

Comparison of Cytogenetic and Molecular Cytogenetic Detection of Chromosome Abnormalities in 240 Consecutive Adult Patients With Acute Myeloid Leukemia

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Purpose: To prospectively compare cytogenetic and molecular cytogenetic analysis for the detection of the most relevant chromosome abnormalities in a large series of patients with acute myeloid leukemia (AML).

Patients and Methods: Two hundred forty consecutive adult patients with AML entered onto the multicenter treatment trial AML HD93 were studied. Chromosome banding and fluorescence in situ hybridization (FISH) applying a comprehensive set of genomic DNA probes were performed in a single reference laboratory.

Results: Two cases of *inv*(16), three cases of *t*(11q23), and three cases of *t*(8;21)var were only detected by molecular cytogenetics. By FISH, aberrations were identified in three cases with normal karyotypes: *inv*(16), -Y (in a patient with low metaphase yield on chromosome banding) and a 12p microdeletion. Additional aneuploidies, in particular +8q and +11q, were diagnosed by FISH; however, virtually all these aber-

rations occurred in patients with complex karyotypes or as an additional abnormality in leukemias with an AML-specific translocation. Finally, aberrations were detected by FISH in eight of 14 patients with no assessable metaphases.

Conclusion: In most cases of AML, conventional cytogenetic study reliably detects chromosomal abnormalities, and this method should not be replaced by FISH. FISH should be used as a complementary method for the detection of more subtle abnormalities, such as *inv*(16) and *t*(11q23), in all patients with newly diagnosed AML and for suspected *t*(8;21)var. Furthermore, molecular cytogenetics using this comprehensive set of DNA probes provides a valuable diagnostic tool for patients with poor chromosome morphology, low or no yields of metaphase cells, or both.

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IN ACUTE MYELOID leukemia (AML), clonal chromosome aberrations constitute markers of diagnostic value, and the molecular characterization of numerous abnormalities has greatly improved the understanding of the biology of distinct subtypes of the disease.¹⁻⁴ Moreover, cytogenetic data are particularly useful for determining prognosis or predicting response to certain therapeutic approaches.⁵⁻⁷ Thus, because modern risk-adapted therapy of patients with AML is based on cytogenetic characteristics, the rapid and reliable identification of chromosome aberrations is of utmost importance for the clinical management of patients with this malignancy.

Conventional chromosome banding study allows a comprehensive analysis of the karyotype and is still a fundamental component of modern tumor cytogenetics. Nevertheless, despite continuous improvements in cytogenetic

methodology, in almost all studies of patients with AML, substantial numbers of patients without adequately banded metaphase cells have been reported.^{1,2}

With the increasing availability of a large variety of specific DNA probes due to the developments in the Human Genome Project (<http://www.ncbi.nlm.nih.gov>), studies that use fluorescence in situ hybridization (FISH) are no longer limited to the detection of numerical aberrations or the identification of certain structural abnormalities in a small number of patients.⁸ In 1996, our group reported on the use of FISH for a comprehensive analysis of the most relevant AML-associated chromosome aberrations in a pivotal series of 105 patients with adult AML.⁹ FISH proved to be a rapid and sensitive technique that complemented conventional chromosome banding studies. We present the results from a comparative study of cytogenetic and molecular cytogenetic analysis in a prospective series of 240 patients with adult AML entered onto the German multicenter treatment trial AML HD93.

