Leukemia Research Vol. 16, No. 8, pp. 789–796, 1992.
Printed in Great Britain.

0145-2126/92 $5.00 + .00

MORPHOLOGIC, IMMUNOLOGIC AND CYTOGENETIC STUDIES IN ACUTE MYELOID LEUKEMIA FOLLOWING OCCUPATIONAL EXPOSURE TO PESTICIDES AND ORGANIC SOLVENTS

ANTONIO CUNEO, FRANCA FAGIOLI, ISABELLA PAZZI, ANTONELLA TALLARICO, RITA PREVIATI, NADIA PIVA, M. GRETEL CARLI, MASSIMO BALBONI and GIANLUIGI CASTOLDI
Institute of Hematology, Via Savonarola 9, University of Ferrara, Italy

(*Received* 10 *February* 1992. *Revision accepted* 31 *March* 1992)

Abstract—In order to analyze the correlation between environmental exposure and the clinicopathological picture in acute myeloid leukemia (AML), cytogenetic, cyto-immunologic and clinical studies were performed in 70 newly diagnosed AML patients, 30 of which were anamnestically exposed to pesticides (21 cases) or to organic solvents (9 cases). Clonal chromosome aberrations, with involvement of chromosome 5 and/or 7 were more frequently encountered among exposed patients. While the classical t(15;17), t(8;21) and t(9;11) were detected more frequently among non-exposed patients, other recurring chromosome changes in the exposed group were: rearrangements leading to total or partial monosomy 17p (5 cases), structural aberrations involving the band 16q22 (4 cases), trisomy 11q (2 cases), breaks involving bands 6p23, 7p14, 11q13 (2 cases each). Cytologically, trilineage myelodysplasia was observed in 21 exposed patients, whereas morphologic aberrations of the non-blast cell population were confined to a minority of cells in most patients non-exposed. Immunologic studies revealed positivity for the CD34 stem cell marker in 80% exposed patients vs 22% in the non-exposed group. Conventional chemotherapy achieved complete remission in 3/21 patients exposed and in 16/32 patients non-exposed. Median survival was 2 months in the former group and 9 months in the latter group. These findings show that AML following occupational exposure to pesticides and organic solvents may represent a distinct cytogenetic and clinicopathological entity.

Key words: AML, cytogenetics, pesticides, organic solvents.

INTRODUCTION

A DISTINCT pattern of chromosome aberrations has been shown to be associated with acute myeloid leukemia (AML) following exposure to organic solvents, petroleum products and to pesticides [1–3]. In these patients the frequent involvement of the long arms of chromosomes 5 and/or 7 recall the cytogenetic picture of so called 'secondary leukemia' (SL) [4], suggesting that both toxic agents present in the environment and cytotoxic drugs or radiation therapy may preferentially involve some chromosome regions [5].

However, description of cytogenetic findings in correlation with cytologic and clinical parameters in patients environmentally exposed has not been reported previously and it is not known whether AML following exposure to toxic agents represents a distinct cytogenetic and clinicopathological entity with respect to AML in patients non-exposed.

Therefore, we retrospectively analyzed cytogenetic, cytoimmunologic and clinical findings in 70 consecutive AML patients, 30 of which were anamnestically exposed to pesticides or to organic solvents. The aim of the present report is two-fold: (1) to describe cytogenetic findings in patients exposed to pesticides and organic solvents and (2) to compare the cytogenetic and clinicopathologic findings in patients exposed to myelotoxic agents and in patients non-exposed.

Abbreviations: *AML*, acute myeloid leukemia; *SL*, secondary leukemia; *BM*, bone marrow; *TMD*, trilineage myelodysplasia; *PB*, peripheral blood; *MAKA*, major karyotype aberrations; *MDS*, myelodysplastic syndrome; *WBC*, white blood cells.

PATIENTS AND METHODS

Patient selection

Eighty-six consecutive patients with *'de novo'* AML, admitted to our Institution during a 6-year period (1986–1991) were routinely submitted to immunologic and cytogenetic analysis.

Seventy patients with BM smears available for cytologic review and with evaluable immunologic and cytogenetic data form the basis of the present report.

