-FISH shows two fused signals in a normal cell, but one separates or breaks apart in cells with a translocation or inversion [6]. The sensitivity of these methods varies significantly. For example, in 500 nuclei >1% neoplastic cells can be detected by D-FISH, 3% by ES-FISH, 3% by BAP-FISH and 10% by S-FISH.¹ The sensitivity of B-FISH studies can be increased to 0.079% by analyzing 6000 nuclei [15].

In BAP-FISH the probes hybridize to a specific region on only one chromosome. The probes are so designed that a red signal occurs on one side of the breakpoint and a green signal on the other side. In a fluorescent microscope, red and green signals merge to form a yellow fusion signal. The reciprocal translocation causes the fusion signal to "break apart" and form a red signal on one chromosome and a green signal on the other chromosome. In interphase, one red, one green, and one yellow signal are observed.

D-FISH uses red and green probes that hybridize to different loci [2]. The hybridization sites span the breakpoint on each chromosome. The reciprocal translocation produces adjacent red and green hybridization sites on each abnormal chromosome. Thus, in interphase one red, one green, and two yellow signals are observed.

ES-FISH uses red and green probes that hybridize to different loci. The hybridization site spans the breakpoint on one of the chromosomes. The hybridization site of the other chromosome is relegated to one side of the breakpoint. The reciprocal translocation produces a yellow fusion signal on one abnormal chromosome, and a small residual red signal on the other abnormal chromosome. In interphase two red, one green, and one yellow signal is observed.

S-FISH uses red and green probes that hybridize to different loci [18]. The green hybridization site occurs on one side of the breakpoint on one chromosome, and the red hybridization site occurs on the other side of the breakpoint on the other chromosome. The reciprocal translocation produces a yellow fusion signal on one of the abnormal chromosomes. In interphase one

red, one green, and one yellow signal is observed.

Multiple probes can be applied to any specimen as a "panel FISH test." For example, a set of probes for chromosomes 5, 7, 8, 11, 13 and 20 allows detection of common anomalies in myelodysplasia [14].

For metaphase, whole chromosome paints can be used to identify most major structural anomalies [19]. However, this method allows analysis of only a few chromosomes at a time and is expensive to perform. A variant of whole chromosome paints is called "multi-color FISH" because it paints each of the 24 sets of human chromosomes a different color. This method is particularly useful to accurately characterize chromosome anomalies in metaphases with complex karyotypes [13].

1.3. Myelodysplasia

The myelodysplastic syndromes (MDS) are a clinically heterogeneous group of hematological disorders [5]. Cytogenetic studies are important in the work up of patients with MDS as this method can provide both diagnostic and prognostic information [20]. A chromosomally abnormal clone can be detected in approximately 60% of patients with aggressive RAEB or RAEB-1 [21,22]. In contrast, a chromosomally abnormal clone can be detected in approximately 35% of patients with RA and RARS. The most common chromosome anomalies in MDS involve chromosomes 5, 7, 8, 11, 13, 17, 20, 21 and X [21,22]. Patients with MDS that have a complex karyotype (defined as ≥ 3 anomalies) or anomalies of chromosome 7 generally have a poor prognosis [20]. Patients with 5q- alone or 20q- alone generally have a good prognosis. 20 Patients with -Y alone or a normal karyotype generally have an intermediate prognosis [20].

A reasonable panel FISH test for MDS would utilize probes to detect numeric and/or structural anomalies of chromosome 5, 7, 8, 11, 13 and 20 (Table 1). These anomalies occur in approximately 85% of patients with MDS. We used both conventional cytogenetic and interphase-FISH studies for 42 patients enrolled in two ECOG MDS protocols [23]. For each patient, the percentage of neoplastic nuclei in 200 consecutive cells for each probe was established. Chromosome studies were done on the same specimens by analyzing up to 25 metaphases. FISH studies were done on 34 fixed cell pellets and 8 bone marrow aspirate smears that were available from morphology studies. The patients included 5 that were enrolled in a high risk MDS protocol (E3996) and 37 enrolled in a low risk MDS protocol (E1996).

