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Clinical and Cytogenetic Correlations in 63 Patients With Therapy-Related Myelodysplastic Syndromes and Acute Nonlymphocytic Leukemia: Further Evidence for Characteristic Abnormalities of Chromosomes No. 5 and 7

By Michelle M. Le Beau, Kathy S. Albain, Richard A. Larson, James W. Vardiman, Elizabeth M. Davis, Richard R. Blough, Harvey M. Golomb, and Janet D. Rowley

Clinical, histologic, and cytogenetic features in 63 patients with a therapy-related myelodysplastic syndrome (t-MDS) or acute nonlymphocytic leukemia (t-ANU) following cytotoxic chemotherapy or radiotherapy for a previous disease were analyred. Eleven patients had received only radiotherapy for the primary disorder. In most cases, high doses had been administered to treatment ports that included the pelvic or spinal bone marrow. Twenty-one patients had received only chemotherapy for their primary disease, all for more than 1 year and all but one with an alkylating agent, either alone or in combination with other drugs. Thirty-one patients had received both radiotherapy and chemotherapy, either concurrently or sequentially. A clonal chromosomal abnormality was observed in marrow or blood cells from 61 of the 63 patients (97%). Fifty-five patients (87%) had a clonal abnormality of chromosomes no. 5 and/or 7 consisting of loss of all or part of the long arm of the chromosome. The critical chromosome region that was consistently deleted in all 17 patients with del(5q) comprised bands q23 to q32. In addition to nos. 5 and 7, five other chromosomes (no. 1, 4, 12, 14, and 18) were found to be nonrandomly involved. Both t-MDS and t-ANLL ore late complications of cytotoxic therapies thot have distinctive clinical and histologic features and are associated with characteristic aberrations of chromosomes no. 5 and 7. It seems likely that these two chromosomes contain genes involved in the pathogenesis of these hematopoietic neoplasms.
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A DISTINCTIVE disorder of bone marrow morphology and function that terminates in a myelodysplastic syndrome (MDS) or in acute nonlymphocytic leukemia (ANLL! has been recognized as a late complication of cytotoxic therapy used in the treatment of both malignant and nonmalignant diseases.[1–15]

Therapy-related MDS (t-MDS) and acute nonlymphocytic leukemia (t-ANLL) represent distinct clinical syndromes that exhibit important differences from ANLL that arises de novo.[4–6,10] In patients with no prior history of exposure to known mutagens, ANLL de novo usually appears suddenly. whereas t-ANLL is often preceded by a secondary MDS (or preleukemic phase) that may persist for several months. Frequently. all three hematopoietic cell lines. er>-throid. myeloid, and megakaryocytic. are involved in this myelodysplastic process. and patients characteristically manifest an initial pancytopenia.[6,16] Infection, bleeding. and anemia are common complications and may prove fatal even before overt leukemia evolves. Survival times of patients with t-ANLL are often short because this disorder has been less responsive to current forms of leukemia treatment than has ANLL de novo.[18] In the future, as a greater fraction of patients survive treatment with cytotoxic drugs or ionizing radiation for their primary cancer. the incidence of t-MDS or t-ANLL arising secondary to such treatment may be expected to increase

Characteristic nonrandom chromosome abnormalities are commonly observed in bone marrow cells of patients with t-MDS or t-ANLL.[4,5,19–22] These abnormalities differ in their type and frequency from those noted in ANLL

From the Departments of Medicine and Pathology, and The Franklin McLean Memorial Research Institute, The University of Chicago

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Address reprint requests in Michelle M. Le Beau, PhD, Box 420 University of Chicago Medical Center, 5841 S Maryland Ave Chicago, IL 60637.

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developing de novo. We reported previously that part or all of chromosomes no. 5 and or 7 was lost in cells from 23 of 26 (88%) t-MDS t-ANLL patients.' and this has been confirmed by others.'''' In contrast, only about 16% of patients with ANLL de novo have a similar abnormality of chromosomes no. 5 or 7 or both.': Moreover. the latter patients frequently have had significant occupational exposure to potential environmental carcinogens such as chemicals. solvents. or pesticides.[24,25] Another major difference between the karyotypic pattern of t-ANLL and that of ANLL de novo involves the frequency of nonrandom structural chromosome rearrangements. In t-ANLL, one seldom finds the specific rearrangements that are closely associated with the distinct morphologic subsets of ANLL de novo such as the t(15;17) in acute promyelocytic leukemia. the t(8;21) in acute myelogenous leukemia with maturation. or the inv(16) in acute myelomonocytic leukemia with abnormal eosinophils.[26,27]

This report details the clinical. histopathologic, and cytogenetic data on 63 patients with t-MDS and/or t-XNLL. thus expanding the findings on our earlier series of 26 patients.'.' Based on these data. we have asked the following questions: (1) Was there a correlation between the type or dose of radiotherapy or chemotherapy and the development of t-MDS/t-ANLL? (2) What was the median time from primary therapy to bone marrow dysfunction'? (3) Was the high frequency of abnormalities of chromosomes no. 5 and 7 confirmed in these cases and. if so. how were these abnormalities related to the development of t-ANLL'? (4) Was there a relationship between the chromosomal changes and either the primary disease or the type of primary therapy'? (5) Were abnormalities of chromosomes other than no. 5 or 7 observed more frequently in these patient, with t-ANLL than in patients with ANLL de novo in a concurrently analyzed series? (6) Did specific chromosomal abnormalities correlate with response to antileukemia therapy or with median survival after the diagnosis of t-ANLL? (7) By analysis of the chromosomal breakpoints in cells with a deletion of no. 5 or 7, could the smallest region that is consistently deleted in every patient. ie. the critical region. be defined? The identification of this region is of crucial importance because it may contain genes whose altered function can result in hematopoietic neoplasia.

MATERIALS AND METHODS

Clinical and Morphologic Analysis

The 63 patients described here include all those who were first seen at or were referred to the University of Chicago Medical Center with a diagnosis of t-MDS or t-ANLL that could be confirmed by morphologic study of bone marrow specimens. Each patient had received cytotoxic therapy (chemotherapy. radiotherapy. or some combination of these) for an antecedent disease. Patients no. 2001 to *2026* and *2036* have been reported previously.[4,5,15] Thirty-nine patients had been treated for their primary disease at the University of Chicago. whereas 24 had been treated at other institutions. Therefore. the total number of similarly treated patients at risk for developing t-ANLL in each primary-disease category is not available. Only one of the 24 patients referred from other institutions was also treated at the Cniversity of Chicago for the subsequent leukemic process: the other 23 were examined at our institution on a consultative basis. or clinical information on these cases was forwarded to the University of Chicago when a specimen was sent for cytogenetic analysis.

Clinical data were collected. and details concerning the primary treatment were obtained from a review of each patient's medical history For the referral cases. clinical data were gathered by direct communication uith the referring physician(s). Radiation therapy pons. the dose of each treatment course in rad. and the doses and duration of each chemotherapy course were determined whenever possible for each patient. Patients were monitored until death or through February 1985. The duration of the secondary myelodysplastic phase. the type of treatment given for the leukemic process and the response. and the survival from the time of initial bone marrow dysfunction were noted

The diagnosis of t-MDS was made when the patient's peripheral blood and bone marrow showed features of dyspoiesis as defined by the French-.American-British (FAB) criteria for MDS and described by us and others as characteristic of the changes seen in t-MDS.[6,27-30] Patients classified as having t-MDS had < *30%* blasts in the marrow. whereas the diagnosis of overt t-ANLL was made when the percentage of blasts were > 30%. as determined from marrow aspirates or as judged from marrow biopsy sections when increased reticulin prevented aspiration. As noted in other studies.[6,27-30] a substantial number of cases could not readily be classified according to FAB criteria or were somewhat atypical: for the purpose of this report. unless otherwise noted. all of these cases are simply listed as t-ANLL

Cytogenetic Analysis

Cytogenetic analyses were performed with quinacrine fluorescence and trypsin-Giemsa banding techniques on bone marrow cells from aspirates or biopsy specimens or on peripheral blood cells obtained at the time of diagnosis. The metaphase cells examined were obtained from direct preparations. from 24- or 18-hour unstimulated cultures. or from methotrexate-synchronized cells cultured for 24 or 48 hours uith phytohemagglutinin-stimulated leukocyte-conditioned medium. Chromosome abnormalities are described according to the International Sys-

tem for Human Cytogenetic Nomenclature.[31] The criteria adopted at the First International Workshop on Chromosomes in Leukemia were used for the identification of abnormal clones.[32]

Statistical Methods

The relationships of chromosomal abnormalities to variables describing clinical features were investigated. For evaluations involving only uncensored variables, ue used primarily scatter plots. comparisons of frequency distributions, and comparisons of means. Censored variables were studied with the techniques of survival analysis Survival curves and their associated medians were computed by the actuarial method, with grouping intervals of 2 months. Descriptive statistics. correlations. chi-squared statistics, and actuarial survival curves and their comparisons were computed with programs of SPSS (Statistical Package for the Social Sciences). When cell sizes were too small for a valid approximation of the chi-squared distribution. Fisher's exact test or the analogous exact test developed by Pagano and Halvorsen was used.[33] All data processing and computations were done on a DEC-20 computer (Digital Equipment. Marlboro, Mass), accessed through the Biomedical Computation Facilities at the University of Chicago.

