

# Application of Fluorescence In Situ Hybridization in Hematological Disorders

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**ABSTRACT:** In the present study, chromosome changes in bone marrow (BM) or peripheral blood (PB) cells from 13 patients with malignant hematologic disorders were analyzed by classical cytogenetic techniques (G-banding) and fluorescence in situ hybridization (FISH) procedures using centromere specific probes for chromosomes 1, 6, 7, 8, 9, 12, 18, 13/21, and X, and a DNA probe specific for the long arm of chromosome Y. The cytogenetic data obtained with G-banding were in accord with those obtained by FISH to metaphase chromosomes. Most significantly, FISH to interphase nuclei offered reliable results and in some cases provided important information concerning crucial chromosome anomalies which were not or could not be completely detected by analyzing metaphase chromosomes.

Our results indicate that FISH could be clinically valuable in five major areas: 1) marker chromosome identification; 2) identification of trisomy consistent with certain specific hematological neoplasms; 3) clonal evaluation post observation of a single cell with trisomy; 4) clonal evaluation post-sex-mismatched bone marrow transplantation (BMT); and 5) residual disease detection following clinical remission.

## INTRODUCTION

To date, a number of chromosome changes have been reported as acquired anomalies in hematologic neoplasms correlating with specific clinical, morphologic, and immunophenotypic features [1, 2]. The recognition that cytogenetic analysis can play an important role in the clinical diagnosis, prognosis prediction, and management of patients with malignant hematologic disorders has been well established [1, 2]. However, conventional cytogenetic studies sometimes cannot provide complete information concerning the interpretation of the chromosome banding patterns due to insufficient metaphases and/or poor chromosome morphology. In addition, standard cytogenetic analyses are performed on dividing cells, thus excluding from analysis the majority of cells that remain in interphase, cells which are terminally differentiated (e.g., segmented neutrophils) or have a low mitotic rate (e.g., chronic lymphocytic leukemia cells).

Recently developed molecular hybridization techniques, i.e., fluorescence in situ hybridization (FISH) with the use of nonradioactive chromosome-specific DNA probes, have permitted the cytogenetic analysis of nondividing

cells as well as metaphase cells [3, 4] and are generally referred to as "interphase cytogenetics". Three of the important characteristics of FISH are: 1) the ability to provide a rapid analysis based on nondividing cells without the requirement of *in vitro* culture; 2) the ability to evaluate interphase nuclei of terminally differentiated cells; and 3) the ability to screen large numbers of cells (e.g., 1000) within a very short period of time. Thus, FISH greatly expands the capabilities of clinical cytogenetic laboratory in the study of malignant hematologic disorders.

In order to characterize the cogent application of interphase cytogenetic studies to clinical hematology, we analyzed bone marrow (BM) or peripheral blood (PB) specimens from 13 patients with malignant hematological disorders by FISH procedures using chromosome-specific DNA probes. Our results suggest that FISH may be a very useful adjunct to conventional cytogenetics for clinical purposes.

## MATERIALS AND METHODS

BM or PB samples from 13 patients with malignant hematologic disorders were included in the present study. The diseases of these patients were determined according to FAB criteria, based on BM cell characteristics of morphology, cytochemical staining, and immunophenotype [1]. Cell preparations for FISH were made with freshly obtained samples.

## Cytogenetic Analysis

In 11 cases, the chromosome studies were performed on BM cells processed from a 24-hour nonstimulated culture

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**Table 1** Chromosome-specific DNA probes used in this study

Chromosome	Probe	HGM Designation	Source	Reference
1	pSD21-1	D1Z5	Oncor, Inc. (Gaithersburg, MD)	
6	308	D6Z1	Jabs, E (The Johns Hopkins Hospital)	[6]
7	p7tet1	D7Z1	Oncor, Inc.	
8	pJM128	D8Z1	Donlon, T (Stanford University Medical Center)	[7]
9	pHuR98	D9Z?	Moyzis, R (Los Alamos National Laboratory)	[8]
12	NA	D12Z3	Oncor, Inc.	
18	L1.84	D18Z1	Devilee, P (Rijksuniversiteit)	[9]
13/21	NA	D21Z1/D13Z	Oncor, Inc.	
X	pBamX7	DXZ1	Oncor, Inc.	
Yq	pY3.4	NA	Lau, Y	[10]

Abbreviation: NA, not available.

in RPMI 1640 medium with 20% FCS, penicillin:streptomycin (50 U/ml:50 mg/ml), and 2 mM L-glutamine, and a 24-hour synchronized culture using a methotrexate block and thymidine release [1]. In one case (Case 2) PB cells were incubated for 24 hours without phytohemagglutinin (pHA) for chromosome study. In Case 11, BM or PB cells were not available for chromosome analysis. Chromosomes were characterized by a trypsin G-banding method [1]. Karyotypes were described according to the ISCN nomenclature [5].