PATIENTS AND METHODS

Patients

Between December 1993 and January 1998, 240 consecutive patients (aged 16 to 60 years) with AML (de novo and secondary after a primary malignancy) diagnosed according to French-American-British Cooperative Group criteria¹⁰ were studied by chromosome banding analysis and FISH in our laboratory, which is the central reference

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Table 1. Designation, Chromosomal Localization, and Gene/Locus of the DNA Clones Selected for the Detection of AML-Associated Aberrations by FISH

Aberration	DNA Clone	Localization	Gene/Locus
-5/5q-	773_D_3	5q31	<i>IL9, D5S89</i>
	yPR411	5q33	<i>CSF1R</i>
-7/7q-	HSC7E506	7q22	<i>RELN, D7S240</i>
	HSC7E124	7q35	<i>D7S688</i>
+8/+8q	935_A_12	8q24	<i>D8S508</i>
	464_H_8/72_H_9	21q22	<i>AML1</i>
t(8;21)	+P1 164 phage	8q22	<i>ETO</i>
	253_F_8	9q13-q21	<i>D9S15</i>
-9/9q-	933_G_6	9q22.3	<i>D9S280</i>
	13HH4	11q23	<i>MLL</i>
t(11q23)	785_C_6/856_B_9	11q23	<i>MLL, Thy 1</i>
	+48/55 cosmid pool	9p22	<i>AF9</i>
t(9;11)	964_C_10	12p13	<i>TEL</i>
	t(12p13)/12p-	13q14	<i>RB1</i>
13q-/+13q	RB1 phage pool	13q14	<i>RB1</i>
	804_D_9	15q22	<i>PML</i>
t(15;17)	356_C_12	17q21	<i>RARA</i>
	+RARAcos121/124		
inv(16)	Cosmid pool		
	854_E_2	16p13	<i>MYH11</i>
-17/17p-	+LA2-2/LA4-1 cosmid pool	16q22	<i>CBFβ</i>
	TP53 cosmid pool	17p13	<i>TP53</i>
-20/20q-	808_C_05	20q13	<i>D20S99</i>
+21/+21q	464_H_8/72_H_9	21q22	<i>AML1</i>
	2D3	21q, proximal half	
+22/+22q	D107F9	22q11	<i>BCR</i>
	361_D_9	22q11	<i>BCR</i>
-/+Xq	A24CH4	Xq28	<i>DXS304</i>
	IE018	Xq28	<i>DXS304</i>
-/+Yq	yOX-57	Yq11	<i>DXYS1</i>

laboratory for cytogenetic and molecular diagnostics within the AML Study Group Ulm. All patients were entered into the multicenter treatment trial AML HD93, in which postremission therapy was stratified according to the karyotype¹¹: low risk [t(8;21), inv(16)/t(16;16), t(15;17)], intermediate risk (normal karyotype), and high risk (all other chromosome aberrations). Conventional chromosome banding studies were performed using standard techniques and chromosomal abnormalities were described according to the International System for Cytogenetic Nomenclature.¹² The cytogenetic risk classification defined all patients with t(8;21), inv(16)/t(16;16), or t(15;17) as favorable, regardless of additional abnormalities. Complex karyotypes were defined as the presence of three or more clonal aberrations.

The study was approved by the institutional review boards of the participating institutions. Informed consent was obtained from all patients according to the guidelines set forth by institutions participating in the study.

Molecular Cytogenetic Analysis

The DNA clones selected for the detection of the most relevant AML-associated chromosome aberrations by FISH (Table 1), the criteria required for the identification of AML-specific gene fusions, and the cutoff levels for the diagnosis of aneuploidies or deletions were

described previously.⁹ In the course of this study, the DNA probe set was subjected to several modifications: because of the heterogeneity of the breakpoints at 9q reported in the literature,^{1,2} yeast artificial chromosome (YAC) clone 933_G_6 was included to identify deletions telomeric to 9q21. The *RB1* phage pool used to detect deletions at 13q14 was replaced by YAC clone 804_D_9 because this probe yielded a greater fluorescence signal intensity. YAC clone 2D3 mapping to the proximal half of 21q¹³ was added to the probe set to distinguish between translocations involving the *AML1* gene at 21q22, as detected by the 464_H_8/72_H_9 YAC pool and trisomy 21q. YAC clone 361_D_9 was used to detect disruption of the *BCR* gene at 22q11 because a greater proportion of Philadelphia chromosome-positive patients exhibited splitting of one fluorescence signal with this probe than with D107F9. In addition to the DNA clones listed in Table 1, centromere-specific probes were used to distinguish between monosomies or trisomies, and partial aneuploidies.