RESULTS

Clinical Characteristics

The clinical characteristics of the 63 patients are listed in detail in Tables 1 to 3 and are summarized in Tables 4 and 5. There were 31 men and 32 women. ranging in age from 6 to 76 years at the time of diagnosis of treatment-associated bone marrow dysfunction. Based on morphologic findings. t-MDS without subsequent t-ANLL developed in 19 patients (30%), 29 patients (46%) had t-MDS followed by t-ANLL, and 15 patients (24%) had t-ANLL only. It is not known whether any of the latter group had had an antecedent t-MDS because the population at risk had not been monitored prospectively.

Table 4 lists the type of primary disease, the mode of primary therapy. and the time from primary therapy to initial bone marrow dysfunction. There were 23 patients *(37%)* with Hodgkin's disease, ten (16%) with non-Hodgkin's lymphoma, six (10%) with other hematologic malignant diseases. 21 (33%) with various solid tumors. and three (5%) renal transplant recipients. Altogether. 21 patients (33%) had received chemotherapy alone for their primary disease. 11 (17%) had received radiotherapy alone. and 31 (49%) had 'received both modalities.

Of the patients who had received only chemotherapy, all had undergone more than 12 months of treatment. and all but one had received an alkylating agent (most often cyclophosphamide. nitrogen mustard. procarbazine. chlorambucil, or methyl CCNU) either alone or in conibination with other cytotoxic drugs. The single exception was a patient (no. 2052) who received oral doses of 5-fluorouracil (5-FU) daily for 5 years for squamous carcinoma of the rectum. In many cases. the primary chemotherapy doses had been sufficient to cause moderate to severe myelosuppression: but in ten cases. cyclophosphamide. melphalan. or chlorambucil had been taken orally for a period of many months or years only at low daily doses that do not commonly cause significant myelosuppression. Three patients with colorectal carcinoma had received multiple courses of methyl CCNU and 5-FU for a period of 15 to 27 months. Three renal transplant recipients had received cyclophosphamide with or without azathioprine for 1.5 to 16 years.

Nine of the 11 patients who received only primary radiotherapy had had the major portion or all of the pelvic bone marrow included within the initial treatment ports. Two patients with Hodgkin's disease (2.000 rad. 4,000 rad) and one with non-Hodgkin's lymphoma (4.000 rad) had received total nodal irradiation. Two patients with prostate carcinoma had received 5.000 rad to the entire pelvis plus an additional 2,000 rad to the prostate. Two patients with endometrial carcinoma and one with cervical carcinoma received radiotherapy to the entire pelvis by external-beam techniques in addition to radium implants. A second patient with carcinoma of the cervix was treated with a radium implant alone. Of the two radiotherapy patients who did not receive pelvic irradiation, one with laryngeal carcinoma was treated with a laryngeal port only; the other, with metastatic carcinoma of the lung, was treated with mediastinal, hepatic, and femoral ports.

The 31 patients who had received both radiotherapy and chemotherapy were a heterogeneous group. In 16 cases, the radiotherapy and chemotherapy had been administered approximately concurrently. In another nine cases, the radiotherapy had been administered as initial treatment. and chemotherapy was administered only later. at the time of relapse of the primary disease. In most of these cases. high doses (3.000 rad to 6.000 rad) had been administered to bone marrow–containing regions over total nodal fields or pelvic or spinal ports. In the remaining six patients, radiotherapy followed initial che-

Table 1. Clinical Characteristics of 21 Patients With Bone Marrow Dysfunction Following Chemotherapy Only

Patient	Age†/	Primary Disease	Primary Chemotherapy Drugs (Months of Treatment)	Time to BMD (mo)	t-MDS (mo)	t-ANLL	Treament	Response	Survival From BMD (mo)
		, MC, CS IIIB	Multiple combination* (36)	80	no	yes	CA + TG		2
			MOPP (6) Bleo + Vcr + Pred (2)	51	yes (5	yes	CA	—	9
2010	51/F	PDL, N, PS IVA	Vcr + Pred (2) COPP (9) Cy (36, 100 mg/d)	50	(11)	yes	CA + TG	PR	15
2012	60/M	HD, MC, CS IIIA	MOPP (2) Cy-Bleo-Proc (18)	46	(15)	yes	CA + TG + Dnr	no	20
2014	47/F	HD, MC, PS IVB	COPP (9) CCNU (21)	40	(3)	no	none		3
2021	54/F	PDL, D, PS IVB	COPP (18) COPP (6)	26	(8)	no	none	—	8
2022	69/F	myeloma	melphalan + pred (27)	43	(5)	no	none	—	5
2026	63/M	Ca lung, PS IIIM$_1$	CAMP (32)	42	(3)	yes	CA + TG × 3	PR	7
2027	28/F	renal transplant	Cy + Pred (18)	192		yes	CA + TG + Dnr	no	2
2028	40/M	renal transplant	azathioprine + pred (24) Cy + pred (168)	52	es (12)	no	none	—	12
2031	69/F	Ca colon	5FU + MeCCNU (27) melphalan (33)	67	o	yes	CA + G + Adr × 2	CR	20
2034	49/F	Ca breast	chlorambucil (26, daily)	26	es (4)	no	none	—	4
2036	63/M	hairy-cell leukemia	melphalan + 5FU (16)	22	es (10)	yes	Vcr + pred	no	13
2038	54/F	Ca breast	melphalan + pred (40)	40	o	yes	CA + Adr	CR	6
2043	64/M	myeloma	chlorambucil (72, intermittent)	67	es (5)	yes	CA + Dnr × 2	no	7
2049	51/F	Ca ovary	5FU (60, daily)	108	es (4)	yes	CA + Adr	no	5
2052	67/F	Ca anus	chlorambucil (36, daily)	36	es (17)	no	none	—	17
2053	76/F	PDL, D	5FU + MeCCNU (24)	69	es (0)	no	none	—	0
2054	65/M	Ca colon	Cy (36, daily)	173	es (13)	no	retinoic acid	no	14
2058	62/F	myeloma	melphalan + pred (36) VMCP (14)	51	es (8)	yes	high-dose CA	no	
2059	57/M	Ca colon	5FU + MeCCNU (15)	67	no	yes	CA + TG + Dnr × 2	no	9
2064	25/M	HD, MC, PS III$_2$B	methotrexate + MOPP (6) MOPP (9)				CA + Adr	CR	15

Abbreviations: HD, Hodgkin's disease; MC, mixed cellularity; PDL, poorly differentiated lymphocytic lymphoma; N, nodular; D, diffuse; CS, clinical stage; PS, pathologic stage; Ca, carcinoma; MOPP, nitrogen mustard, Oncovin (Lilly, Indianapolis procarbazine, preddnisone; Bleo, bleomycin; Vcr, vincristine; Pred, prednisone; COPP, cyclophosphamide, Oncovin, procarbazine, prednisone; Cy, cyclophosphamide; Proc, procarbazine; CCNU, lomustine; CAMP, cyclophosphamide, Adriamycin, methotrexate, procarbazine; 5FU, 5-fluorouracil; MeCCNU, semustine; VMCP, vincristine, melphalan, cyclophosphamide, prednisone; BMD, bone marrow dysfunction; CA, cytarabine; TG, thioguanine; Dnr, daunorubicin; Adr, Adriamycin; PR, partial remission; CR, complete remission.

*Patient numbers 2001 to 2026 have been previously reported on in part as cases 1 to 10 and 11 to 26.[5,6] Patient 2036 was previously reported.[15]

†Age at time of bone marrow dysfunction.

Table 2. Clinical Characteristics of 11 Patients With Bone Marrow Dysfunction Following Radiotherapy

Patient No.	Age/ Sex	Primary Disease	Radiotherapy Port and Dose (rad)	Time to BMD (mo)	t-MDS (mo)	t-ANLL	Treatment	emission	urvival From BMD (mo)
2006	68/F	HD, MC, CS IIIA	TN, 2,000 larynx, 4,000	10	yes (19)	yes	none	—	21
2008	51/F	PDL, N, PS IIIA	TN, 4,000	18	yes (20)	yes	CA + TG	no	23
2019	55/M	HD, MC, PS IIIA	TN, 4,000	83	yes (4)	yes	CA + TG + Adr	no	10
2025	46/F	Ca cervix	pelvis, dose unknown, radium implants × 2	9	no	yes	CA + TG × 3	no	4
							CA + Dnr × 2	no	
							COAP × 2	no	
2033	60/F	Ca endometrium	pelvis, 7,000	34	yes (5)	no	none	—	5
2037	54/M	Ca prostate	prostate, 7,000 pelvis, 5,000	13	yes (43)	no	none	—	3
2041	47/F	Ca endometrium	pelvis, 4,600 radium implant	23	no	yes	CA + AG	no	0
2047	63/F	Ca cervix	radium implant	136	yes (4)	yes	CA + TG	no	5
2048	68/M	Ca prostate	prostate, 7,000 pelvis, 5,000	19	yes (3)	no	CA + TG + Dnr	no	3
2057	63/M	Ca lung	chest, 5,000 liver, 3,000 femur, 3,000	56	no	yes	CA + TG + Dnr	CR	9
2061	74/M	Ca larynx	neck, dose unknown	183	yes (2)	no	CA + TG + Dnr high-dose CA		

Abbreviations: TN, total nodal; COAP, cyclophosphamide, vincristine, cytarabine, prednisone. Other abbreviations are the same as in text.