#### Probes and In Situ Hybridization

The alpha-satellite DNA probes specific for the centromeres of chromosomes 1, 6, 7, 8, 9, 12, 18, 13/21, and X, and a probe specific for the long arm of chromosome Y (Yq) were used in this study (as summarized in Table 1). Some probes were commercially available in biotin-labeled form from Oncor (Gaithersburg, MD). Other probes were kindly provided by those persons listed in Table 1. Unlabeled probes were first nicked using DNase, then labeled with biotin-11-dUTP (Enzo Diagnostics, New York) by the random primer method of Feinberg and Vogelstein [11]. The hybridization protocol followed that of Pinkel et al. [3] with modifications. Slides were denatured in 70% formamide, 2 × SSC (standard saline citrate), pH 7.0 at 70°C for 2 minutes, then dehydrated in a cold ethanol series and air dried. The hybridization mixture (55% formamide, 10% dextran sulfate, 50 µg/ml carrier DNA, and 80 ng/ml probe DNA) was denatured for 5 minutes at 70°C and applied to prewarmed (37°C) slides under a coverslip at a volume of 0.02 µl per cm<sup>2</sup>. Three 2-minute post-hybridization washes at 46°C or 48°C in 50% formamide, 2 × SSC, pH 7.0, were followed by successive washes at room temperature in 2 × SSC, pH 7.0, and PN buffer (0.1 M NaPO<sub>4</sub>, pH 8.0, 0.1% NP-40). The probe was detected by fluorescein-avidin DCS (Vector Laboratories, Burlingame, CA) [5 µg/ml in PNM buffer (PN buffer with 5% nonfat dry milk, 0.2% NaN<sub>3</sub>)] and the signal was amplified by using biotinylated goat antiavidin (Vec-

tor) (5 µg/ml in PNM buffer) followed by another layer of fluorescein-avidin. Coverslips were mounted with an anti-fade solution [12] containing propidium iodide (1 µg/ml) as a counterstain. Slides were viewed through a Nikon fluorescent microscope equipped with a fluorescein filter package and photographed using Ektachrome 400 film (Eastman Kodak, Rochester, NY).

The standard FISH protocol was also modified for use with dual-color fluorescence of centromere-specific probes [13]. One probe was labeled with biotin-11-dUTP, and the other with digoxigenin-11-dUTP (Boehringer Mannheim Biochemicals). Hybridization and washing were performed as described above. The biotin-labeled probe was detected with Texas Red avidin DCS (Vector) (5 µg/ml in PNM buffer) and the signal was amplified by using biotinylated goat antiavidin followed by another layer of Texas Red avidin to give a red signal. The digoxigenin-labeled probe was detected with anti-digoxigenin-fluorescein (Boehringer Mannheim Biochemicals) [20 µg/ml in diluting buffer (PBS with 0.5% bovine serum albumin, pH 7.4)] to give a green signal. The 4,6-diamino-2-phenyl-indole (DAPI, 1 µg/ml) was used as a counterstain which stains the nuclei blue. By using a double band-pass filter set (Omega Optical), the Texas Red and fluorescein isothiocyanate (FITC) were viewed microscopically at the same time. Photographs required a double exposure.

#### Evaluation of FISH results

Evaluation of the preparations was performed by counting 500 to 1000 nuclei/probe. Means and standard deviations (SD) of the percentages of nuclei with 0, 1, 2, 3, and 4 hybridization signals were calculated for the control specimens from human PB and BM of hematologic disease-free individuals who were known by cytogenetic analysis to be normal (Table 2). A decision of monosomy or trisomy for a particular chromosome was based on the percentage of cells with one or three signals, respectively, being greater than the mean +2 × SD of the controls [14].