Cosmid and P1 phage DNA was prepared using the Plasmid Midi Kit (Qiagen, Hilden, Germany). Human sequences from YAC clones were generated by an inter-Alu polymerase chain reaction (PCR) protocol.¹⁴ The sequence-independent amplification technique¹⁵ was used to amplify DNA from YAC clone 854_E_2 and from *RB1* phage clones. PCR products were labeled by nick translation with biotin-16-dUTP or digoxigenin-11-dUTP (Roche Diagnostics, Mannheim, Germany).

Dual-color FISH was performed as previously described.⁹ The hybridization mixture contained approximately 250 ng labeled cosmid, P1 phage or YAC DNA, 10 μg Cot-1 DNA fraction (BRL/Life Technologies, Gaithersburg, MD) and 10 μg herring sperm DNA (Roche Diagnostics). FISH signals were visualized by a Zeiss Axioskop epifluorescence microscope (Oberkochen, Germany), and images were captured by a cooled charged coupled device camera (Xillix Technologies, Richmond, Canada).

RESULTS

Two hundred forty patients were studied by cytogenetics and FISH. One hundred five of these patients have been reported previously.⁹ Results from the two methods were compared with regard to the detection of the most relevant AML-specific gene fusions and the most frequent aneuploidies or deletions. Because karyotype was used for selecting postremission therapy within the AML HD93 treatment trial, results of all patients were compared, irrespective of success of the assays. The distribution of individual aberrations is listed in Table 2. The combined results of cytogenetic and molecular cytogenetic analysis are provided in Table 3. Assessable metaphases were obtained in 226 (94%) of 240 patients. Of the 226 assessable patients, 124 (55%) exhibited clonal chromosome aberrations; 102 patients (45%) had a normal karyotype. In contrast to chromosome banding analysis, assessable interphase preparations were obtained in all 240 patients. Clonal aberrations were detected in 125 patients (52%).

Translocations and Inversion

All patients with a t(8;21)(q22;q22) on banding analysis and four additional cases of an *AML1/ETO* fusion were detected by FISH; three of these four patients had a variant

Table 2. Distribution of AML-Specific Aberrations in 240 Consecutive Adult Patients: Comparison of Cytogenetic and Molecular Cytogenetic Analysis

Aberration	No. of Aberrations		
	Chromosome Banding	FISH	Chromosome Banding or FISH
Translocations/inversion			
t(8;21)/t(8;21)var	13	17	17
t(9;11)	11	11	11
t(11q23) other	6	9	9
t(12p13)	2	2	2
t(15;17)	18	18	19
inv(16)/t(16;16)	22	25	25
Aneuploidies/deletions			
−5/5q−	14	14	15
−7/7q−	18	17	19
+8/+8q	16	25	25
−9/9q−	8	7	8
+11/+11q	6	12	12
−12/12p−	4	4	6
−13/13q−	4	4	5
−17/17p−	5	8	8
−20/20q−	4	3	4
+21/+21q	8	8	10
+22/+22q	5	8	8
−X/Xq−	6	8	8
−Y/Yq−	4	4	5

t(8;21), designated add(2)(p13), add(21)(q22), t(8;9;21)(q22;q36;q22), and t(4;21)(p12;p11) on banding analysis; in one case, there were no assessable metaphases.

Inv(16)(p13q22) or t(16;16)(p13;q22) was identified in 22 patients by cytogenetics and in 25 patients by molecular cytogenetics. The three additional patients identified by FISH included one patient with a normal karyotype, a second patient with numerical aberrations of chromosomes Y, 13, and 22 but without abnormality of chromosome 16, and a third patient without assessable metaphases. Of the 18 patients with t(15;17) found on banding analysis, one was not detected by molecular cytogenetics; and one patient without assessable metaphases demonstrated a t(15;17) by FISH.