Table 3. Clinical Characteristics of 31 Patients With Bone Marrow Dysfunction Following Both Radiotherapy and Chemotherapy

Patient No.	Age/ Sex	Primary Disease	Radiotherapy Port and Dose (rad)	Chemotherapy Drugs (Months of Treatment)	Time to BMD (mo)	t-MDS (mo)	t-ANLL	Treatment	Remis-sion	Survival From BMD (mo)
2001	37/F	HD, NS, CS II	mantle, 3,000 left chest, 4,000	chlorambucil (72, daily)	132	no	yes	CA + TG CA + Dnr	no	8
2003	37/M	HD, NS, PS IIIA	TN, 4,000	COPP (12)	42	no	yes	none	—	3
2004	27/F	HD, NS, PS IIIB	TN, 4,000	COPP (17)	70	yes (6)	yes	HU	no	9
2005	50/F	HD, NS, PS IVB	right neck, 2,300 left neck, 1,500	COPP (18)	74	no	yes	none	—	0
2007	31/M	HD, MC, PS IIIA	EM, 4,000 Inv Y, 4,000	MOPP (3) COPP (3)	60	yes (3)	yes	none	—	3
2009	57/F	PDL, N, CS IIIA	left mantle, 4,000	COPP (21)	59	yes (2)	yes	Dnr	no	3
2011	52/M	PDL, N, PS IIIA	TN, 4,000	CVP (6)	60	yes (12)	no	Vcr + Pred	no	12
2013	32/F	HD, NS, PS IVA	EM, 3,500	MOPP + Bleo (6) CCNU + Vlb + Pred + Proc (21)	45	yes (4)	yes	CA + Dnr	no	9
2015	55/M	HD, MC, PS III_EA	TN, 4,000 thorax, 4,500	COPP (6)	100	yes (13)	no	none	—	13
2016	28/M	HD, NS, PS IIA	EM, 4,000 left chest, 4,000	MOPP (9)	72	yes (4)	yes	CA + TG + Dnr	no	5
2017	25/M	HD, MC, PS IIIA	TN, 4,000	MOPP (6)	47	yes (5)	yes	none	—	5
2018	64/M	HD, MC, PS IIB	epigastric, 750 periaortic + splenic, 2,400	MOPP (13)	20	yes (2)	yes	none	—	3
2020	34/M	HD, MC, PS IIIA	EM, 4,000 Inv Y, 2,800	MOPP (3)	46	yes (19)	yes	HU	—	19
2023	62/F	myeloma	lumbar spine, 3,000	Cy + Pred (24) melphalan + pred (24)	51	yes (7)	yes	none	—	8
2024	62/M	myeloma	sacrum, 3,000 hips, 2,400, 2,500	Cy + Pred (24) melphalan + Pred (15)	66	no	yes	CA + TG + Dnr	PR	5
2029	30/M	HD, NS, PS IIIA	TN, 4,400	MOPP (10)	59	yes (6)	yes	CA + TG + Dnr	CR	57 +
2030	53/F	HD, NS	multiple local	MOPP (10)	108	no	yes	CA + TG + Dnr	no	0
2032	40/F	HD, NS, CS IVB	mantle, 2,400	MOPP (9) ABVD (6)	35	yes (3)	yes	high-dose CA	PR	10
2035	55/M	HD, NS, PS IIIB	local, 3,250	MOPP (6) MOPP-Bleo (6) chlorambucil (27, daily) B-DOPA (2) CVPP (4)	82	yes (4)	yes	none	—	7

Table 3. Clinical Characteristics of 31 Patients With Bone Marrow Dysfunction Following Both Radiotherapy and Chemotherapy (Cont'd)

Patient No.-	Age/ Sex	Primary Disease	Radiotherapy Port and Dose (rad)	Chemotherapy Drugs (Months of Treatment)	Time to BMD (mo)	t-MDS (mo)	t-ANLL	Treatment	Remis-sion	Survival From BMD (mo)
2039	66/M	PDL, N, PS IV	multiple local	COPP (8)	84	yes (11)	no	none	—	11
2040	65/F	Ca breast	chest, 6,000 rib, 1,700 rib + pelvis,—	CMF (30) Vcr + Adr + Tamox (6) CMF (6)	119	yes (2)	yes	none	—	3
2042	67/F	Ca breast	local,—	melphalan (10) CMF (23)	59	yes (3)	yes	none	—	3
2044	64/F	MCL, D, PS IVB	spine, 2,000	COPA (6) CCNU + Adr (6)	24	yes (1)	no	none	—	1
2045	51/M	HD, PS IIB	TN, 4,000	C-MOPP (7) CVPP (6)	48	yes (6)	yes	CA + TG + Dnr × 3 high-dose CA	PR	25
2046	57/M	PDL, N, PSIV	mantle, 2,000 pelvis, abdomen, 3,000	CVP (38)	117	yes (5)	yes	none	—	6
2050	6/M	lymphoma	mediastinum,—	LSA_2-L_2 (26)	36	no	yes	CA + Dnr + Pred × 2 Asp + Vcr + Pred	no no	14
2051	58/M	Ca rectum	abdomen, 2,000	5FU (48) mitomycin C + DTIC (6) 5FU + MeCCNU (16)	105	yes (22)	yes	low-dose CA retinoic acid high-dose CA	no no no	22
2055	76/F	Ca ovary	pelvis, 2,100	CMF + hexamethylmelamine (13)	46	yes (15)	no	none	—	15
2060	64/M	glioblastoma	brain,—	CCNU (7) vinblastine + Pred (14)	20	yes (2)	yes	none	—	2
2061	26/M	HD, NS, PS III_2A	inv Y, 2,000 mediastinum, 800 spine, 1,000 rib, 3,000	COPP/MOPP (9) ABVD (7) BCAVe (8) COPP (6) CCNU + VM-26 + Mtx (11)	72	yes (4)	no	none	—	4
2063	60/M	renal transplant	kidney, 1,350	Cy + azathioprine (19)	69	no	yes	none	—	1

Abbreviations: NS, nodular sclerosis; MCL, mixed large- and small-cell lymphoma; EM, extended mantle; Inv Y, inverted Y; CVP, cyclophosphamide, vincristine, prednisone; B-DOPA, bleomycin, dacarbazine, Oncovin, prednisone, Adriamycin; CVPP, cyclophosphamide, vinblastine, procarbazine, prednisone; CMF, cyclophosphamide, methotrexate, 5-fluorouracil; Tamox, tamoxifen; ABVD, Adriamycin, bleomycin, vinblastine, dacarbazine; BCAVe, bleomycin, cyclophosphamide, Adriamycin, vinblastine; Mtx, methotrexate; Vlb, vinblastine; DTIC, dacarbazine; HU, hydroxyurea; Asp, asparaginase. Other abbreviations are the same as in Table 1.

Table 4. Primary Disease, Type of Therapy, and Time to Bone Marrow Dysfunction

Primary Disease	No. of Patients	Primary Therapy			Mean Time to Bone Marrow Dysfunction (mo)
		Chemotherapy	Radiotherapy	Both	
Hodgkin's disease	23	4	4	17	63
Nan-Hodgkin's lymphoma	10	3	1	6	53
Other hematologic malignant diseases*	6	4	0	2	64
Solid tumors†	21	8	8	5	65
Renal transplants	3	2	0	1	101
Total	63	21	11	31	64
Mean time to bone marrow dysfunction (mo)		64	59	65	
Median time to bone marrow dysfunction (mo)		50	34	60	56
Range (mo)		22–192	10–183	20–132	

*Hairy-cell leukemia, one; multiple myeloma, five.

†Lung carcinoma, two; breast, four, colorectal, four; cervical/endometrial, four; ovarian, two; prostate, two; anus, one; glioblastoma, one; larynx, one.

motherapy. and more commonly. only local fields were irradiated.