Environmental exposure

On admission each patient was interviewed about his

occupational history and his hobbies. All patients were questioned on previous contacts with organic solvents, pesticides, chemicals, metals and petroleum products and were subsequently categorized as 'exposed' or 'non-exposed'. Exposed subjects were carefully questioned on the type and duration of exposure and were asked as to whether or not they employed protection measures (masks, gloves, pressurized cabins) in the workplace. An 'exposure index' was calculated as follows: hours/day × days/year × years.

Cytology

Besides recommended procedures for the classification of each patient in the FAB system [6, 7] bone marrow (BM) smears were reviewed to assess the presence of associated myelodysplastic features. According to previously proposed criteria [8], trilineage myelodysplasia (TMDS) was defined by the presence of more than 25% dysplastic erythroblasts and more than 50% abnormal granulocytes and megakaryocytes.

Immunophenotyping

BM cytospin preparations and/or peripheral blood (PB) smears were stained by an immunocytochemical method using alkaline phosphatase anti-alkaline phosphatase (APAAP) complexes [9]. In order to minimize Fc aspecific binding the slides were previously incubated with rabbit serum (Dakopatts). Reactivity to a panel of monoclonal antibodies purchased from various firms was tested: HPCA-1(CD34), My9(CD33), My7(CD13), My4(CD14), GpIIb/IIIa(CD41), Leu12(CD19), OKBCalla(CD10), OKT16(CD7), OKT11(CD2). The cut-off point for positivity was 20%.

Cytogenetics

Chromosome analysis was performed at leukemia presentation in all patients. BM samples were cultured for 24 and 48 h without mitogens. Synchronization with methotrexate and thymidine was carried out. Metaphases were G banded with Wright stain [10]. At least 10 karyotypes were studied in each patient and chromosome aberrations were described according to the ISCN [11].

RESULTS

Environmental exposure

Thirty patients (21 farmers, 5 factory painters, 2 shoe workers, 2 hairdressers) were categorized as 'exposed', based on interviews revealing unequivocal contact pesticides (21 cases) and with organic solvents (9 cases). All patients with a history of exposure to pesticides were farmers who had been spraying carbamates and organophosphates [12, 13] for several years without effective protection measures. Pesticides were usually dissolved into water, and no significant exposure to organic solvents could be documented in these patients. The mean 'exposure index' for patients exposed to pesticides and to organic solvents was 20 000 h and 38 000 h, respectively. Nineteen patients were actively working when AML was diagnosed, while in 11 patients direct exposure had ceased 3–9 years before referral to our center. In 40 patients (16 housewives, 14 white-collar workers, 2 students, 2 teachers, 3 operators and 3 farmers), here referred to as 'non-exposed', no evidence of exposure to myelotoxic agents was anamnestically documented.

Clinical features

The salient clinical data in patients exposed to pesticides and to organic solvents and in patients non-exposed is summarized in Table 1. Overall, patients categorized as exposed had a median age (68 years) similar to that of non-exposed patients (64 years), while the male/female ratio was 3.5 in the former group and 0.9 in the latter group. Except for median age, no important difference emerged when comparing clinical features in patients exposed to pesticides and to organic solvents (see Table 1).

As compared with the non-exposed group, exposed patients presented with lower leukocyte counts and with lower blast cell percentage in the bone marrow. Erythroleukemia and megakaryoblastic leukemia were encountered more frequently among exposed patients, while other FAB subtypes of AML were almost uniformly distributed in both categories of patients.

Outcome of remission induction therapy and survival in exposed and non-exposed subjects is reported in Table 1. Of 21 exposed patients treated with conventional myeloablative chemotherapy 3 achieved complete remission, with a median survival of 2 months. Complete remission was obtained in 50% non-exposed subjects with a median survival of 9 months.

Cytology and immunophenotype

(a) *Morphologic findings.* Among subjects exposed to pesticides and to organic solvents morphologic abnormalities affecting multiple cell lineages were apparent in 21 patients and were not evaluable in 9 patients because of overwhelming blast infiltrate. Abnormal cytologic features included the classical signs of dysmyelopoietic syndrome [14], i.e. nuclear irregularities and defective hemoglobinization in the red cell series, neutrophil hypogranulation, pseudo-Pelger forms, micromegakaryocytes, large mononuclear megakaryocytes. According to the stringent criteria by Brito-Babapulle *et al.* [8], 15 patients in this group could be classified as AML with trilineage myelodysplasia.