Among these 42 patients, results of conventional cytogenetics were abnormal in 14 (33.3%) and normal in 28 (66.7%). By comparison, results of interphase-FISH studies on 41 of these same specimens were abnormal in 11 (26.8%), normal in 30 (73.2%), and failed in 1 (2.4%). The results of conventional cytogenetics were abnormal in 1/5 patients with RA, 3/16 with RARS, 8/15 with RAEB, 2/4 with RAEB-T, and 0/1 with CMMoL. By comparison, interphase-FISH detected

≥ 1 anomalies of chromosome 5, 7, 8, 11, 13 or 20 in 2, 3, 4, 0, 0 and 4 patients respectively. Chromosome anomalies were detected in metaphases of three patients that were not identified by interphase-FISH. In two of these cases the FISH strategy was not designed to detect the chromosome anomalies. In the other case, at (11;17)(q23;q21) was detected by cytogenetic studies, but the hybridization process for MLL failed on three attempts. Each of the 28 patients with only normal metaphases also had only normal interphase cells. The FISH method failed for all probes in 1/34 fixed cell specimens, for chromosome 11 in 1/8 smears, and for chromosome 20 in 3/8 smears.

Results of this study dispel the argument that patients with MDS often have chromosome anomalies that are missed by conventional chromosome studies due to sampling errors. The results indicate that approximately 70% of patients with low risk MDS do not have chromosome anomalies that are detectable by either conventional chromosome studies or panel FISH testing. The results suggest that the sensitivity of interphase-FISH studies for MDS is nearly as good as conventional cytogenetic studies. Thus, interphase-FISH may be useful in clinical practice to study bone marrow specimens when conventional chromosome studies are not successful or when only smears are available.

Recently, we studied 32 patients with primary MDS and a normal karyotype by conventional chromosome studies to determine if interphase-FISH testing and/or whole chromosome paint (M-FISH on metaphase cells) testing could detect chromosome anomalies that were not apparent by conventional cytogenetic studies [24]. We found that one patient had a chromosome 13q-arm deletion, while the remaining 31 patients had normal results. A retrospective metaphase-FISH study of the patient with a 13q-arm deletion in interphase nuclei, confirmed the presence of a subtle interstitial deletion in the q-arm of a chromosome 13 in metaphases. These findings confirm that standard cytogenetics is an excellent technique to identify the common chromosomal anomalies associated with MDS and suggest limited utility for either a FISH panel test or M-FISH in primary MDS. Moreover, these results indicate that many patients with MDS have a normal karyotype. We now suspect that patients with MDS and a normal karyotype owe their disease to submicroscopic gene mutations or other biological cause.

Some reports suggest that interphase FISH may be useful to detect very low levels of t-MDS in bone marrow prior to bone marrow transplantation even when results of conventional chromosome studies are normal [25,26]. In an attempt to confirm these observations, we used interphase-FISH to study bone marrow from seven patients that underwent bone marrow transplant for lymphoma and then subsequently developed t-MDS. Our study indicated that when cytogenetics detected an anomaly in t-MDS, so did FISH. The results of both FISH and cytogenetics were normal in bone marrow just prior to the bone marrow transplantation. We concluded that interphase-FISH does not detect t-MDS prior to trans-

plantation when results of cytogenetic studies are normal. The outcome of this study is also consistent with our efforts to compare conventional cytogenetics and FISH for primary MDS [23,24].

The FISH strategy and normal cutoffs of previous publications that deal with FISH detection of t-MDS differed from our investigation. We used state-of-the-art dual colored probes for detection of anomalies of chromosomes 5 and/or 7, and non-parametric statistical methods based on 20 normal individuals to compute the normal cutoff for false-positive nuclei. In contrast, previous studies used unusually low normal cutoff values and/or single probe strategies which may have led the investigators to confuse false-positive nuclei with evidence for low levels of t-MDS or t-AML. In any case, the discrepancy between the few investigations suggests that a good deal more investigation of the efficacy for FISH to detect t-MDS in pre-bone marrow transplant specimens would be valuable.

1.4. Acute Myeloid Leukemia

Some investigators recognize four broad cytogenetic risk categories of AML: favorable, intermediate, poor, and unknown (Table 3). At least two clinical trials have demonstrated the clinical utility of cytogenetic risk categories to manage patients with untreated AML [27,28]. Consequently, it is important to perform appropriate cytogenetic and FISH studies to establish the correct cytogenetic risk category.

The favorable group includes patients with *de novo* AML that have translocations, such as t(8;21)(q22;q22) or inv(16)(p13q22) which involve one of the subunits of the core binding factor (alpha or beta) or translocations such as t(15;17)(q22;q21) which involve the retinoic acid receptor alpha locus. These patients are generally younger and have a good response to chemotherapy and a favorable duration of remission. For patients in the intermediate cytogenetic risk group, the natural history of the disease is less clear, but is generally associated with an intermediate prognosis. With further technological advances, it is likely that many of the patients with normal cytogenetics will turn out to have recurring molecular and/or chromosomal anomalies that will allow their disease to be better characterized. Already, a subset of these patients have been identified with a tandem

duplication of the long arm of chromosome 11, a finding not generally detected by routine cytogenetic analysis [29].