Table 4 shows the mean times from initial treatment to subsequent bone marrow dysfunction for all patients (median. 56 months) and for the subgroups based on primary disease and primary therapy. Neither the comparison according to primary disease nor that according to primary-therapy modality was statistically significant despite the unexpected trend of a shorter time to bone marrow dysfunction for the radiotherapy-alone group than for either the chemotherapy-alone group or the group receiving both modalities. These latency intervals. however. may not be accurate because of the variable observation of these patients after the completion of primary therapy. The median age at the time of bone marrow dysfunction was 55 years overall. but was lower for the patients with Hodgkin's disease (37 years) and for the renal transplant recipients (40 years): this observation probably reflects the lower ages of these patients at the time of primary treatment.

In Table 5. the three secondary bone marrow disorders (t-MDS. t-MDS→t-ANLL. t-ANLL) are categorized according to the primary disease and the primary therapy. There were no statistically significant differences in these distributions. The intervals between primary treatment and subsequent bone marrow dysfunction were 43, 51, and 67 months for patients with t-MDS alone. t-MDS evolving into t-ANLL. and t-ANLL alone. respectively. Although interesting. this trend did not reach statistical significance. The median duration of the myelodysplastic phase in the 29 patients in whom t-ANLL subsequently developed was 5 months.

Table 5. Primary Disease, Primary Therapy, and Secondary Disorder

	Number of Patients With Secondary Disorder		
	t-MDS only	t-MDS ↓ t-ANLL	t-ANLL only
Primary disease			
lymphoma	7	19	7
Other hematologic malignant disease	3	1	2
Solid tumor	9	8	4
Renal transplant	0	1	2
Primary therapy			
Chemotherapy only	8	8	5
Radiation only	4	4	3
Both modalities	7	17	7
Total	19	29	15

Response to Treatment and Survival After Bone Marrow Dysfunction

Of the 19 patients with t-MDS only. most died of infection. hemorrhage. or some other complication of pancytopenia. Treatment was supportive only: none of the patients were given intensive antileukemia chemotherapy. Two patients received daily oral doses of *cis*-retinoic acid. and one received daily subcutaneous injections of cytarabine at low doses: none of these patients showed any evidence of response.

The 44 patients in whom t-ANLL developed were treated in various ways. depending in part on the year of diagnosis and the treating institution. Thirteen patients received supportive care only. either because they refused treatment or because of a moribund state at the time of diagnosis. Twenty-eight patients received conventional ANLL remission induction chemotherapy with cytarabine and either 6-thioguanine or an anthracycline drug. and five patients received high-dose cytarabine therapy (3 gm/m^2 even 12 hours). Five patients had a complete remission: four of these had not had an antecedent t-MDS. Three of the complete responders subsequently relapsed and died. one died in remission after bone marrow transplantation. and one is alive with no evidence of disease after 57 months. None of the patients with partial or no response to treatment are alive. Most patients died of infection or bleeding during prolonged periods of bone marrow hypoplasia following intensive chemotherapy.

The median survival time for all 63 patients from the time of initial bone marrow dysfunction was **8** months. No significant differences in survival were noted based on age. sex. primary disease. or primary therapy. There was. however, a statistically significant difference ($P < .05$) in median survival among patients with t-MDS only (6 months). those with t-MDS that evolved into t-ANLL (9 months). and those with t-ANLL only (5 months). This result may be influenced. of course. by the possibility that an antecedent myelodysplastic phase had been undetected in the last group of patients. who had frank t-ANLL at diagnohs.

Cytogenetic Analyses

The results of the cytogenetic analyses are shown in Table 6. None of the 62 patients had received antileukemia therapy before chromosomal analysis. Analyses were performed on bone marrow cells in 54 cases and on cultured unstimulated peripheral blood cells in the remaining nine cases. Clonal chromosomal abnornialities were observed in 61 of the 63 patients (97%). Consistent clonal abnormalities leading to loss of the whole chromosome or loss of par?of the long arm of chromosomes no. 5 or 7. or both of these chromosomes. were observed in 55 of the 61 patients (90%) with abnormal karyotypes (87% of all patients). Abnormalities of chromosome no. 5 occurred in 14 patients; six had a loss of one chromosome no. 5. and eight others had a deletion of the long arm of this chromosome [del(5q)]. Loss of all or part of the long arm of one chromosome no. 7 was noted in 24 patients; 21 had monosomy 7. Patient 2.039 had an unbalanced translocation resulting in monosomy of the long arm of no. 7. and patients 2027 and 2029 had deletions of the long ann. An additional 17 patients had abnormalities of both no. 5 and no. 7. In nine of these 17 patients. the aberration of no. 5 was a del(5q); in four patients. loss of **7q** resulted either from a deletion (two patients) or from an unbalanced translocation (two patients). Thus. among the 55 patients. 14 patients had a −5. 17 patients had a del(5q), 34 patients had a −7. and seven patients had a loss of the long ami of no. 7 that resulted from an unbalanced translocation in three patients. A del(5q) was the most common structural aberration in our series.

Although abnormalities of nos. 5 and **7** were frequently observed in the same clone. these abnormalities may in fact occur independently. That is. the number of cases in which aberrations of both nos. **5** and 7 were observed was similar to that expected to occur by chance. given the high frequency with which abnormalities of either one or the other of these chromosomes were present in these patients. The most common single abnormality was loss of one no. 7: this karyotype was observed in eight patients. On the other hand. of the 31 patients with abnormalities of no. 5. only one (patient no. *2022)* had an alteration of this chromosome [del(5q)] as the sole karyotypic change.

In eight patients it was possible to determine whether an abnormality of no. 5 or no. 7 occurred first because we were able to study chromosome evolution in sequential samples (three

Table 6. Results of Cytogenetic Analysis of 63 Patients With Therapy-Related MDS or ANLL

Patient No.	Specimen	Method*	No. of Metaphase Cells	Percentage Abnormal	Koryotype
2001	BM	direct	18	90	46,XX/44,XX, − 5, − 7, − 16, − 18, + 2mar
2002	BM PB	24 h 24 h, 48 h	11	100	48,XX, − 5, − 16, − 21,t(8;?)(p23;?),t(15;?)(p11;?), + mar1, + mar2, + 2mar3, + mar4
2003	BM	direct	17	100	46,XY,t(1;17)(p36;q21)
2004	PB	24 h, 48 h	10	30	46,XX/45,XX, − 7
2005	BM	direct	12	92	46,XX/45,XX, − 4, − 5, − 14,t(21;?)(q22;?), +2mar
2006	BM	24 h	4	100	43,XX, − 5, − 7, − 12,del(6)(q15orq21), + min
2007	BM	24 h	6	75	46,XY/45,XY, − 5, − 7, − 20, + 2mar
2008	PB	24 h, 48 h	14	93	46,XX/45,XX, − 5, − 7, − 12, − 17, − 22,t(1;?)(p3?6;?),t(13;?)(q34;?),t(14;?)(p11;?),del(15)(q2?4), + 4mar
2009	BM	direct	9	100	46,XX, − 5, − 7, − 17, + 8,del(6)(q13), + 2mar
2010	BM	direct, 24 h	12	77	46,XX/43,XX, − 5, − 13, − 18 (15%)/44,XX, − 5, − 18,t(13;?)(p11;?) (62%)
2011	PB	24 h	13	30	46,XY/46,XY, − 5, + mar
2012	BM	24 h	13	93	46,XY/47,XY, + 8
2013	EM	24 h	21	90	46,XX/46,XX, − 7, − 16, − 21, + t(2;7)(2q7q),del(2)(q33),del(11)(q22), + t(11;?)(p15;?),t(14;?)(p11;?), + der(21)t(21;21;21;16)(21pter→q22::21q11→q22::21q11→q22::16p13→qter)
2014	BM	24 h	23	80	46,XX/45,XX, − 7,inv(1)(p36q13)
2015	BM PB	24 h 24 h, 48 h	13	100	45,XY, − 7
2016	BC	24 h	10	60	46,XY/44,XY, − 7, − 16,t(17;?)(p11;?),del(20)(q11q13) (10%)/43,XY, − 5, − 7, − 12, − 16,t(4;?)(p16;?),t(17;?),del(20), + mar (20%)/43,X,t(Y;?)(q12;?),same (30%)
2017	BM	24 h	12	100	45,XY, − 5, − 7,del(3)(p13), + der(5)t(5;17)(q1?1;q11)
2018	BM	24 h	16	94	46,XY/45,XY, − 7,del(5)(q13q33) (38%)/45,XY,same,t(12;17)(q1?3;p1?2) (4406)
2019	BM	24 h	13	100	45,XY, − 7,del(3)(p1?3),del(8)(q22),del(16)(p12)
2020	BM	24 h	8	88	46,XY/44,XY, − 2, − 3, − 6, − 12, − 15, − 21, − 22,del(2)(p11),del(11)(p1?4),t(14;?)(q32;?),t(17;?)(p13;?),del(22)(q1?1), + 5mar
2021	BM	direct, 24 h	6	0	46,XX
2022	BM	direct	6	100	45,XX, − 13, − 14, + t(13;14)(p13;q11),del(5)(q13q33)†
2023	PB	24 h	22	100	45,XX, − 7,t(3;9)(q29;p21) (73%)/44,X, − X,same (27%)
2024	BM	direct	25	100	51,XY, − 7, − 14, + 1, + 2, + 6, + 8, + 15, + 21, + mar
2025	BM	24 h	5	100	45,XX, − 7,t(3;3)(q21;q26)
2026	BM	24 h	30	100	45,XY, − 7,t(2;3)(p21;q27)
2027	BM	24 h	7	100	44,XX, − 15, − 16, − 19,del(7)(q11), + der(19)t(19;15;8)(19pter→q13::15q11→q26::8q21→qter)
2028	BM	direct, 24 h	19	100	59,XY, + X, − 7, − 12, + 1, + 2, +4, + 11, + 15, + 18, + 19, + 20, + 21, + 21, + del(2)(q2?3q3?1), + t(8;?)(q2?4;?), + 2mar
2029	BM	24 h	15	13	46,XY/46,XY,del(7)(q34)
2030	BC	24 h	20	100	45,XX, − 7
2031	PB	24 h, 48 h	8	100	43,XX, − 7, − 8, − 10, − 22, + mar (50%)/43,XX,same,t(17;?)(p13;?) (37%)/43,XX,same,t(17;?),t(4;12)(q22orq23;p13) (13%)