Table 3. Incidence of Clonal Chromosome Aberrations by Cytogenetics and Molecular Cytogenetics in 240 Consecutive Adult Patients With AML*

Aberration	Chromosome Banding		FISH		Chromosome Banding or FISH	
	No.	%	No.	%	No.	%
Not assessable	14	6	None		None	
No clonal aberrations	102	42	115	48	105	44
Clonal aberrations	124	52	125	52	135	56

*Percentages are based on all 240 cases.

Rearrangements involving band 11q23 were identified in three additional patients by molecular cytogenetics: one patient had a ?del(11)(q23) on banding analysis, whereas interphase and metaphase FISH demonstrated a t(11;16)(q23;p13). The second patient had an add(10)(q26) on banding analysis; molecular cytogenetic analysis using DNA probes recognizing band 11q23 (13HH4, 785_C_6/856_B_9 pool) displayed three fluorescence signals in 49% of the cells, and metaphase FISH revealed a t(10;11). In the third patient without abnormality of chromosome 11 on banding analysis, FISH that used the 785_C_6/856_B_9 pool and a chromosome 6-specific painting probe demonstrated that 11q23 material was inserted into band 6q28. Finally, both patients with balanced translocations involving chromosome band 12p13 were identified by cytogenetic and molecular cytogenetic analysis.

Aneuploidies/Deletions

FISH identified all cases of −5 and one additional case of 5q− in a patient without assessable metaphases. One patient with a 5q− within a complex karyotype was not detected by molecular cytogenetics using YAC clones 773_D_3 and yPR411. Metaphase FISH demonstrated that a translocation of chromosome 5 material to chromosome 19 had occurred: the *D5S89* locus (identified by clone 773_D_3) was retained on the der(5), whereas the *CSFR1* gene (identified by clone yPR411) was translocated to the der(19). Additional experiments that used YAC clone 802_D_5 mapping distally to 773_D_3 to band 5q31 revealed that this translocation was associated with a 5q− that was missed by the DNA probes selected for this study.

Of the six patients with −7 found on banding analysis, one with a subclone of −7 within a complex karyotype was not identified by FISH. One patient with a del(7)(q22q32) present in two of 16 metaphase cells analyzed also had no aberrations on molecular cytogenetic analysis. In one patient without assessable metaphases, a 7q− was detected by FISH. All patients with +8/+8q found on banding analysis and nine additional patients were identified by FISH: in two patients, a subclone of +8q was masked in a complex karyotype, one patient exhibited an i(10q) and a t(11q23), one patient had an add(7)(q3?1) and a t(15;17), and in five of these nine patients, no metaphase cells were obtained. In two of the five patients without assessable metaphases, +8/+8q was the sole cytogenetic abnormality.

Molecular cytogenetic analysis detected all cases of +11/+11q found on banding analysis and six additional cases: in three patients without abnormalities of chromosome 11 on banding analysis and three patients without assessable metaphase spreads, FISH experiments demonstrated a +11q. In five of the six additional cases, the

+11/+11q was part of a complex karyotype; one patient had a t(15;17) as the primary cytogenetic abnormality. Similarly, two additional cases of 12p- were detected by FISH: one patient had a normal karyotype, and one patient had a t(11q23) as the sole cytogenetic abnormality on banding analysis. Of the three patients who had an add(12p) within a complex karyotype, only one was identified by FISH.

Deletion of the short arm of chromosome 17 was detected in five patients by banding analysis and in eight patients by FISH. The three additional patients included two with complex karyotypes and one without assessable metaphases. The latter patient exhibited a complex karyotype on molecular cytogenetic analysis.

Of the eight patients with trisomy 21 detected by chromosome banding analysis, two with subclones of +21 were missed by FISH: one patient had trisomy 8 and a t(11q23) as the leading abnormalities; one patient exhibited an inv(16). In contrast, two additional patients with +21q were identified by FISH: one patient had a complex karyotype without abnormality of chromosome 21, and in one patient, there were no assessable metaphases.