Certain chromosome anomalies are associated with MDS or AML arising from chemotherapy or radiotherapy, environmental or occupational exposure such as pesticides containing arsenic, organic solvents, petroleum, or benzene. These hematological malignancies in elderly individuals have similar morphologic and cytogenetic characteristics as secondary AML and MDS. The most common clonal chromosome anomalies involve chromosomes 5, 7, 8, 11, 13 and 17. The karyotype of the neoplastic clone is usually complex (≥ 3 chromosome anomalies). These patients have a similar poor response to chemotherapy as t-AML or AML arising from MDS.

Many chromosome anomalies associated with AML cannot be detected by current FISH or molecular genetic methods. For patients with these anomalies, conventional chromosome studies are the sole method of their detection. Some chromosome anomalies that are associated with AML can be detected by cytogenetics and FISH. In these cases, the genetic test of choice should be selected according to cost, turnaround time, and clinical situation, i.e. at the time of diagnosis versus quantitation of residual disease following therapy. Since all genetic tests are relatively expensive and genetic laboratories are busy, it is not reasonable to routinely perform all available genetic tests.

At diagnosis of AML, quantitative cytogenetic studies are useful to establish the presence and kind of chromosome anomalies as well as the percentage of mitotic neoplastic cells. If a FISH test is available to detect the primary chromosome anomaly, this method can be performed on bone marrow and blood at diagnosis as well to establish a benchmark for percentage of neoplastic cells. After treatment, quantitative cytogenetic studies and/or FISH can be used to establish the percentage of neoplastic cells and help assess the effectiveness of therapy. If the percentage of neoplastic cells by FISH is less than the sensitivity of the test, then more sensitive procedures such as RT-PCR should be employed. For AML, this approach is useful for t(8;21), 1(9;22), 1(11;val), inv(16), -515q-, -7/7q- and some others (Table 1).

For some hematological malignancies, the primary chromosome anomaly is difficult to detect; e.g., the 15;17 translocation associated with AML-M3. If AML-M3 is strongly suspected, the initial genetic study could be FISH or RT-PCR to detect PML/RAR α fusion. An ES-FISH test is available to detect cells with PML/RAR α when these cells exceed 3% of all cells. Most untreated patients with AML-M3 have 50 to 91% cells with PML/RAR α fusion in their bone marrow. Rare patients with AML-M3 have a translocation other than 15;17 (Table 2). This FISH test can usually detect these variants as the hybridization site for RAR α breaks apart, but no PML/RAR α fusion is observed. This FISH test is particularly valuable because it can be done overnight and applied to cells prepared for standard cytogenetic studies. If the results of FISH are normal, then conventional cytogenetic studies can be performed to assess

Table 3.

Summary of Cytogenetic Risk Categories for AML [28].

<u>Favorable:</u>	inv(16)/t(16;16)/del(16q)w/any additional anomalies, t(8;21) w/o del(9q) and w/o a complexity ≥ 3 , or t(15;17) w/ any additional anomalies
<u>Intermediate:</u>	+8, -Y, +6, del(12p), normal
<u>Unfavorable:</u>	-5/del(5q), -7/del(7q), inv(3q)/t(3;3), t(6;9), del(9q), t(9;22), abnormal 11q, 20q, 21q or 17p, or complex karyotype defined as ≥ 3 anomalies
<u>Unknown:</u>	All other anomalies

the entire karyotype of the abnormal clone to look for other chromosome anomalies. If AML-M3 is not initially suspected, cytogenetic studies do not reveal a 15; 17 translocation and morphology studies strongly suggest AML-M3, then FISH or RT-PCR studies for PML/RAR α should be performed. In these cases, FISH for PML/RAR α can be done on left-over cells from cytogenetic studies or unstained blood or bone marrow aspirate smears.

Cytogenetic studies are valuable to assess the effectiveness of therapy. For AML, patients have predominantly cells with normal chromosomes in remission. When the disease of these patients relapse, the cells with the original chromosome anomalies are usually observed. The sensitivity of conventional cytogenetic studies is sometimes underestimated. Because cytogenetic studies focus on mitotic cells, they sometimes detect 1 or 2 abnormal metaphases even when other genetic tests are normal. Moreover, cytogenetic studies can detect chromosome anomalies associated with t-AML. Nevertheless, if a good FISH or RT-PCR test is available to quantify disease, these are the genetic tests of choice to monitor remission and predict relapse because they are less expensive and more sensitive. If t-AML is suspected, it is also useful to perform a cytogenetic study.