Table 6. Results of Cytogenetic Analysis of **63** Patients With Therapy-Related MDS or **ANLL** (Cont'd)

Patient No.	Specimen	Method*	No. of Metaphase Cells	Percentage Abnormal	Karyotype
2032	BM	48-h MTX	20	100	45,X, −X, −7, + der(X)t(X;7)(p22;p1?5), t(1;4)(p36;p12), del(5)(q11q34), del(12)(p1?2) (80%)/4 related single cell obnormalities
2033	BM	direct, 24 h	17	24	46,XX/45,X, −X, −12, −16, del(3)(q21), del(5)(q13q33), i(21q), + 2mor
2034	BM	24-h MTX	20	100	47,XX, + 8
2035	BM	24-h MTX	26	77	46,XY/46,XY, −7, + del(1)(p32) (12%)/47,XY, + del(1) (31%)/49,XY, + 8, + 9, + del(1) (19%)/50,XY, same, + 15 (8%)
2036	BC	24 h	2	100	46,XY/47,XY, −5, −12, −15, del(2)(q33), del
	PB	24 h	2	50	(7)(q22q35), del(19)(q12), −del(19), t(12;15)(12q15q), + 2r (25%)/49,XY, −5, del(2), del(7), +mor, + 3r, − Idmin (25%)/45,XY, −5, −12, −15, del(2), del(7), del(19), t(12;15), + r (25%)
2037	BM	48-h MTX	26	73	46,XY 46,XY, del(5)(q13q32orq33) (60%)/45,XY, −1, −13, −18, + del(1)(q1?1), + dic (1;4)(cen or p11;p16), del(2)(q23q36)
2038	PB	24 h, 24-h MTX	13	100	45,XX, −5, −13, −15, + t(13q15q), − mar1 (31%)/45,XX, −5, −13, −15, + t(13q15q), − mar2 (15%)/7 single cell abnormalities, same, + 1 −3mar
2039	BM	24 h, 48-h MTX	19	15	46,XY/46,XY, −7, + der(1)t(1;7)(p11;p11)
2040	BC	24 h	30	100	46,XX, −7, t(21;?)(q22;?), +mar
2041	BM	24 h, 72 h	56	93	46,XX/46,XX, t(15;17)(q22;q21)
2042	PB	24 h	23	96	46,XX/45,XX, −7
2043	BC	24 h	17	76	46,XY/43,XY, −7, − 17, − 18, − 19, t(6;10)(p25;q22), del(9)(q12), inv(11)(p15q13).t(16;?)(p1?1;?), − der(19)t(17;19)(q13;q21) (65%)/44,XY, −7, −17, −19, inv(11), −der(19), t(20;?)(q13;?), del(10)(p13) (12%)
2044	BM	24 h	18	68	46,XX/45,XX, −7 (52%)/45,XX, −7, del(5)(q15q31) (16%)
2045	BM, PB	48-h MTX	22	91	46,XY/45,XY, −7 (82%)/45,XY, −7, del(5)(q23q32) (9%)
2046	BM	24 h, 48-h MTX	25	68	46,XY/48,XY, −4, −7, − 14, − 16, + der(4)t(4;14)(q31;q11), del(5)(q13q33), t(9;?)(p24;?), del(12)(p12p13), t(21;?)(q22;?), + mar1 −5 (36%)/47,XY, −3, same (16%)/47,XY, same, + mar2 −5
2047	BM	direct	34	100	48,XX, + 1, + 11, del(5)(q13q33)
2048	BM	48-h MTX	14	100	46,XY, t(3;4)(q28orq29;q21orq22), del(5)(q23q32), del(9)(q2?2)
2049	BM	24 h	18	100	47,XX, −7, −18, del(4)(q22q31), del(5)(q22q32), del(9)(q13), del(21)(q22) (83%)/43,XX, same, −21 (17%)
2050	PB	24 h, 48-h MTX	20	95	46,XY/45,XY, −7
2051	BM	24 h, 48-h MTX	22	100	45,XY, −7
2052	BM	24 h	23	100	45,XX, −7, −10, −17, −20, −20, + 8, del(5)(q14q34), + der(10)t(10;17)(q22;p1?3), t(12;?)(p11;?), + der(20)t(7;20)(p13;p1?3), + mar1 (57%)/46,XX, −7, −10, −17, −20, −20, + 8, del(5), + der(10), t(12;?), − der(20), + mar2, + mor3 (22%)/5 related single cell abnormalities

Table 6. Results of Cytogenetic Analysis of 63 Patients With Therapy-Related MDS or ANLL (Cont'd)

Patient No.	Specimen	Method*	No. of Metaphase Cells	Percentage Abnormal	Karyotype
2053	BM	24 h, 48-h MTX	20	80	46,XX/44,XX, −7, −18 (25%)/44,XX, −7, −20, del(5)(q12q31) (55%)
2054	BM	24 h, 48-h MTX	22	91	46,XY/46,XY,del(5)(q13q34) (9%)/45, XY, −6,−15, −17, +8, +der(17)t(6;?;17)(q13;?;p13),del(5) (23%)/46,XY,same, +mor
2055	BM	48-h MTX	20	80	46,XX/44,XX, −3, −17, −18, −22,del(5)(q12q31), +der(12)t(12;12)(p11;q21), +der(16)t(16;22)(q24;q13), +der(17)t(3;17)(q11;p13) (35%)/45,XX, same, +mar (45%)
2057	PB	48-h MTX	22	95	46,XY/47,XY, +8,t(15;17)(q22;q21) (55%)/46,XY, −21, −21, +8,t(15;17), +der(21)t(21;21)(p11;q11 or cen;cen) (45%)
2058	BC	24 h	18	100	45,XX, −5, −12, +8,t(4;8)(q3?4;q2?2),dic(4;12)(p14;q13),t(18;?)(p11;?) (39%)/45,XX, some, −6,+der(6)t(6;11)(p22;q13) (61%)
2059	BM	24 h, 48-h MTX	21	90	46,XY/45,XY, −7
2060	BM	24 h, 48-h MTX	21	100	47,XY,t(19;?)(q13;?), +mar1 (19%)/46,XY, −7,t(19;?),t(11;?)(q25;?),del(11)(p13), +mar2 (43%)/44, XY, −7,−21,t(19;?),t(11;?),del (11) (19%)/46,XY, −7,t(19;?),t(11;?), del(11), +mar3 (10%)/2 related single cell abnormalities
2061	BM	24 h	20	90	46,XY/46,XY, −16,del(4)(q21q31),del(5)(q15q31),t(18;19)(p23;p13),t(9;17;13)(q22;p13;q1?4),t(14;?)(p13;?),t(21;21)(p11orp12;q21), +mar1 (40%)/45,XY, −17, some (20%)/45,XY, −17,same, +f(10%)/45,XY, −10, −17,same, +mar2/2related single cell abnormalities
2062	BC	24 h	19	100	45,XY, −21, −21,t(1;6)(q25;q13), del(7)(q22q32 orq11q22),del(9)(q13q22), +mar1 (47%)/45,XY, −21, −21,t(1;6), del(7),del(9), +der(21)t(21;21)(p12;q11), del(5)(q13q33) (37%)/3 reloted single cell abnormalities
2063	BC	24 h	28	0	46,XX
	PB	24 h, 48-h MTX			
2044	BM	24 h	21	90	46,XY/45,XY, −7,(85%)/45, XY, −7,del(6)(q21q24) (5%)

Abbreviations: BM, bone marrow; PB, peripheral blood; BC, bone morrow biopsy.

*Direct refers to direct preporations; 24 h and 48 h refer to cells cultured for 24 or 48 hours; 48-h MTX refers to cells cultured for 48 hours with phytohemagglutinin–leukocyte-conditioned medium and synchronized with methotrexate.