**Table 2** FISH data of BM and PB samples from female and male controls<sup>a</sup> (n = 6)

Probe for Chromosome	Number of Signals per Nucleus					
	0	1	2	3	4	5
1	0.1 ± 0.4	9.1 ± 2.1	87.8 ± 2.6	2.7 ± 0.6	0	0
6	0.1 ± 0.1	6.4 ± 1.1	92.1 ± 1.5	1.2 ± 0.2	0.2 ± 0.3	0
7	0	5.2 ± 0.7	93.9 ± 0.5	0.8 ± 0.2	0	0
8	0	2.9 ± 0.3	96.7 ± 0.5	0.4 ± 0.3	0	0
9	1.0 ± 0.5	17.4 ± 0.5	81.1 ± 1.1	0.5 ± 0.1	0	0
12	0.2 ± 0.2	7.1 ± 1.4	90.4 ± 0.9	1.9 ± 0.1	0.2 ± 0.4	0
18	0.1 ± 0.2	8.4 ± 0.5	91.2 ± 0.6	0.3 ± 0.1	0	0
13/21	0	1.9 ± 0.1	7.8 ± 1.5	16.7 ± 2.9	71.2 ± 0.9	2.7 ± 0.4
X (female) <sup>b</sup>	0	2.4 ± 0.4	97.1 ± 1.1	1.3 ± 0.6	0	0
X (male) <sup>c</sup>	1.8 ± 0.6	96.5 ± 0.7	1.7 ± 0.2		0	0
Yq <sup>c</sup>	0.1 ± 0.1	98.0 ± 0.9	1.9 ± 0.8		0	0

<sup>a</sup> Included are three female controls providing one BM and two PB samples, and three male controls providing one BM and two PB samples.

<sup>b</sup> FISH data from the three female controls (n = 3).

<sup>c</sup> FISH data from the three male controls (n = 3).

## RESULTS

The clinical data, cytogenetic analyses, and FISH results on the 13 patients are listed in Table 3. A comparison of GTG-banded chromosome findings with the FISH results is described in detail for all the 13 patients as follows:

### Case 1

Cytogenetic analysis of BM cells from this patient with AML revealed a karyotype of 46,XX,-7,+mar in all 20 cells studied. FISH on interphase nuclei with the use of

centromeric probe for chromosome 7 indicated that the marker chromosome was not of chromosome 7 origin (99% one spot). In addition, in each of two metaphases examined, only one chromosome showed bright chromosome-7-specific centromeric fluorescence, also supporting the interphase result by FISH.

### Case 2

Chromosomal study performed on PB cells from this patient with "CML" revealed a karyotype of 47,XY,+mar in all 20 cells analyzed. Cytogenetically, the marker chromo-

**Table 3** Clinical data, cytogenetic data, and FISH results on interphase nuclei of BM or PB from 13 patients

Case	Sex/Age (yr)	Diagnosis	Clinical Stage	Samples	Karyotype	FISH Results
1	F/48	AML	D	BM	46,XX,-7,+mar [20]	Monosomy 7 (99%)
2	M/42	CML	D	PB	47,XY,+mar[?der(9)] [20]	Three chromosome 9 centromeres (76%)
3	F/59	AML	D	BM	46,XX [2]/46,XX,-7,t(9;22)(q34;q11.2),+mar [18]	Two chromosome 7 centromeres (90%)
4	F/63	MDS	D	BM	46,XX [19]/47,XX,+mar (76 or 78) [1]	Trisomy 8 (5.2%)
5	M/65	CLL	D	BM	46,XY [13]	Trisomy 12 (16.6%)
6	M/80	MDS	D	BM	46,XY [20]	Trisomy 8 (2.6%)
7	M/86	Anemia	D	BM	46,XY [31]/45,X,-Y [4]/46,XY,+8 [1]	Trisomy 8 (2.0%)
8	F/42	MDS	D	BM	46,XX [19]/47,XX,+18 [1]	Trisomy 18 (2.4%)
9	M/35	CLL	D	BM	46,XY [19]/47,XY,+12 [1]	Trisomy 12 (31.8%)
10	M/68	AML	D	BM	46,XY [19]/47,XY,+21 [1]	Trisomy 21 (4.8%)
11	M/3	SCID	P-BMT	PB	ND	Male cells (about 85%); female cells (about 15%)
12	F/5	MDS	B-BMT P-BMT	BM BM	45,XX,-7 [20] with two chromosome 7's (partially analyzed) [4]	ND Male cells (more than 90%); female cells (less than 10%); all female cells with two chromosomes 7
13	F/21	ALL	D R	BM BM	46,XX [2]/47,XX,14q+,+21 [5] 46,XX, [20]	ND Trisomy 21 (7%)

Abbreviations: F, female; M, male; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; MDS, myelodysplastic syndrome; CLL, chronic lymphocytic leukemia; SCID, severe congenital immunodeficiency; ALL, acute lymphocytic leukemia; D, diagnosis; R, remission; P-BMT, post bone marrow transplantation; B-BMT, before bone marrow transplantation; BM, bone marrow; PB, peripheral blood; ND, not done.

some seemed to be a der(9)t(1;9) (q11;q13). FISH studies validated the der(9) identification with the use of a probe for chromosome 9 centromere (76% three spots). No evidence was obtained suggesting that the chromosome 1 centromere was involved in the der(9) with the use of a chromosome 1 centromere-specific probe (1.4% three spots < mean  $+2 \times SD = 3.9\%$ ).