In the 'non-exposed' group morphologic abnormalities of the non-blast cell population were mostly confined to the granulocytic lineage. A minority of morphologically abnormal megakaryocytes was detected in 5 patients, while dyserythropoiesis involving more than 25% erythroblasts was present

TABLE 1. FAB CLASSES, CLINICAL FEATURES AT PRESENTATION, RESPONSE TO CHEMOTHERAPY AND SURVIVAL IN AML PATIENTS 'EXPOSED' TO PESTICIDES, TO ORGANIC SOLVENTS AND IN PATIENTS ANAMNESTICALLY 'NON-EXPOSED'*

	Exposed		
	Pesticides	Solvents	Non-exposed
FAB subtypes†	M1(1); M2(7); M4(6); M5(4); M6(1); M7(2)	M1(2); M2(2); M3(1); M4(1); M5(2); M6(1)	M1(4); M2(11); M3(2); M4(13); M5(9); M6(1)
Age: (years)	50–82 (69)	26–74 (55)	14–79 (64)
Hb (gr/dl)	5.0–10.5 (8.8)	5.4–9.5 (7.6)	5.6–13.7 (9.6)
WBC ($\times 10^9$/l)	2.1–83.9 (7.7)	1.3–12.3 (4.8)	1.0–249.0 (15.6)
Plts ($\times 10^9$/l)	20.0–488.0 (85)	18–243.0 (85)	26.0–240.0 (71)
% bls (PB)	4–82 (39)	3–70 (30)	2–95 (64)
% bls (BM)	30–90 (50)	34–85 (58)	35–90 (70)
CR‡	3/15	0/6	16/32
Survival (months)	1–78+ (2)	1–8 (2)	1–36+ (9)

* Results are reported as variation range, median value in parentheses.
† Number of cases in each FAB category.
‡ Number of complete remissions/Number of patients treated with myeloablative chemotherapy.

in 6 patients. In this patient group, myelodysplasia affecting more than one cell lineage was seen in 7 patients only, 1 of which fulfilled the cytologic criteria for the diagnosis of AML with trilineage myelodysplasia.

(b) *Immunophenotype.* In general, immunophenotype was in agreement with the cytologic classification according to the FAB criteria [15] (Table 2). Positivity for the CD34 stem cell marker [16] was detected in 20/25 patients in the exposed group and in 7/32 patients in the non-exposed group. Unequivocal positivity for the CD41 platelet antigen was found in a minority of cells (5–10%) in 7 patients categorized as exposed and in 1 patient non-exposed. Inappropriate expression of lymphoid antigens was tested in 25 exposed patients, 6 of which were found to be positive for the CD10 (4 cases), and for the CD7 (2 cases). CD7 and CD19 positivity was also found in 2 and 1 non-exposed patients respectively (32 non-exposed patients tested).

Cytogenetics

Detailed results in each category of patients are reported in Tables 3 and 4. Overall, clonal chromosome aberrations were detected in 17/21 patients exposed to pesticides, in 9/9 patients exposed to organic solvents and in 13/40 patients anamnestically non-exposed. Ten 'exposed' patients (5 to pesticides and 5 to organic solvents) had 3 or more events of translocation or non-disjunction in the same clone, thus fulfilling the definition of 'major karyotype aberrations' (MAKA) [17]. Recurring chromosome aberrations in exposed patients were −5/5q− (8 patients), deletions or translocations of 17p (5 patients), deletion or translocations of 16q22 (4 patients), −7/7q− and trisomy of 21q (3 patients), trisomy of 11q (2 patients). Breaks at bands 6p23, 7p14, 11p14 − 15, 11q13 were found in 2 patients each.

In the non-exposed group, two patients had the t(15;17), 2 patients had aberrations of the long arms of chromosome 3 and 6, and 3 patients had a 5q- chromosome, 1 of which had MAKA.