†Patient no. 2022 had a constitutional chromosomal abnormality consisting of o Robertsonion translocation between chromosomes no. 13 and 14.

patients) or because multiple clones showing evolution were identified in the initial sample (five patients). In six of the eight patients, the abnormality of chromosome no. 7 occurred first.

Clonal abnormalities not involving chromosomes no. 5 or 7 were noted in only six patients. Two of these (patients no. 2041 and 2057) had a t(15;17)(q22;q21) and the characteristic morphologic features of acute promyelocytic leukemia.[11] Patients no. 2012 and 2034 each had only a gain

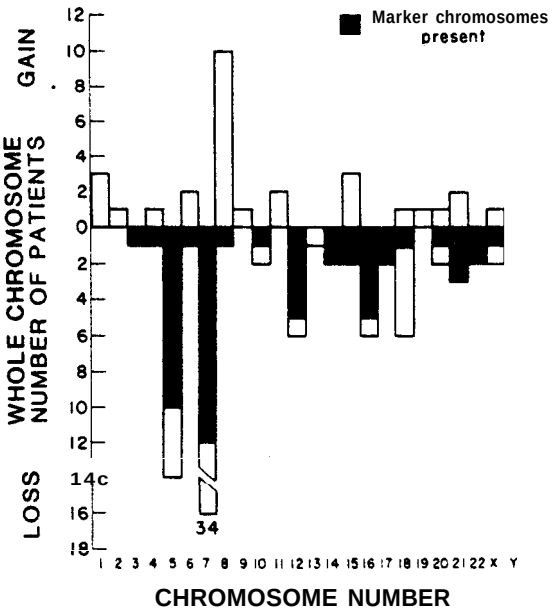

Fig 1. Distribution of the gain and loss of whole chromosomes from the 61 patients with t-MDS or t-ANLL who had clonal abnormalities.

of no. 8. Patient no. 2057 had both a t(15:17) and a gain of no. 8. Various structural rearrangements were noted in the remaining two cases (patients no. 2003 and 2013).

The frequencies of gain or loss of a whole chromosome and of structural rearrangements are illustrated in Figs 1 and 2. With the esception of a gain of no. 8 that was noted in ten patients. a pain of other chromosomes was infrequent. A gain of chromosomes no. 5 or 7 was never seen: however. among patients whose karyotypes shon ed chromosome loss. monosomy for these two chromosomes was observed most frequently. Losses of nos. 12, 16, and 18 were the next most frequent. being observed in five patients each. Chromosomes no. 3. 5. 7. 10. 12. 13. 13. 16. 17. 22. and Y were never gained. and no. 1, 2. 9. 11. 19. and Y were never noted among the losses. Of the structural rearrangements observed (Fig 2), a deletion of 5q was noted in 17 patients. and translocations involving no. 17 were seen in 13 patients. Of the 13 translocations of no. 17. six involved a breakpoint at band p13, and three. a breakpoint at band q21. Rearrangements of chromosomes no. 10, 13, 16, 18. 20, 22. X. and Y were seldom observed.

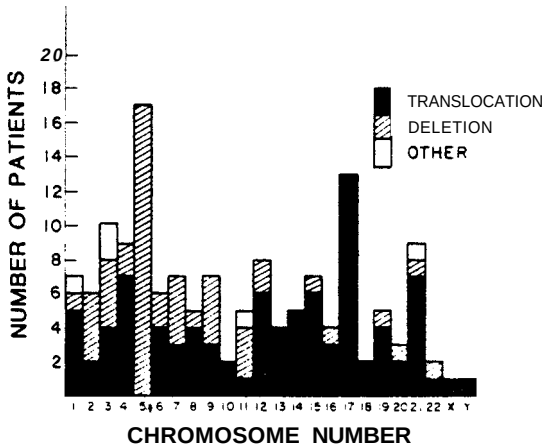

Fig 2. Distribution of the involvement of individual chromosomes in structural rearrangements from the 61 patients with t-MDS or t-ANLL who had clonal abnormalities.

Comparison of the Frequency of Chromosomal Abnormalities in t-ANLL and ANLL De Novo

To determine whether the frequency and types of abnormalities observed in patients with t-MDS and t-ANLL differed from those observed in our own concurrent series of 140 patients with ANLL de novo (79 of whom [56%] had an abnormal karyotype). we compared the number of anomalies of each chromosome in the two groups of patients by using Fisher's exact test.?' The frequency of alterations of seven chromosomes. namely. no. 1, 4, 5, 7, 12, 14, and 18. was found to differ significantly ($P < .05$) between these groups of patients. In each case, abnormalities of these chromosomes occurred more frequently in patients with therapy-related disease (Fig 3). As expected, the greatest differences were found for chromosomes no. 5 and 7. Twenty-eight of the 140 patients (20%) with ANLL de novo had abnormalities of nos. 5 and/or 7, as compared with 55 of 63 (87%) patients with therapy-related disease. Moreover. only 22 of the patients with ANLL de novo (16%) had losses of the long ami or of the entire chromosome no. 5 or 7, whereas these aberrations occurred in all of the 55 patients with therapy-related disease. The frequency of patients with clonal abnormalities involving both nos. 5 and 7 floss or deletion of the long arm) was much higher in t-MDS/t-ANLL (17 of 63. 27%) than in ANLL de novo (five of 140. 4%). The frequency of abnormalities of chromosomes no. 5 and 7 in MDS arising de novo is even lower

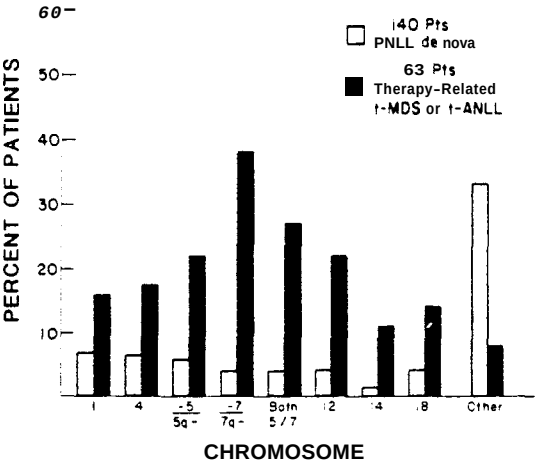

Fig 3. Comparison of the frequency of involvement of specific chromosomes in our patients with ANLL de novo and in those with t-MDS or t-ANLL.

than that seen in patients with ANLL de novo. and thus it is markedly lower than that observed in patients with t-MDS. Of 49 patients with MDS de novo who were evaluated at our institution. 20 (41%) had clonal chromosomal abnormalities, and only six of these patients (12%) had aberrations involving no. 5 or 7.[35] Moreover. two of the six patients had a history of occupational exposure to potential mutagens.

The presence of an abnormality of chromosomes no. 1, 4, 12. 14, or 18 in t-ANLL was not invariably independent of abnormalities of nos. 5 and/or 7. Specifically. most patients with therapy-related disease who had a rearrangement of chromosome no. 4 also had an abnormality of nos. 5 and/or 7 ($P = .04$), and all 14 patients with an abnormality of no. 12 also had rearrangements of no. 5 and/or 7 ($P = .014$). In contrast, the occurrence of an abnormality of chromosomes no. 1, 14, or 18 was not associated with the presence of aberrations of nos. 5 and/or 7. The nature of the abnormalities of nos. 1, 4, 12, 14, and 18 was similar for the patients with therapy-related disease and those with ANLL de novo. with chromosome loss and translocations accounting for most of the aberrations.

Deletions of Chromosomes no. 5 and 7 and the Critical Regions

We have used the karyotypes showing loss of only part of 5q or 7q to define more precisely the location of genes involved in the pathogenesis of t-MDS/t-ANLL. Seventeen patients were found to have a del(5q), which in every case was interstitial rather than terminal. In interstitial deletions, two breaks occur in the long arm, and the intermediate segment is lost. By comparing the chromosomal breakpoints in these patients, one can identify the smallest region that is consistently deleted, ie. the critical region. The breakpoints and the segments that were deleted are illustrated in Fig 4. In most of the patients, the proximal breakpoint was in bands q11 to **q13,** and the distal breakpoint was in band **q33** or **q34;** the critical region consisted of bands **q23** to **q32.** A similar analysis for the critical region of 7q was not possible because only four patients in our series had a del(7q).