#### Case 3

Cytogenetic analysis of BM cells from this patient with AML revealed two cells with a normal female karyotype and 18 cells with a karyotype of 46,XX,-7,t(9;22)(q34;q11.2),+mar. The marker chromosome was a very small lightly stained "dot", presumably consisting of a chromosome 7 centromere and little else. FISH with a probe for chromosome 7 centromere revealed 90% of interphase nuclei with two fluorescent spots (Fig. 1a) and each of six metaphases examined showing fluorescence for the normal chromosome 7 as well as the marker, suggesting the tiny marker was of chromosome 7 origin.

#### Case 4

Cytogenetic analysis of BM cells from this patient with MDS showed 19 metaphases with a normal female chromosome complement and one single cell with a karyotype of 47,XX,+mar. The marker chromosome could not be clearly identified due to poor morphology of the metaphase; however, it did appear very similar to a chromosome 6 or 8. The single-target FISH results obtained with chromosomes 6- and 8-specific centromere probes revealed 1.2% (< mean  $+2 \times SD = 1.6\%$ ) and 5.2% (> mean  $+2 \times SD = 1.0\%$ ) of interphase nuclei with three fluorescent spots, respectively, suggesting the marker chromosome to be a chromosome 8.

#### Cases 5 and 6

Cytogenetic analysis of BM cells showed a normal karyotype in each of the two cases (patient 5 with CLL and patient 6 with MDS). FISH studies using a probe for chromosome 12 (often +12 is observed in CLL) or 8 (often +8 is observed in MDS) centromere were performed on the cells of these two patients. The results showed a significant number of cells with three signals (16.6%) using the probe for chromosome 12 centromere in Case 5 (Fig. 1b), and with three signals (2.6% > mean  $+2 \times SD = 1.0\%$ ) with the probe for chromosome 8 centromere in Case 6, therefore confirming these diagnoses.

#### Cases 7-10

Cytogenetic analyses of BM cells from these four patients (patient 7 with anemia, patient 8 with MDS, patient 9 with CLL, and patient 10 with AML) showed single abnormal cells in each case: trisomy 8 in one of 36 cells, trisomy 18 in one of 20 cells, trisomy 12 in one of 20 cells, and trisomy 21 in one of 20 cells, respectively. Based on these chromosomal results, we could not make an evaluation of a possible clone for these single-cell trisomies. FISH on interphase nuclei from these patients using appropriate probes showed significant aberrations for chromosome 8 in Case 7 (2.0% three signals > mean  $+2 \times SD = 1.0\%$ ), chromo-

somes 18 in Case 8 (2.4% three signals > mean  $+2 \times SD = 0.5\%$ ), and chromosome 12 in Case 9 (31.8% three signals). Only a cross-target probe for chromosomes 13/21 centromeres was available for FISH study in Case 10. The FISH result in combination with the standard cytogenetic finding confirmed the clonal nature of trisomy 21 in this patient (4.8% five signals > mean  $+2 \times SD = 3.5\%$ ).

#### Case 11

Cytogenetic analysis of BM cells from this male patient with severe congenital immunodeficiency (SCID) were not done before and after the sex-mismatched BMT due to the lack of available samples. Two years post BMT, hybridization to ficoll isolated mononuclear cells from the uncultured blood specimen showed 15.8% of cells with two spots and 83.8% of cells with one spot using a chromosome-X-specific centromere probe and 85.8% of cells with one spot using a probe specific for the long arm of chromosome Y (Yq), suggesting the coexistence of a male (XY) and female (XX) cell population in the blood of this patient.