Patients With Normal Karyotypes

One hundred two patients had a normal karyotype on banding analysis. In three of these patients, single chromosome aberrations were detected by FISH: inv(16), -Y, and 12p-. In retrospective analysis of the first patient, the inv(16) was likely present but was missed as a result of poor chromosome morphology. In the patient with loss of the Y chromosome, only five metaphase spreads could be obtained; by FISH, 31% of the cells exhibited -Y. In the patient with 12p-, metaphase FISH demonstrated a normal size of the short arm of chromosome 12 but loss of the sequences detected by probe 964_C_10, indicating a microdeletion that was not detectable by chromosome banding analysis.

Patients Without Assessable Metaphase Cells

In 14 patients, no assessable metaphase preparations were obtained. In eight of these patients, chromosome aberrations were detected by FISH: t(8;21); +8, +9q, inv(16); t(15;17); +8 (two patients); +7q, +11q, 13q-, +Y; 7q-, +8q, +11q, 17p-, +21q; and 5q-, +8, +11q.

DISCUSSION

The results from this study demonstrate the applicability of FISH for the detection of the most relevant AML-associated chromosome aberrations in a large prospective series of patients with adult AML entered onto a multicenter treatment trial. The study was initiated to improve the

accuracy of cytogenetic diagnostics within the AML HD93 trial in which pretreatment cytogenetics was used for selecting risk-adapted postremission therapy. All experiments were performed in a central reference laboratory, and FISH proved to be a rapid (results were available within 5 working days) and reliable (experiments were successful in all patients) technique. FISH was particularly sensitive for detecting the AML-specific translocations and inversion and some aneuploidies and deletions. Furthermore, FISH identified chromosome aberrations in three patients with normal karyotypes and eight of 14 patients in whom chromosome banding analysis was unsuccessful.

Results from several trials suggest that two of the chromosomal rearrangements involving components of the core-binding factor transcription factor complex, inv(16) and t(8;21), confer a favorable prognosis, and it has been demonstrated that the outcome of patients with these aberrations can be significantly improved by the use of high-dose cytarabine in postremission therapy.^{1,2,5,6,16} In our study, three additional cases of inv(16)(p13q22) were identified by FISH. These included one patient without assessable metaphases and two patients that had been missed on banding analysis, presumably because of insufficient chromosome morphology. In consequence of the low number of G bands within the inverted region of chromosome 16, high quality of metaphases is required for the definitive cytogenetic diagnosis of an inv(16). Therefore, the detection of this abnormality may be more difficult in the setting of a multicenter trial where the specimens are transported to a reference laboratory.

Four additional cases of an *AML1/ETO* fusion were detected by FISH: in one patient, no assessable metaphases were obtained, and in three patients, the chimeric gene resulted from a variant t(8;21). Thus, because the classical t(8;21)(q22;q22) is relatively easy to detect at the cytogenetic level, FISH that uses probes for the identification of the *AML1/ETO* hybrid gene should be performed only in cases exhibiting breakpoints in bands 8q22 and/or 21q22, which may result from a variant t(8;21). Our findings are strikingly consistent with the results from a study of the Cancer and Leukemia Group B comparing cytogenetics and reverse transcriptase-PCR for the detection of t(8;21)(q22;q22) and inv(16)(p13q22)/t(16;16)(p13;q22) in 284 patients with adult AML¹⁷: in this study, cytogenetic results were considered to be false negative in three *AML1/ETO*-positive patients with variants of t(8;21) and in three *CBFB/MYH11*-positive patients with, respectively, an isolated +22; del(16)(q22), +22; and a normal karyotype.