DISCUSSION

This report extends previous observations on the correlation between environmental exposure and cytogenetic patterns in AML [1, 2], confirming that (a) patients exposed to organic solvents and to pesti-

TABLE 2. IMMUNOLOGIC FINDINGS IN AML PATIENTS CLASSIFIED ACCORDING TO THE FAB SYSTEM (BENNETT 1985): 'EXPOSED' SUBJECTS AND 'NON-EXPOSED' SUBJECTS*

Immunologic markers	FAB subtype M1	M2	M3	M4	M5	M6	M7
CD34							
Exposed	2/2	8/8	0/1	6/7	2/4	1/1	1/2
Non-exposed	1/3	5/9	0/1	1/10	0/8	0/1	0/0
CD33							
Exposed	2/2	8/8	1/1	7/7	3/4	1/1	2/2
Non-exposed	2/3	9/9	1/1	10/10	3/8	1/1	0/0
CD13							
Exposed	2/2	8/8	1/1	7/7	2/4	1/1	2/2
Non-exposed	2/3	6/9	1/1	10/10	3/8	1/1	0/0
CD14							
Exposed	0/2	2/8	0/1	7/7	3/4	0/1	1/2
Non-exposed	0/2	0/6	0/1	9/9	7/7	0/1	0/0
CD41a†							
Exposed	1/2	2/6	0/1	4/7	0/2	0/1	2/2
Non-exposed	1/3	0/6	0/1	0/9	0/7	0/1	0/0
Lymphoid							
Exposed	1/2	2/8	0/1	3/7	1/4	0/1	0/2
Non-exposed	0/3	1/9	0/1	1/10	1/8	0/1	0/0

* Number of positive patients/Number of patients tested.

† Patients with 5–10% unequivocally positive blasts (minor megakaryoblastic component) are classified as positive cases in this table. Only AML-M7 had more than 20% positive cells.

cides have a higher incidence of clonal aberrations than patients anamnestically non-exposed; (b) chromosomes 5 and 7 are non-randomly involved in AML following occupational exposure to toxic agents; (c) complex karyotypes of the 'MAKA' type (defined by the presence of at least 3 aberrant events in the same clone) are found not only in SL, but in patients exposed to environmental hazards as well.

In addition, our study shows that other recurring chromosome changes, whose presence was not emphasized before, may be frequently encountered in exposed patients.

In 5 cases we could detect aberrations involving the short arm of chromosome 17 (either translocations of total/partial monosomy). Chromosome 17p aberration was the sole anomaly in 1 patient and was seen in all abnormal cells in patients with multiple related clones, thus outruling the possibility that this chromosome change may represent a late event in the cytogenetic evolution of highly abnormal cell lines. At present the molecular defect associated with 17p aberrations is unknown; it should be outlined, however, that a tumor suppressor gene encoding the p53 protein was mapped to this chromosome region [18]. The detection of *p53* gene point mutations in some patients with hematologic neoplasias [19] suggests the possibility that this molecular event, in association with the loss of the normal allele on the deleted chromosome 17, may play a role in leukemogenesis, as already demonstrated for the retinoblastoma gene [20]. Interestingly, the short arm of chromosome 17 has recently been added to the list of chromosomal sites non-randomly involved in SL [21].

Four patients in this series showed structural aberrations involving chromosome 16, with breakpoint at band q22. Unlike the classical inv(16) [22] aberrations of 16q22 were observed in the context of complex karyotypes and were associated with trilineage myelodysplasia, in the absence of abnormal eosinophils. The association of 16q22 breaks and professional or iatrogenic exposure was not documented previously, however the existence of clinicopathological differences between AML with the classical inv(16) and with del(16)(q22) were recently emphasized, the latter chromosome change having been found in AML preceded by myelodysplasia without bone marrow eosinophilia [23].

Other recurring cytogenetic aberrations in exposed subjects were structural changes of chromosome 6p and 7p, trisomy 11q, breaks at band 11q13. These chromosome regions are commonly involved in a spectrum of myeloid stem cell disorders, including MDS, SL and myeloproliferative syndromes [24–27] and may thus be related to the transformation of an early progenitor cell rather than being specifically associated with a subset of AML [28]. Interestingly, both in this study and in the literature [1–4] the classical t(8;21), t(15;17) and t(9;11) were detected unfrequently in exposed patients. Globally, the suggestion can be drawn from these data that specific chromosome regions may be the target both of myelotoxic agents present in the workplace and of cytotoxic therapy [29]. According to recent findings, however, 11q23 rearrangements may be associated with a clinicopathological subset of SL [30] whereas no patient with breaks involving band 11q23 was detected in our exposed patients. At present it is unclear whether this finding reflects true heterogeneity in the cytogenetic pattern of SL and environmentally induced AML, or whether 11q23 breaks, the presence of which was not emphasized in the former studies of SL, will become obvious in environmentally induced AML as more cases will be studied.