Because the tumor burden following bone marrow transplantation is very small or absent, the best genetic method to monitor disease associated with AML may be RT-PCR. This method has proven particularly useful to detect PML/RAR α fusion in the post bone marrow transplantation period and is useful to detect relapse of disease. FISH can also be used to monitor the tumor burden after bone marrow transplantation. Various FISH strategies are available to monitor patients after bone marrow transplantation. Probes for the X and Y chromosome can be used for opposite-sex bone marrow transplantation as XY and XX nuclei are easy to detect by the red and green signals [9]. This method can detect less than 0.3% XY cells in females and less than 0.6% XX cells in males. This method defines the ratio of host versus donor cells, but does not directly establish the percentage of neoplastic cells. If an appropriate FISH test is available for any given patient with a hematological malignancy, it can be used to establish the percentage of neoplastic cells. Depending on the FISH test, these methods can detect disease when it exceeds 1 to 3% of all cells.

FISH and RT-PCR both require prior knowledge of the primary genetic anomaly for any given patient and do not detect other anomalies. To overcome this weakness some investigators have begun to use panel FISH tests or multiplexed RT-PCR for hematological disorders. For AML, a panel FISH test to detect t(9;22), 1(8;21), 1(15;17), t(11;var), inv(16) and common anomalies of chromosomes 5 and 7 may be useful (Table 1). Given the karyotype diversity of AML, these methods are not generally a substitute for cytogenetic studies at diagnosis. Nevertheless, these methods hold promise as evidence is emerging to suggest that multiplex RT-PCR and panel FISH testing may detect neoplastic clones in

a few patients with AML when results of cytogenetic studies are normal, not possible, or too complex to reveal a classical chromosome anomaly.

1.5. Chronic Lymphocytic Leukemia

The prognosis and clinical course of patients with B-CLL are highly variable. This makes the care of patients with B-CLL very challenging. Studies with conventional cytogenetics, FISH, molecular genetics, immunophenotyping, and mutational analysis of the immunoglobulin heavy chain variable regions (IgV_H) have all been used to study B-CLL. Each of these approaches has helped to assess prognosis in this disease, but the oncogenic events that lead to the origin and progression of B-CLL are unknown [3,4,30-33].

The most common chromosome anomalies in B-CLL involve chromosomes 6, 11, 12, 13 and 17. These anomalies have been associated with differing prognoses and lengths of survival [3,30,34-36]. Using various B-cell mitogens, conventional cytogenetic studies detect abnormal clones in many patients with progressive B-CLL [30,37]. Conventional cytogenetic studies have been much less successful for early and indolent B-CLL even when mitogens have been used to stimulate neoplastic B-cells to divide [38].

New FISH methods permit detection of anomalies involving chromosomes 6, 11, 12, 13, 14 and 17 in neoplastic non-dividing cells (interphase nuclei) in patients with B-CLL [4,39,40]. These FISH-detected chromosome anomalies have also been associated with different clinical outcomes [4]. Recent reports indicate that IgV_H gene mutational status and CD38 expression levels also predict disease course and/or response to therapy. The studies of mutations in the variable regions of Ig genes indicate that approximately 50% of patients with B-CLL have mutated IgV_H genes (somatic mutation-type clones) [32] and these patients have a better prognosis than patients with no mutation of IgV_H genes (germline-type clones) [33]. Several other investigators have also shown that expression of surface membrane CD38 in >30% of B-CLL cells may be an important indicator of a poor prognosis in B-CLL [41-44].

We compared FISH-detected chromosome anomalies with known prognostic features of B-CLL. To accomplish this work, we selected a set of fluorescent-labeled DNA probes to detect anomalies of chromosomes 6, 11, 12, 13, 14 and 17 in B-CLL (Table 1) [39]. We then used these FISH probes to study two groups of patients: those with stable B-CLL who did not require treatment and those with progressive B-CLL who did require treatment. FISH results were compared with Rai stage, stable versus progressive disease, percent abnormal nuclei in blood and bone marrow, conventional cytogenetic studies, CD5⁺/CD19⁺ cells, CD38 expression and IgV_H mutational status.

The results indicated that chromosome anomalies were frequent in B-CLL even in Rai stages 0-1, but were more frequent among patients with progressive disease than patients with stable disease. In addition, chromo-

some anomalies were more frequently detected by interphase FISH analyses than by conventional cytogenetic studies, and the percentage of abnormal nuclei by FISH was similar in bone marrow and blood. Although chromosome anomalies did not correlate strongly with either germline or somatic IgV_H mutation-type clones, 11q-, +12 and 17p- appeared to be associated with CD38 expression in >30% of B-cells whereas 13q- alone was found only in CD38⁻ B-CLL clones.