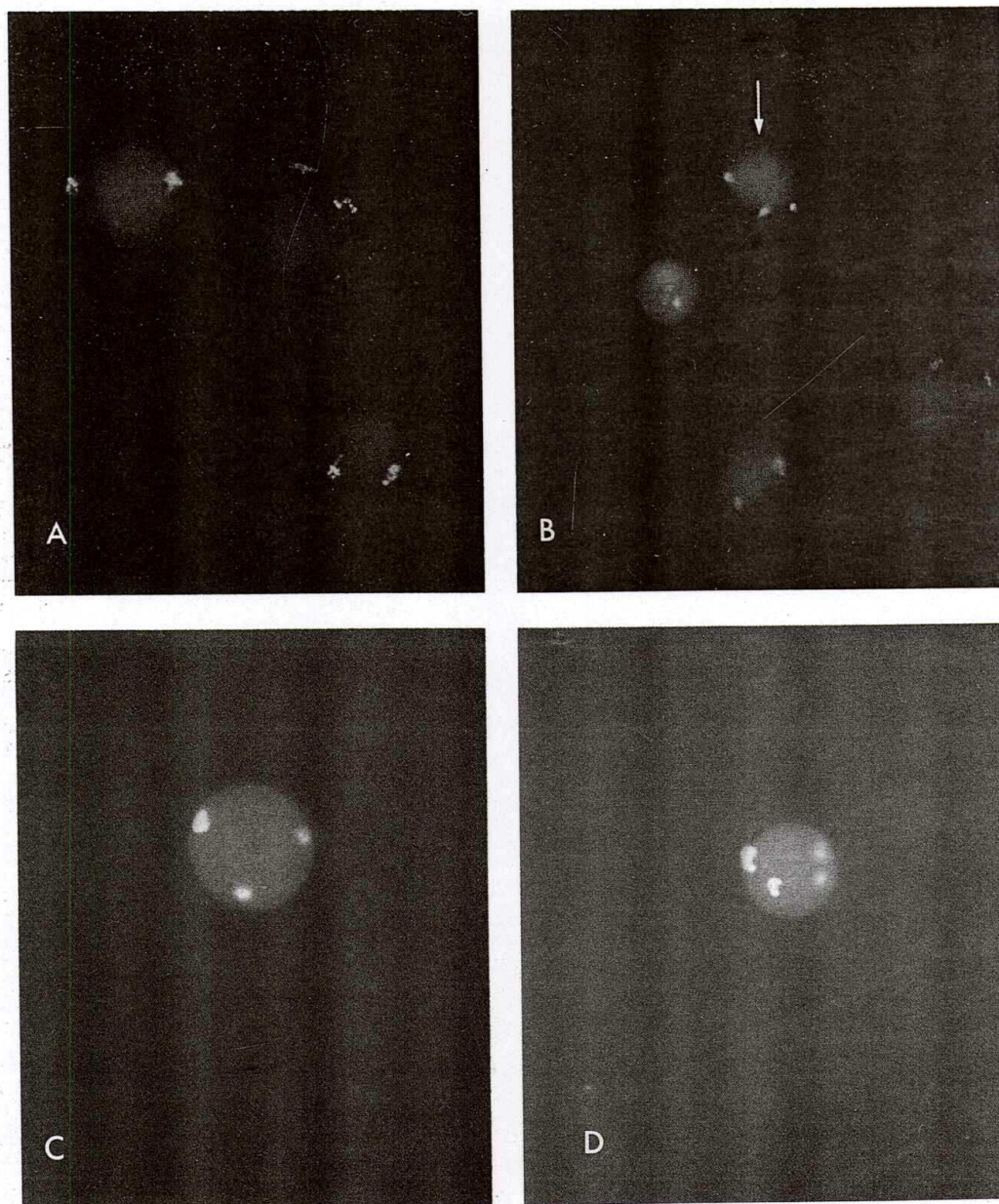
#### Case 12

This female patient with MDS with the previously identified monosomy 7 in the BM cells underwent a sex-mismatched BMT. Five months following the BMT, only four metaphases were partially analyzed and all had two chromosomes 7. There were insufficient metaphases to complete the standard cytogenetic study. The single-target FISH studies on BM cells obtained with a probe for chromosome X centromere and a probe for Yq showed one spot per nucleus in 93.2 and 91% of the cells, respectively, indicating a male (XY) cell population (donor cells). Two spots per nucleus were also found in 6.8% of the cells using the chromosome-X-specific centromere probe, indicating a female cell population (host cells). The observations suggested a BM chimerism of host and donor cells. Furthermore, a two-color FISH analysis was performed on the BM cells from this patient. The probe for chromosome 7 centromere was labeled with digoxigenin and detected with fluoresceinated anti-digoxigenin to give a green signal, whereas the probe for the chromosome X centromere labeled with biotin was detected with Texas-Red-conjugated avidin to give a red signal. The FISH results showed 92.6% of cells with three spots [two green, one red—representative of normal male (donor) cells] and 7.4% of cells with four spots [two green, two red—representative of normal female (host) cells] (Figs. 1c,d). Of interest was that we could not find a single cell showing one green and two red spots [representative of female (host) cells with monosomy 7] in 500 interphase nuclei examined. Thus, a good correlation between the two FISH analytical procedures could be demonstrated.