Among exposed patients with an abnormal karyotype, only abnormal metaphases were detected in 67% (6/9) patients exposed to organic solvents and in 29% (5/17) patients exposed to pesticides, thus suggesting that an entirely abnormal karyotype may be frequently associated with a history of exposure

TABLE 3. CLONAL CHROMOSOME ABERRATIONS IN 30 AML PATIENTS CATEGORIZED AS 'EXPOSED' (4 PATIENTS EXPOSED TO PESTICIDES HAD NORMAL KARYOTYPE)

Patient, age*	FAB	Karyotype	Abnormal cells/ normal cells
Pesticides			
(1) 64	M7	46,XY,del(7)(q22q35)/46,XY,del(7)(q22q35),−9	12/0
(2) 73	M7	45,XY,−17	4/6
(3) 61	M2	45,XY,−5,i(17q)	7/3
(4) 61	M4	47,XY,+8	5/5
(5) 51	M4	45,XX,−5,t(22;?)(q11;?)	6/6
(6) 73	M2	46,XY,del(11)(q13q23)	8/4
(7) 74	M4	46,XY,t(11;?)(p15;?)	2/10
(8) 69	M4	46,XX,del(6)(q22)	14/6
(9) 67	M4	46;XX,i(1q+)/46,XX,i(1q+),i(11q)	8/3
(10) 74	M4	44,XY,del(5)(q11q34),−7,−14,t(16;?)(q22;?)	8/3
(11) 82	M1	45,XY,t(1;7)(p14;p11),del(3)(p11p15),−5, +der(5)t(5;17)(q31;q11),−17,t(17;?)(p?;?)/ same without the der(5)chromosome	10/0
(12) 50	M4	44,XY,−6,+der(6)t(6;7)(p23;p14),−7,−16, +der(16)t(11;16)(q13;q22),−19,+M1/ same without M1	10/0
(13) 69	M2	47,XY,+M(E-size)	10/0
(14) 56	M2	44,XY,−3,−5,t(15;?)(q21;?),i(21q)/45,XY,−3,t(15;?)	12/0
(15) 72	M2	43,XY,−5,del(11)(q23),t(15;12;13)(p11;q13;q13), del(16)(q22),t(17;?)(p12;?),−18,−21	10/4
(16) 68	M5	46,XY,t(8;?)(q23;?)	3/11
(17) 77	M5	47,XY,+20	3/15
Organic solvents			
(18) 70	M3	47,XY,ins(14;2)(q23,q13q37),t(5;?)(q31;?),t(11;12) (p14;q12),+21	10/0
(19) 63	M5	47,XY,+10	12/0
(20) 33	M6	49,XY,+11,t(13;?)(p11;?),+M1,+M2/50,XY,+11, t(13;?)(p11;?),+M1,+M2,+M3	10/7
(21) 55	M2	44,XX,del(5)(q13q33),del(16)(q22),t(17;?)(p12;?),−18,t(20;?) (p11;?),−21/45,XX,del(5)(q13q33),t(17;?)(p12;?),t(20;?) (p11;?),−18,−21,+M	10/0
(22) 43	M2	46,XY,t(6;9)(p23;q34)	10/0
(23) 40	M6	46,XY,t(13;?)(q14;?),del(20)(q11q13),−8	12/5
(24) 74	M5	47,XY,+21	10/0
(25) 26	M2	47,XX,t(8;21)(q22;q22),del(15)(q11q15),+20	10/0
(26) 71	M1	45,XX,−21	3/10

* Years.

to organic solvents. Since *in vitro* culture conditions were the same in all patients studied, the difference of the karyotype status in patients exposed to organic solvents and to pesticides is unlikely to represent a technical artifact and it may in fact reflect heterogeneity of cytogenetic evolution of preleukemic/leukemic clones in the two groups of patients.