In our experience, FISH is also useful to distinguish patients with B-CLL from patients with the leukemic phase of mantle cell lymphoma who have an 11, 14 translocation by testing for CCND1/IgH fusion [45]. The results of FISH also provide important prognostic information. In one investigation of 325 patients studied by FISH the median survival for patients with 17p deletion, 11q deletion, trisomy 12, normal or 13q deletion was 32, 79, 114, 111 and 133 months, respectively, and the treatment-free interval was 9, 13, 33, 49 and 92 months, respectively [4]. Similar information can be obtained by conventional cytogenetics, but in a study of patients with indolent B-CLL we 13 detected chromosome anomalies by interphase FISH in 73% of specimens compared with only 7% by conventional cytogenetic studies.

We believe that interphase FISH studies provide important cytogenetic information for evaluation of patients with newly diagnosed B-CLL and should be used in lieu of conventional chromosome studies. Panel FISH testing is informative for many patients with B-CLL and is cost effective. One particularly important clinical outcome of our studies and others is that regardless of disease status (stable or progressive), patients with 11q-, +12 and/or 17p- and a high CD38 expression level may require more urgent counseling to manage their disease. We recognize that further studies involving larger cohorts of patients with B-CLL are necessary to consolidate the correlations between interphase FISH studies and key biological features. Nevertheless, we believe the results of FISH studies taken together with other newer biological features of B-CLL clones that are under study, such as CD38 expression, IgV_H mutation status and gene profiling, will help identify sources of heterogeneity among patients with B-CLL.

1.6. Lymphoma

The World Health Organization recognizes that genetic anomalies are one of the most reliable criteria for classification of malignant lymphomas [46]. New genetic methods using FISH are valuable for detection of genetic anomalies [47]. The application of FISH methods to lymphomas has been hindered by the wide use of paraffin-embedded tissue to study and store lymphoid tissue. FISH studies of paraffin-embedded tissue sections are difficult because the paraffin and standard fixatives interfere with hybridization of DNA probes to target loci. In addition, the high cellularity and truncated cells in standard thin-tissue sections interferes with accurate scoring of individual nuclei.

Exciting new techniques are emerging to isolate individual nuclei from paraffin-embedded tissue by using xylene, proteinase K, citric acid and pepsin [6,12,45,48]. Recently, we experimented with a new method to extract individual nuclei from paraffin-embedded tissue using tissue core samples. This method requires minute quantities of tissue, two hours to extract nuclei and consistently produces successful FISH studies. We tested the efficacy of this method by studying 6 normal lymph nodes or tonsils and 32 malignant lymphomas including 5 mantle cell, 5 follicular, 5 Burkitt, 5 extranodal marginal zone lymphomas of mucosa associated lymphoid tissue, 5 anaplastic large cell, and 7 diffuse large B-cell. FISH studies were successful for each of the 38 specimens. The expected chromosome anomalies were detected in each malignant specimen, and the results for normal lymphoid tissue were normal.

This study demonstrated the efficacy of extracting nuclei from paraffin-embedded lymphoid tissues to perform successful interphase FISH studies. This method worked on a variety of lymphomas including mantle cell, follicular, Burkitt, MALT, anaplastic large cell and diffuse large B-cell. The results for BCL6, BCL2, c-MYC, CCND1, ALK, IgH, MALT1 and API2 were consistent with the type of lymphoma investigated (Table 1). Each of the mantle cell, follicular, MALT and anaplastic large cell lymphomas, and two Burkitt lymphoma specimens were known to have anomalies by previous genetic and FISH studies [12,45,49]. For these cases, the sensitivity of the method was 100%. Since each of the six normal tissues studied had a normal FISH pattern with each probe indicates this method has a high specificity as well. In addition, a variant FISH pattern associated with c-MYC breakapart was detected in one patient with Burkitt lymphoma due to a t(2:8)(p12;q24) and was confirmed by cytogenetic studies. Thus, FISH has the potential to detect variant chromosome anomalies associated with malignant lymphomas.

We believe that the application of this method to extract individual nuclei from paraffin-embedded specimens and then studying specimens with appropriate FISH probes can detect one or more chromosome anomalies with high specificity and high sensitivity in many lymphomas. This method should be helpful to hematopathologists to diagnose and classify lymphomas.

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