#### Case 13

At diagnosis of ALL, cytogenetic analysis of BM cells revealed a karyotype of 46,XX in two cells and 47,XX,14q+,+21 in five cells. A subsequent BM examination at the stage of clinical remission 4 years following presentation revealed a normal female chromosome com-





**Figure 1** Single-target FISH results obtained with centromere probes showed: a) two signals for chromosome 7 in Case 3; and b) three signals for chromosome 12 in Case 5. In Case 12, the two-color FISH results obtained with a digoxigenin-labeled chromosome 7 centromere probe and a biotin-labeled probe for chromosome X centromere showed: c) three signals (two green, one red—representative of normal male cells), and d) four signals (two green, two red—representative of normal female cells).



plement in 20 cells analyzed. At the time, hybridization to interphase nuclei using a cross-target centromere probe for chromosomes 13/21 showed a significant percentage of cells with five spots ( $7\% > \text{mean} + 2 \times \text{SD} = 3.5\%$ ), indicating the presence of trisomy 21 in a small portion of nondividing BM cells.

## DISCUSSION

Recently, a few studies have been published concerning interphase cytogenetic analyses in comparison with standard cytogenetic studies of neoplastic cells [14–23]. However, no single paper deals with the general application of FISH in clinical hematologic cytogenetics. In the present study, we analyzed a group of 13 patients with malignant hematologic disorders by using the FISH procedures and compared these results with conventional cytogenetic findings. This study showed the effective application of FISH analysis to clinical hematologic cytogenetics as follows:

**1. Identification of Marker Chromosome:** Although in conventional cytogenetics a number of specialized techniques, such as C-banding, silver stains for nucleolar-organizing regions (AgNOR), anti-centromere staining, and distamycin/DAPI staining have already been developed to facilitate such identifications, some marker chromosomes still cannot be determined based on these techniques due to insufficient metaphases or inconclusive staining results. In this study, we showed a crucial use of FISH technique in identifying (or excluding) the origin of marker chromosomes in four cases (Cases 1–4). In Case 1, FISH results excluded the possibility of the marker being a chromosome 7 in origin, suggesting the existence of monosomy 7 in this patient. This observation was clinically significant because monosomy 7 is frequently associated with secondary AML with an increased susceptibility to bacterial infections and with a rapidly fatal outcome [1, 24]. FISH study in Case 2 confirmed the der(9) identification. In Case 3, the tiny marker was identified to be of chromosome 7 origin. Normally, this would have been impossible to determine with conventional cytogenetic techniques. In Case 4, FISH not only identified the marker chromosome to be a chromosome 8, but also confirmed the clonality of trisomy 8 in this patient. It provided crucial evidence supporting the clinical diagnosis of MDS because trisomy 8 is one of the most common chromosomal anomalies found in MDS [1]. Thus, FISH using a chromosome-specific satellite DNA probe provides an accurate means for marker chromosome identification.

**2. Identification of Trisomies Consistent with Certain Specific Hematologic Neoplasms:** Some hematologic neoplasias are closely associated with chromosomal aneuploidy, e.g., trisomy 12 in CLL [25, 26] and monosomy 5, monosomy 7, and trisomy 8 in MDS [1]. However, due to limitations of conventional cytogenetic techniques, such as the small number of analyzable metaphases, the requirement of dividing cells and the selection of certain highly proliferative tumor cells, the interpretation of the G-banding chro-

mosome aneuploidy sometimes is very difficult, if not impossible, or just yields normal information. FISH greatly expands the capabilities of conventional cytogenetics in reliably detecting chromosomal aneuploidy. The present study confirmed the advantages of this technique.

In Cases 5 and 6, FISH analyses revealed a significant number of cells with trisomy 12 and trisomy 8 which were not detected by conventional cytogenetic studies. It provided crucial evidence for the clinical diagnoses of CLL and MDS, respectively, in these two cases. Recently, a FISH study in CLL found two of 13 CLL patients with a normal karyotype in G-banding study having trisomy 12 by FISH analyses using a probe for the chromosome 12 centromere, and suggested that trisomy 12 is more common than expected on the basis of standard chromosomal analysis [21]. We confirmed this by using the FISH procedure to detect trisomy 12 in one of five CLL patients who were known to have a normal G-banded karyotype (unpublished observations).

**3. Clonal Evaluation of a Single Cell with Trisomy:** One single cell with trisomy is a not-infrequent cytogenetic phenomenon found in G-banding studies. In order to exclude an in vitro artifact in this case, normally it would be very laborious in analyzing another 20 or more metaphases if available, and even so, it might yield a negative result. However, using the FISH technique with an appropriate chromosome-specific centromere probe, one can easily analyze up to 500 cells, and thus provide a more sensitive and quantitative method for clonal trisomy evaluation.