Comparative analysis of morphologic, immu-

TABLE 4. CLONAL CHROMOSOME CHANGES IN 40 AML PATIENTS CATEGORIZED AS 'NON-EXPOSED' (27 PATIENTS HAD A NORMAL KARYOTYPE)

Patient, age*	FAB	Karyotype	Abnormal cells/ normal cells
(1) 47	M4	47,XX,+4	6/4
(2) 68	M4	46,XY,del(5)(q14q21)	5/8
(3) 16	M3	46,XY,t(15;17)(q22;q12)/46, XY,t(15;17)(q22;q12),+22	10/0
(4) 25	M1	45,XY,inv(3)(q21q26),−8/46, XY,inv(3)(q21q26),del(8q)	9/2
(5) 21	M3	46,XX,t(15;17)(q22;q12)	10/0
(6) 62	M2	46,XY,t(8;21)(q22;q12)	4/10
(7) 71	M2	45,XX,−4,del(5)(q21q32),+10,−14,del(17p),+M	8/3
(8) 24	M2	46,XY,del(3)(q21q26),dup(17)(q12→q14)	12/4
(9) 64	M2	46,XX,del(6)(q22)/46,XX,del(6)(q22),+22	10/0
(10) 60	M4	46,XX,t(6;?)(q15;?)	7/7
(11) 31	M5	46,XX,t(9;11)(p21;q23)	7/10
(12) 63	M1	46,XX,del(5)(q13q14)/same with +21	11/12
(13) 56	M2	46,XX,del(4)(p15)/same with del(6)(q12q15)	5/15

* Years.

nologic and clinical data in the two patient groups documents that AML following environmental exposure to pesticides and organic solvents may show distinct clinicopathological features as compared with AML in non-exposed subjects.

Morphologically, patients exposed to pesticides and to organic solvents had unequivocal signs of disordered maturation of the non-blast cell population. Since identification of myelodysplasia in leukemic bone marrow may be somewhat arbitrary, we classified our patients according to previously published criteria for the diagnosis of AML with TMDS [8]. While over 60% of patients exposed fulfilled the stringent criteria for the diagnosis of AML with TMDS, morphologic aberrations were confined to a minority of erythroid, myeloid and megakaryocytic cells in the majority of patients non-exposed. These findings in AML following environmental exposure recall the cytologic picture of therapy-related leukemia, a disorder commonly regarded as a panmyelosis [31] and seem to indicate that AML in exposed patients may frequently be associated with a pre-clinical myelodysplastic phase, possibly due to the involvement of a multipotent progenitor cell retaining the capability to differentiate along multilinage pathways [32].

The frequent positivity for the CD34 stem cell marker in exposed patients (80% of cases tested), along with the presence of a minor megakaryoblastic component documented immunologically, seem to support this argument. The occurrence of multipotent stem cell involvement with multilineage differentiation of the leukemic clone has been documented previously in secondary leukemia and therapy related MDS [33].

According to our data, not only AML in exposed patients represents a cytologic and cytogenetic entity, but may show distinct clinical features as well. Globally, these patients may present with lower white blood cell count and lower percentage of bone marrow blasts than patients non-exposed. Interestingly, one of these patients, reported in detail elsewhere [34], showed a classical MDS with the features of refractory anemia with excess of blasts after remission induction with conventional chemotherapy. This pattern of relapse was previously described in some patients with AML with TMDS [35].

The difference observed in this series in terms of complete remission rate and overall survival in the two groups of patients is striking, especially when considering that other prognostic indicators such as FAB subtype, age at presentation and some hematologic parameters were similar in the two groups. Because cases so far reported are few in number the interpretation of these findings must be cautious; however since chromosome findings represent an independent prognostic indicator [36] the association

of karyotype anomalies, frequently of the MAKA type, with occupational exposure, may have influenced clinical outcome in our study.

Acknowledgements—Work supported by CNR, Grant No. 88.00573.44 and by Fondi regionali.