Reciprocal translocations involving the retinoic acid receptor alpha (*RARA*) gene include the t(15;17)(q22;q21), resulting in the juxtaposition of the *PML* gene and the *RARA*

gene and the less frequent t(11;17)(q23;q21), t(11;17)(q13;q21), and t(5;17)(q35;q21), whereby *RARA* is fused to the *PLZF*, *NuMA*, and *NPM* genes, respectively.¹⁸ The majority of AML cases with morphologic and clinical features of acute promyelocytic leukemia (FAB M3) have a t(15;17). Detection of a t(15;17) is crucial in determining which individuals will derive therapeutic benefit from all-*trans* retinoic acid induction or maintenance therapy.¹⁹ In our hands, FISH offered no additional benefit for the detection of a t(15;17) in patients with successful banding analysis.

Compared with inv(16), t(8;21), and t(15;17), the outcome of patients with chromosomal rearrangements involving the *MLL* gene at chromosome band 11q23 is less well defined. Complete remission rates of adult patients with abnormalities of 11q23 were reported to vary between 25% and 83%, possibly reflecting the marked heterogeneity of 11q23 aberrations, and overall survival of the majority of patients is poor.^{1,2,4} In the present study, all cases of t(9;11) were detected by FISH. This aberration is regarded to confer a more favorable prognosis than other 11q23 translocations, especially in patients who receive intensive postremission therapy with high-dose cytarabine or allogeneic bone marrow transplantation.^{6,20} In addition, three cases of other rearrangements involving band 11q23 were only detected by FISH using a YAC clone containing the entire *MLL* gene and a contig of YAC clones recognizing more than 1.0 Mb of DNA sequences distal to *MLL*.

By FISH, more cases of genomic imbalances, in particular +8q and +11q, were detected. However, almost all aneuploidies or deletions that were missed on conventional cytogenetics were detected in patients with complex karyotypes or as additional abnormalities in patients with at least one leukemia-specific primary chromosome change—for example, inv(16), t(8;21), t(15;17), or t(11q23). In addition, some aneuploidies or deletions were identified in patients with normal cytogenetics or in patients without assessable metaphases. Considering that the majority of studies associate complex cytogenetic abnormalities with an adverse prognosis^{1,2} and that additional aberrations did not have a deleterious effect on outcome in a large series of patients with low-risk abnormalities entered into the 10th United Kingdom Medical Research Council trial,⁶ our data imply

that routine FISH that uses DNA probes for the detection of chromosomal aneuploidies or deletions does not add prognostic information to that gained from pretreatment cytogenetics in patients in whom banding analysis is successful. On the contrary, patients without assessable metaphases should be screened for 5q–, 7q–, +8q, 12p–, 17p–, and 20q– by molecular cytogenetics, particularly with regard to the dismal prognosis associated with these aberrations.^{1,2}

FISH identified clonal aberrations in three of 102 patients with normal cytogenetics: inv(16); –Y; and 12p–. In the patient with inv(16), chromosome morphology was poor; in the –Y patient, only five assessable metaphases could be obtained. Accordingly, the 12p– was the only submicroscopic aberration detected by FISH in 102 patients with normal cytogenetics. These data indicate that molecular cytogenetics using the comprehensive probe set of our study does not add relevant information to that gained from conventional chromosome banding in patients with normal cytogenetics. Considering the heterogeneity of these leukemias at the molecular level,^{21–23} other techniques, such as Southern blot analysis, PCR, and DNA sequencing should be used to detect genetic abnormalities not resolvable by conventional banding analysis.

Our study demonstrates that in the majority of cases, chromosome banding analysis, provided that sufficient metaphase cells are assessable, reliably yields the leukemia karyotype. We propose to use FISH as a complementary method for the identification of inv(16) and t(11q23) in all patients with newly diagnosed AML because these abnormalities may be difficult to detect and in patients suspected of having a variant t(8;21). Furthermore, molecular cytogenetics using the probe set presented in this study provides a valuable tool for patients with poor chromosome morphology and those with a low or no yield of assessable metaphase cells. Our study does not support routine screening by FISH of patients with normal karyotypes.

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