In Cases 7–10, the single-target FISH results showed a significant number of cells with trisomy 8, 18, 12, and 21, respectively. Of interest is that in Case 7, which was clinically diagnosed as having anemia of unknown nature, the FISH study revealed a small population of trisomy 8 cells, thus suggesting that this patient had MDS [1]. In Cases 8–10, the FISH results further confirmed the clinical diagnoses of the disorders [1, 25].

**4. Clonal Evaluation Post Sex-Mismatched BMT:** When results are based only on standard cytogenetic study, limited and skewed information concerning the clonal evaluation post BMT may be available due to a limited number of metaphases suitable for analysis. By FISH using probes for the chromosome X centromere and Yq, one can rapidly screen large numbers of cells and identify the donor and host cells. In the present report we presented two cases (Cases 11 and 12) demonstrating this point.

In Case 11, FISH analysis showed peripheral blood chimerism of XY (male) and XX (female) cells. The presence of XX cells (about 15%) indicated persistence of the donor cells 2 years after the BMT.

In Case 12, the single-target FISH results showed a majority of cells as male (donor cells, more than 90%) and a minority being female (host cells, less than 10%) 5 months following the BMT. In addition, two-color FISH results indicated that the host cells were cytogenetically normal.

Thus, in the two patients, FISH provided an efficient method for clinicians to monitor the BMT effect and proved



very useful in documentation of donor marrow engraftment and in accurate detection of minimal residual disease.

**5. Detection of Residual Disease Following Clinical Remission:** One of the advantages of the FISH technique is that it can be used for rapid screening of large numbers of cells, thus providing a sensitive method in the search for residual disease in patients with leukemia in clinical remission. If a standard cytogenetic study is available at disease presentation, a subsequent FISH analysis will be used for monitoring the disease using appropriate probes indicated by the initial standard cytogenetic results. In Case 13, the initial cytogenetic analysis showed trisomy 21. The patient has been in clinical remission after intensive chemotherapy for almost 4 years. Recently, standard cytogenetic analysis of the BM cells showed a normal karyotype consistent with the clinical remission. However, FISH analysis revealed the existence of residual leukemic cells in this patient. Thus, the FISH result provided important information for clinicians to monitor the course of the disease, and also raised a question concerning the redefinition of clinical remission, challenging the standard criteria for remission based on clinical, hematologic, morphologic, and cytogenetic features.

In conclusion, FISH techniques provide a potentially very sensitive and effective adjunct to standard metaphase analysis in studies of hematologic disorders. FISH significantly expands the capabilities of the clinical cytogenetics laboratory for marker chromosome and chromosomal aneuploidy identification, donor marrow engraftment assessment, and residual disease detection. As techniques become more refined and more extensive and chromosome-specific library probes become available, it is expected that FISH will offer the opportunity to study chromosomal structural aberrations, thus lending itself to routine analytic techniques in the clinical cytogenetics laboratory.