REFERENCES

1. Mitelman F., Nilsson P. G., Brandt L., Alimena G., Gastaldi R. & Dallapiccola B. (1981) Chromosome pattern, occupation, and clinical features in patients with acute non-lymphocytic leukemia. *Cancer Genet. Cytogenet.* **4,** 197.
2. Golomb H. M., Alimena G., Rowley J. D., Vardiman J. W., Testa J. R. & Sovik C. (1982) Correlation of occupation and karyotype in adults with acute nonlymphocytic leukemia. *Blood* **60,** 404.
3. Narod S. A. & Dube' I. A. (1989) Occupational history and involvement of chromosomes 5 and 7 in acute nonlymphocytic leukemia. *Cancer Genet. Cytogenet.* **38,** 261.
4. Whang-Peng J., Young R. C., Lee E. C., Longo D. L., Schecter G. P. & DeVita V. T. (1988) Cytogenetic studies in patients with secondary leukemia/dysmyelopoietic syndrome after different treatment modalities. *Blood* **71,** 403.
5. Brandt L. (1992) Exposure to organic solvents and risk of hematological malignancies. *Leukemia Res.* **16,** 67.
6. Bennett J. M., Catovsky D., Daniel M. T., Flandrin G., Galton D. A. G., Gralnick H. R. & Sultan C. (1985) Proposed revised criteria for the classification of acute myeloid leukemia: a report of the French–American–British Cooperative group. *Ann. Int. Med.* **103,** 626.
7. Bennett J. M., Catovsky D., Daniel M. T., Flandrin G., Galton D. A. G., Gralnick H. R. & Sultan C. (1985) Criteria for the diagnosis of acute leukemia of the megakaryocytic lineage (M7): a report of the French–American–British Cooperative group. *Ann. Int. Med.* **103,** 460.
8. Brito-Babapulle F., Catovsky D. & Galton D. A. G. (1987) Clinical and laboratory features of *de novo* acute myeloid leukemia with trilineage myelodysplasia. *Br. J. Haemat.* **66,** 445.
9. Erber W. N., Jacobs A., Oscier D., O'Hea A. M. & Mason D. Y. (1987) Immunocytochemical detection of cells of megakaryocytic lineage in haematological disorders. In *Leukocyte Typing III* (McMichael, Ed.), p. 779. Oxford University Press, Oxford.
10. de la Maza M. & Sanchez O. (1976) Simultaneous G and C banding of human chromosomes. *J. Med. Genet.* **13,** 235.
11. ISCN (1985) *An International System for Human Cytogenetic Nomenclature* (Harnden D. G. & Klinger H. P., Eds): in collaboration with *Cytogenet. Cell Genet.*, Kargen, Basel, 1985; also in *Birth Defect: Original Article Series*, **21,** (No. 4) March of Dimes Foundation, New York.
12. Worthing C. R. & Walker S. B. (1991) *The Pesticide Manual.* The British Corp Protection Council, London.
13. Nicholas A. H. & Van Den Berghe H. (1982) Sister chromatid exchange and pesticides, with emphasis on organophosphates. *Sist. Chromat. Exchange* **20,** 327.
14. Bennett J. M., Catovsky D., Daniel M. T., Flandrin G., Galton D. A. G., Gralnick H. R., Sultan C. & the French–American–British (FAB) Cooperative Group (1982) Proposals for the classification of the myelodysplastic syndromes. *Br. J. Haemat.* **51,** 189.
15. Drexler H. G. (1987) Classification of acute myeloid leukemias: a comparison of FAB and immunophenotyping. *Leukemia* **1,** 697.
16. Katz F. E., Tindle R., Sutherlands D. R. & Greaves M. F. (1985) Identification of a membrane glycoprotein associated with haemopoietic progenitor cells. *Leukemia Res.* **9,** 191.
17. Sandberg A. A. (1990) *The Chromosome of Human Cancer and Leukemia* 2nd ed. Elsevier North Holland, New York.
18. Levine A. J., Momand J. & Finlay C. A. (1991) The *p53* tumour suppressor gene. *Nature* **351,** 453.
19. Gaidano G., Ballerini P., Gong J. Z., Inghirami G., Neri A., Newcomb E. W., Magrath I. T., Knowles D. M. & Dalla Favera R. (1991) p53 mutations in human lymphoid malignancies: Association with Burkitt lymphoma and chronic lymphocytic leukemia. *Proc. natn. Acad. Sci. U.S.A.* **88,** 5413.
20. Benedict W. F., Xu H. J., Hu S. X. & Takahashi R. (1990) Role of retinoblastoma gene in the initiation and progression of human cancer. *J. clin. Invest.* **85,** 988.
21. Pedersen-Bjergaard J., Philip P., Larsen S. O., Jensen G. & Byrsting K. (1990) Chromosome aberrations and prognostic factors in therapy-related myelodysplasia and acute nonlymphocytic leukemia. *Blood* **76,** 1083.
22. LeBeau M. M., Larson R. A., Bitter M. A., Vardiman J. W., Golomb H. M. & Rowley J. D. (1983) Association of an inversion of chromosome 16 with abnormal marrow eosinophils in acute myelomonocytic leukemia. *New Engl. J. Med.* **309,** 630.
23. Ohyashiki K., Ohyashiky J. O., Kondo M., Ito H. & Toyama K. (1988) Chromosome change at 16q22 in nonlymphocytic leukemia: clinical implication on leukemia patients with inv(16) versus del(16). *Leukemia* **2,** 35.
24. Cuneo A., Kerim S., Vandenberghe E., Michaux J. L., Bosly A., Boogaerts M., Zachee P., Dal Cin P. & Van Den Berhghe H. (1989) Translocation t(6;9) occurring in acute myelofibrosis myelodysplastic syndrome, and acute nonlymphocytic leukemia suggests multipotent stem cell involvement. *Cancer Genet. Cytogenet.* **42,** 209.
25. Mecucci C., Van Orshoven A., Boogaerts M., Michaux J. L. & Van Den Berghe H. (1989) Characterization of deletion of chromosomes 7 short arm occurring as primary karyotypic anomaly in acute myeloid leukemia. *Br. J. Haemat.* **71,** 13.
26. Mecucci C., Van Orshoven A., Vermaelen K., *et al.* (1987) 11q-chromosomes are associated with abnormal iron stores in myelodysplastic syndromes. *Cancer Genet. Cytogenet.* **27,** 39.
27. Takasaki N., Kaneko Y., Maseki N. & Sakurakai M. (1988) Trisomy 11 in chronic myelomonocytic leukemia: report of two cases and review of the literature. *Cancer Genet. Cytogenet.* **30,** 109.
28. Castoldi G. L., Cuneo A. & Tomasi P. (1989) Phenotype-related chromosome aberrations and stem cell involvement in acute myeloid leukemia. *Haematologica* **74,** 525.