Relationship of Clinical Characteristics to Chromosomal Abnormalities

Analysis of the involvement of specific chromosomes among male and female patients revealed no significant associations with sex, nor

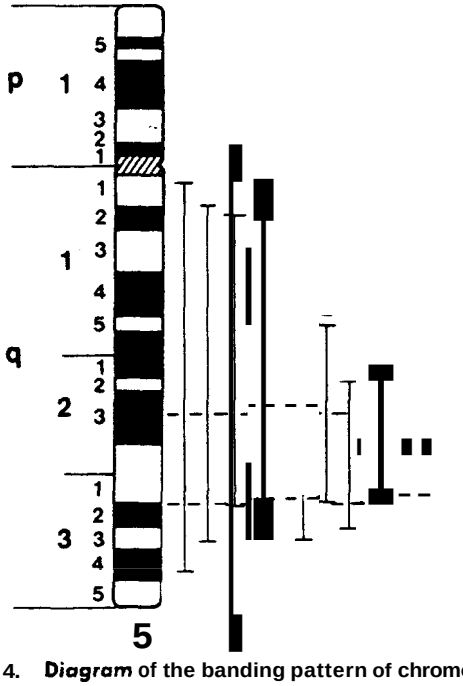

Fig 4. Diagram of the banding pattern of chromosome no. 5 illustrating the chromosomal breakpoints and deletions in 17 patients, Each vertical bar represents the region that was deleted; the numbers above the lines indicate the number of patients with this deletion. The dashed horizontal lines indicate the critical region, ie, the smallest overlapping region that was deleted in each of the patients.

Table 7. Primary Disease, Primary Therapy, and Chromosome Abnormality

	Chromosome Abnormality (n = 61)				
	Abnormality of No. 5	Abnormality of No. 7	Abnormality of Nos. 5 and 7	Other Abnormality, Not No. 5 or 7	Normal (n = 2)
Primary disease					
Lymphoma	4	11	14 (7)	3	1
Other hematologic malignant disease	2 (1)	3	1	0	0
Solid tumor	8 (7)	8	2 (2)	3	0
Renal transplant	0	2	0	0	1
Primary therapy					
Chemotherapy only	6 (2)	8	4 (3)	2	2
Radiation only	5 (5)	2	2	2	0
Both modalities	3 (1)	14	11 (6)	2	0
Total	14 (8)	24	17 (9)	6	2

NOTE: Number in parentheses designates number of patients with a deletion of no. 5.

were there significant associations with patient age. The types of chromosomal abnormalities are shown in Table 7 with the various primary diseases and prima?-therapy categories. There were statistically significant differences in the distribution of abnormalities of no. 5 or 7 or both of these chromosomes based upon the primary disease. Specifically, of the 17 patients with abnormalities of both nos. 5 and 7. 15 (88%) had previously had a hematologic malignant disease ($P = .05$). Sixteen of the 21 (76%) patients whose initial disease was a solid tumor had an abnormality of either chromosome no. 5 or 7. but not both. whereas 14 of the 33 (42%) patients whose primary disease was lymphoma had an abnormal clone involving both nos. 5 and 7. When only patients with a del(5q) were considered, this rearrangement was observed more frequently in patients whose initial disease had been a solid tumor than in patients with a hematologic Primary disease. Thus, of ten patients with prior carcinoma and an abnormality of no. 5, nine had a del(5q) and one had a -5. whereas only eight of 21 patients with a prior hematologic malignant disease and an abnormality of no. 5 had a deletion ($P = .02$).

There were no significant associations between primary-therapy subgroups and chromosomal abnormalities. although aberrations of chromosome no. 7 were seen somewhat more frequently in parients who had received chemotherapy or both modalities than in those who had received radiotherapy (lower section of Table 7). The type of initial therapy was not associated with the subsequent occurrence of abnormalities of no. 5. nor with the nature of the abnormality (loss v deletion). The intervals between the start of therapy for the primary disease and the subsequent development of bone marrow dysfunction were similar when patients were grouped according to the type of chromosomal abnormality (median. no. 5 only, 56 months: no. 7 only, 63 months; both no. 5 and no. 7, 48 months: other abnormalities, 44 months).

The clinical aspects of the secondary disorder were also analyzed with respect to the type of chromosomal abnormality observed (Table 8). Aberrations of no. 5, or 7, or both, were more often associated with the presence of t-MDS. with or without subsequent t-ANLL, than with t-ANLL alone ($P = .03$). Moreover, abnormalities of no. 5 were associated more commonly with t-MDS than with t-ANLL ($P = .02$). The eight patients with a normal karyotype or with aberrations of chromosomes other than nos. 5 and 7 were more likely than the rest to have acute leukemia as opposed to t-MDS with or without leukemia ($P = .03$); four of the six patients with other abnormalities already had leukemia at initial diagnosis. The two patients with a t(15;17)(q22;q21) had the characteristic clinical and morphologic features of acute promyelocytic

Table 8. Secondary Bone Marrow Disorder and Chromosome Abnormalities

	Chromosome Abnormalities (n = 61)			
Secondary Disorder	Abnormality of No. 5	Abnormality of No. 7	Abnormality of Nos. 5 and 7	Other Abnormality Not No. 5 or 7
t-MDS only	9	5	4	0
t-MDS→t-ANLL	3	12	12	2
t-ANLL only	2	7	1	4
Total	14	24	17	6

leukemia de novo and may reflect the development of acute leukemia unrelated to their prior cytotoxic therapies.

Of the five patients who had a complete response to remission induction therapy for leukemia. two had an abnormality of no. 7 only, one had a −7 plus additional complex changes, one had a +8. and the remaining patient had a +8 and a t(15;17)(q22;q21). The two patients with normal karyotypes were not treated. None of the 18 treated patients with an abnormality of no. 5 had a complete remission. The median survival from the time of onset of bone marrow dysfunction did not differ among the 61 patients with the various chromosomal abnormalities (no. 5, 6 months; no. 7.9 months: nos. 5 and 7.6 months: other chromosomes. 10 months).

When patients were grouped according to the complexity of their karyotype (abnormality of nos. 5 andior 7 alone. or with additional rearrangements) and according to the total number of abnormalities. there were no significant differences in the type of primary disease. the type of initial therapy, the clinical course of the secondary disorder. or the survival time. Patients in whom both normal and abnormal (AN) metaphase cells were present at diagnosis did not have a significantly longer median survival (9 months) than did patients who had only abnormal (AA) metaphase cells (6 months. $P = .68$). The clinical characteristics and disease course of patients with abnormalities of chromosomes no. 1, 4, 12, 14, or 18 (the remaining chromosomes involved more frequently in therapy-related leukemia than in ANLL de novo) did not differ from those of patients with other abnormalities or with a normal karyotype. Two unexpected correlations of clinical interest were noted. however. First, 13 of the 16 patients with an abnormality of no. 8 had received chemotherapy as the primary treatment. either alone (ten patients) or together with radiotherapy (3 patients). Of the ten patients with a +8, only a single patient (no. 2057) had received radiotherapy alone. Second. the 13 patients who had an abnormality of chromosome 21 had a shorter median survival from the time of initial bone marrow dysfunction (5 months) than did the patients without an aberration of this chromosome (9 months. $P = .02$).

DISCUSSION

In this study. we have confirmed our earlier observations as well as those of other investigators that a secondary MDS is often the initial disorder seen in patients in whom therapy-related ANLL develops.[4-6,10,16,17] Our data confirm that t-MDS/t-ANLL (ie, either alone or in sequence) is a distinctive therapy-related hematopoietic disorder that is characterized by the presence of recurring clonal abnormalities of chromosomes 5 and 7. This bone marrow disorder is not part of the natural history of any of the primary diseases for which these patients were treated.

We have observed t-MDS and/or t-ANLL both in patients receiving treatment either with a single modality (radiotherapy or chemotherapy) and in those receiving combined or sequential treatment with these modalities. We want to emphasize that patients who had received radiotherapy alone. particularly to the pelvic area. shared an increased risk for developing leukemia because other investigators have suggested that t-ANLL does not occur in such patients.".''Similar to our findings. the data of Greene et al indicated that the volume of irradiated marrow may be related to this late complication."

Among our 21 patients who had received chemotherapy alone, all but one had undergone pro-

longed treatment with alkylating agents. Our data, therefore, confirm the high leukemogenic potential of long-term alkylating agent therapy.[8,11,15] For example. none of our patients developed therapy-related bone marrow dysfunction after receiving only a standard 6-month course of MOPP. Our study included a single patient who had received only an antimetabolite drug. 5-FU. orally for 5 years. Our patients who received both radiotherapy and chemotherapy were a heterogeneous group. Sixteen received combined-modality therapy concurrently as initial treatment. whereas the other 15 received sequential courses of the two modalities, in several cases on multiple occasions. We were unable to show an association between the type of primary therapy and the interval between initial treatment and the onset of bone marrow dysfunction. Thus. the latency period may depend primarily on the intensity of mutapenic damage and on the time required for clonal expansion and not on the precise modality that inflicts the damage. The influence of possible genetic predisposition is unknown.

Among our 37 most recent patients. there was a striking increase in the number of patients with solid tumors and a decrease in the number of those with Hodgkin's disease compared with the relative frequency among our 26 previously published cases. There are two possible reasons for this observation. The first is the growing recognition of the late complications of intensive cytotoxic therapy and. thus, the more judicious use of such treatment for patients with Hodgkin's disease. The second is the success of recent therapeutic trials with solid tumors. which has resulted in longer survival of these patients.