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## REFERENCES

- Sandberg AA (1990): *The Chromosomes in Human Cancer and Leukemia*, Second Ed. Elsevier Science Publishing Co., New York, pp. 104–112, 217–218, 269–272, 282–289, 292–303, 316, 388–392, 522–533, 584.
- Stass SA (1987): *The Acute Leukemias: Biologic, Diagnostic, and Therapeutic Determinants*. Marcel Dekker, Inc., New York and Basel, pp. 153–201.
- Pinkel D, Straume T, Gray JW (1986): Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. *Proc Natl Acad Sci USA* 83:2934–2938.
- Lichter P, Cremer T, Borden J, Manuelides L, Ward DC (1988): Delineation of individual human chromosomes in metaphase and interphase cells by in situ hybridization using recombinant DNA libraries. *Hum Genet* 80:224–234.
- ISCN (1985): *An International System for Human Cytogenetic Nomenclature*, Harnden DG, Klinger HP, eds.; published in collaboration with Cytogenet Cell Genet (Karger, Basel, 1985); also in *Birth Defects: Original Article Series*, Vol. 21, No. 1 (March of Dimes Birth Defects Foundation, New York, 1985).
- Jabs E, Perisco M (1987): Characterization of human centromeric regions of specific chromosomes by means of alphoid DNA sequences. *Am J Hum Genet* 41:374–390.
- Donlon T, Wyman AR, Mulholland J, Barker D, Bruns G, Latt S, Botstein D (1986): Alpha satellite-like sequences at the centromere of chromosome 8. *Am J Hum Genet* 39: A196.
- Moyzis R, Albright K, Bartholdi M, Cram S, Deaven L, Hildebrand E, Joste N, Longmire J, Meyne J, Schwarzscher-Robinson T (1987): Human chromosome-specific repetitive DNA sequences: Novel markers for genetic analysis. *Chromosoma* 95:375–386.
- Devilee P, Cremer T, Slagboom P, Bakker E, Scholl H, Hager H, Stevenson A, Cornelisse C, Pearson P (1986): Two subsets of human alphoid repetitive DNA show distinct preferential localization in the pericentric regions of chromosomes 13, 18, and 21. *Cytogenet Cell Genet* 41:193–201.
- Lau Y, Huang J, Dozy A, Kan YW (1984): A rapid screening for antenatal sex determination. *Lancet* i:14–16.
- Feinberg AP, Vogelstein B (1983): A Technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Analytical Biochem* 132:6–13.
- Johnson GD, Noguera TGM (1981): A simple method of reducing the fading of immunofluorescence during microscopy. *J Immuno Methods* 43:349–350.
- Tkachuk DC, Westbrook CA, Andreeff M, Donlon TA, Clearly ML, Suryanarayan K, Homge M, Redner A, Grey J, Pinkel D (1990): Detection of bcr-abl fusion in chronic myelogenous leukemia by in situ hybridization. *Science* 250:559–562.
- Anastas J, LeBeau MM, Vardiman JW, Westbrook CA (1990): Detection of bcr-abl fusion in chronic myelogenous leukemia by in situ hybridization. *Science* 250:559–562.
- Poddighe PJ, Moesker O, Smeets D, Awwad BH, Ramaeker FCS, Hopman AHN (1991): Interphase cytogenetics of hematological cancer: Comparison of classified karyotyping and in situ hybridization using a panel of eleven chromosome-specific DNA probes. *Cancer Res* 51:1959–1967.
- Nederlof PM, Van der Flier S, Raap AK, Tanke HJ, Van de Ploeg M, Kornips F, Geraedts JPM (1989): Detection of chromosome aberrations in interphase tumor nuclei by non-radioactive in situ hybridization. *Cancer Genet Cytogenet* 42:87–98.
- Van Dekken H, Hagenbeek A, Bauman JGJ (1989): Detection of host cells following sex-mismatched bone marrow transplantation by fluorescent in situ hybridization with a Y-chromosome specific probe. *Leukemia (Baltimore)* 3:724–728.
- Van Dekken H, Pizzolo JG, Reuter VE, Melamed MR (1990): Cytogenetic analysis of human solid tumors by in situ hybridization with a set of 12 chromosome-specific DNA probes. *Cytogenet Cell Genet* 54:103–107.
- Wullich B, Morgan R, Berger C, Jarzabek V, Sandberg AA (1991): Nonradioactive in situ hybridization: A rapid approach for the identification of marker chromosomes: study of a case of acute leukemia with a Yq specific DNA probe. *Cancer Genet Cytogenet* 52:165–172.
- Chen Z, Berger C, Morgan R, Roth D, Sandberg AA (1992): Cytogenetic and FISH studies of abnormal X chromosomes in a patient with ANLL. *Cancer Genet Cytogenet* (in press).
- Losada AP, Wessman M, Tiainen M, Hopman AHN, Willard HF, Solé F, Caballin MR, Woessner S, Knuutila S (1991): Trisomy 12 in chronic lymphocytic leukemia: an interphase cytogenetic study. *Blood* 77:775–779.
- Anastasi J, Thangavelu M, Vardiman JW, Hooberman AL, Bian ML, Larson RA, LeBeau MM (1991): Interphase cytogenetic analysis detects minimal residual disease in a case of acute

- lymphoblastic leukemia and resolves the question of origin of relapse after allogenic bone marrow transplantation. *Blood* 77:1087-1091.
23. Cremer T, Lichter P, Borden J, Ward DC, Manuelidis L (1988): Detection of chromosome aberrations in metaphase and interphase tumor cells by in situ hybridization using chromosome specific library probes. *Hum Genet* 80:235-246.
24. Borgström GH, Teerenhovi L, Vuopio P, de la Chapelle A, van den Berghe H, Brandt L, Golomb HM, Louwagie A, Mitelman F, Rowley JD, Sandberg AA (1980): Clinical implications of monosomy 7 in acute nonlymphocytic leukemia. *Cancer Genet Cytogenet* 2:115-126.
25. Juliusson G, Oscier DG, Fitchett M, Ross FM, Stockdill G, Mackie MJ, Parker AC, Gastoldi GL, Cuneo A, Knuutila S, Elonen E, Gahrton G (1990): Prognostic subgroups in B-cell chronic lymphocytic leukemia defined by specific chromosomal abnormalities. *N Engl J Med* 323:720-724.
26. Juliusson G, Gahrton G (1990): Chromosome aberrations in B-cell chronic lymphocytic leukemia. *Cancer Genet Cytogenet* 45:143-160.