29. Mecucci C., Michaux J. L., Louwagie A., Boogaerts M. & Van Den Berghe H. (1988) The short arm of chromosome 6 is nonrandomly rearranged in secondary myelodysplastic syndrome. *Cancer Genet. Cytogenet.* **31,** 147.
30. Pedersen-Bjergaard J. & Philip P. (1991) Two different classes of therapy-related and *de-novo* acute myeloid leukemia? *Cancer Genet. Cytogenet.* **55,** 119.
31. Foucar K., McKenna R. W., Bloomfield C. D., Bowers T. K. & Brunning R. D. (1979) Therapy-related leukemia: a panmyelosis. *Cancer* **43,** 1285.
32. Cuneo A., Mecucci C., Kerim S., Vandenberghe E., Dal Cin P., Van Orshoven A., Rodhain J., Bosly A., Michaux J. L., Martiat P., Boogaerts M., Carli M. G., Castoldi G. L. & Van Den Berghe H. (1989) Multipotent stem cell involvement in megakaryoblastic leukemia; cytologic and cytogenetic evidence in 15 patients. *Blood* **74,** 1781.
33. Tabilio A., Herrera A., D'Agais M. F., Vaichenker W., Allard C., Clauvel J. P. & Breton-Gorius J. (1989) Therapy-related leukemia associated with myelofibrosis. Blast cell characterization in six cases. *Cancer* **54,** 1382.
34. Cuneo A., Carli M. G., Piva N., Fagioli F., Vandenberghe E., Dal Cin P., Castoldi G. L. & Van Den Berghe H. (1991) Clinicopathological evolution and multilineage involvement in erythroleukemia: report of a case. *Haematologica* **76,** 235.
35. Brito-Babapulle F., Catovsky D. & Galton D. A. G. (1988) Myelodysplastic relapse of *de novo* acute myeloid leukemia with trilineage myelodysplasia: a previously unrecognized correlation. *Br. J. Haemat.* **68,** 415.
36. Yunis J. J., Brunning R. D., Howe R. B. & Lobell M. (1984) High-resolution chromosomes as an independent prognostic indicator in adult acute nonlymphocytic leukemia. *New Engl. J. Med.* **311,** 818.