Our data do not allow us to draw any conclusions regarding optimal treatment for patients with therapy-related bone marrow dysfunction. No consistent treatment approach was taken with the patients in our series because the treatments were given at several institutions over a 12-year period. Recently. both intensive chemotherapy and bone marrow transplantation have been shown to be useful in such patients.[15,36,37]

Among our five patients who attained a complete remission. only one had had a myelodysplastic preleukemic phase. and none of the five had an abnormality of chromosome no. 5. Some authors have suggested that patients receiving a single primary treatment modality have a lower incidence of abnormalities of nos. 5 and/or 7 as well as a better response of their leukemia to subsequent therapy for remission induction.[20] In general. leukemia patients with abnormalities of nos. 5 and/or 7 do very poorly.'".[" In our series, 87% of all patients had abnormalities of nos. 5 and/or 7. including a number who had initially received only single-modality treatment.

The nonrandom involvement of chromosomes no. 5 and 7 in t-ANLL was first recognized by Rowley et al (1977) and was confirmed in several subsequent investigations.[4,5,19–22,27] In the present study. 55 of 61 (90%) patients with karyotypic aberrations had an abnormality of one or both of these chromosomes. Moreover, we have identified several additional chromosomes that may be preferentially involved in aberrations in this therapy-related disorder. namely. nos. 1, 4, 12, 14, and 18.

The frequency of t-ANLL patients with abnormal karyotypes and with aberrations of nos. 5 and 7 has varied among different studies, some of which were based on nonbanded chromosome preparations. Pedersen-Bjergaard et al reported on an updated series of 55 patients?'; of 39 patients studied with banding techniques, 31 (79%) had clonal abnormalities, and 27 (69%) had abnormalities of chromosomes no. 5 and/or 7. The frequency of abnormalities of chromosomes no. 5 and/or 7 was somewhat lower (35%) among 20 patients reported by Sandberg et al.[21] They noted the frequent involvement of two other chromosomes. namely, nos. 3 (four cases) and 17 (five cases). and they suggested that these two chromosomes are also preferentially involved in chromosomal abnormalities in t-ANLL. In addition, these authors have identified 206 previously reported cases of t-ANLL in which cytogenetic analyses were described." Chromosome no. 7 was abnormal in 60 cases (29%), no. 5 in 38 (19%), no. 17 in 34 (17%), no. 3 in 24 (12%), no. 21 in 21 (10%), and no. 8 in 19 cases (9%). Our data have not confirmed their suggestion that nos. 3 and 17 are preferentially involved in t-ANLL. In our series, ten patients had abnormalities of no. 3, and I5 had clonal rearrangements of no. 17. When we compared the frequency of involvement of each chromosome in patients with t-ANLL with that noted in our concurrent series of patients with XNLL de novo.??we de-

termined that the frequency of involvement of no. 3 ($P = .14$) or of no. 17 ($P = .88$) did not differ between these two groups of patients.

Recently. Arthur and Bloomfield suggested that abnormalities of nos. 5 and 7 may be related to a particular form of therapy administered for the primary disease.[20] These authors proposed that abnormalities of nos. 5 and/or 7 may be particularly frequent in patients who initially received combination Chemotherapy. with or without radiotherapy. and that patients who received less intensive therapy were more likely to have abnormalities characteristic of ANLL de novo. In their series of 20 patients with t-ANLL. nine had been treated less heavily: overall. ten of 19 (53%) patients with abnormal karyotypes and only two of the nine with less therapy had abnormalities of no. 5 or no. 7. In contrast. the eight patients in our series who had normal karyotypes or other abnormalities had not received less intensive primary therapy than had the 55 patients with abnormalities of nos. 5 and/or 7. Moreover. nos. 5 and/or 7 were abnormal in 18 of the 21 (86%) patients who had initially been treated with chemotherapy alone and in nine of the 11 (82%) patients who had initially been treared with radiotherapy alone.

Our present findings as well as our observation that abnormalities of nos. 5 and 7 occur frequently in patients with XNLL de novo who had significant occupational exposure have led us to believe that aberrations involving these chromosomes niay be indicators of mutagen-induced malignant disease.[25,38] This association. first noted by Mitelman et al and confirmed by Golomb et al and later by the Fourth Workshop. was based on a correlation between the occupation of adult patients with ANLL de novo and the karyotype of the leukemic cells.'"[25,27] The early findings on this association have been summarized by Golomb et al.[25] Of 236 patients. 68 (29%) were classified as having a history of significant exposure to chemicals. petroleum solvents. pesticides. or industrial metals. The frequency of abnormal karyotypes was higher in the exposed (51 of 68. 75%) than in the nonexposed patients (60 of 168. 36%). More importantly. abnormalities of chromosomes no. 5 and/or 7 were observed in 37% of the exposed patients and in only 12% of the nonevposed patients.:'[38]

Recent evidence suggests that proto-oncogenes. the cellular homologues of the transforming sequences of retroviruses. may play a role in human tumorigenesis. Several proto-oncogenes have been mapped to chromosome regions that are the breakpoints in the specific chromosomal rearrangements in hematologic malignant diseases. In at least one human tumor. Burkitt's lymphoma. transcriptional deregulation of a proto-oncogene (c-*myc*) occurs as the result of a chromosomal translocation, t(8;14)(q24:q32). In a second case. c-*abl* is consistently translocated from the distal long arm of chromosome no. 9 to the long arm of chromosome no. 22. resulting in the Philadelphia chromosome that is characteristic of chronic myelogenous leukemia. As a consequence of this rearrangement. transcription of the fused c-*abl* and bcr (on no. 22) genes results in a larger mRNA[39] and therefore a larger protein. Moreover, this altered protein has tyrosine kinase activity."'

Since abnormalities of nos. 5 and 7 are characteristic of t-MDS/t-ANLL. it is appropriate to consider genes that are present on these chromosomes and their potential role in leukemogenesis. In this regard. analysis of the deleted segments of chromosome no. 5 has enabled us to identify the critical region (bands 5q23 to q32) that is likely to contain the genes whose altered function may result in neoplasia. The results of a similar analysis of the critical region on 7q have been more uncertain because only four patients in this series had a del(7q) and it has been difficult to determine the precise breakpoints. However. if we consider the deletions observed in 3 additional patients with t-MDS or t-ANLL ascertained subsequent to the preparation of this manuscript. the critical region of no. 7 appears to be either in bands 7q32 to q33 or in bands 7q34 to q35.

Two proto-oncogenes (one of which codes for the receptor of a hematopoietic growth factor), one transforming sequence. and one gene encoding a growth factor have been localized to chromosome no. 5 or 7. C-*fms* (McDonough feline sarcoma virus) has been mapped to 5q34. a band that is just below the distal break point on the deleted chromosome"; the protein encoded by this gene has recently been shown to have substantial homology with macrophage colony–stimulating factor (CSF-1).[42] The proto-oncogene c-*erb B* (avian erythroblastosis virus) and

the transforming sequence ***met*** are located on no. 7. The former gene has recently been shown to code for a protein that is a truncated form of the receptor for epidermal growth factor.'? C-erb *B* has been localized to 7p or proximal 7q[41] and. therefore. appears to be at some distance from the deletion of no. 7 in t-MDS.t-ANLL. ***Met*** has been localized to 7q22 (M.M. Le Beau. unpublished observations) and. thus. is closer than c-*erb B* to the critical region on no. **7.** The growth factor gene. *GM-CSF*. has recently been mapped to the critical region on no. 5.[44] Other noteworthy genes on no. ***5*** are the glucocorticoid receptor (5q15 to qter) and the β-adrenergic receptor (5 centromere to q22), both of which are known to influence cell division in specific cell types."

How are abnormalities of nos. ***5*** and/or 7 related to a potential role for oncogenes in leukemogenesis'?At present. it is unknown whether c-erb *B* or *met* are involved in the pathogenesis of t-MDS/t-ANLL; however. data obtained recently suggest that *c-fms* (CSF-I receptor) and *GM-CSF* may be relevant genes in this disease. There are several mechanisms by which chromosomal abnormalities might influence the function of growth factors or their receptors and. thus. lead to malignant transformation. Loss of **a** whole chromosome or part of a chromosome (deletion) may result in the loss of certain genes that regulate cell growth. Alternatively. loss of genetic material (which occurs both in cells with deletions or with loss of a whole chromosome) may allow expression of a recessive mutant gene on the homologous chromosome, **as** is currently proposed for retinoblastoma and Wilms' tumor.[45]

As mentioned above. *c-fms* **was** previously mapped to band q34 of chromosome ***5:*** recent data, however, suggest that this gene may be more proximal. That is, by fusing human bone marrow cells from two patients with a del(5q) with rodent cells. Nienhuis et al found that *c-fms* was within the deleted segment of chromosome no. 5[46]; the distal breakpoint on chromosome 5 was thought to be proximal to band q34. By using in situ chromosomal hybridization. Le Besu et al have localized the *c-fms* gene to band q33; *GM-CSF* has been localized to 5q23 to 5q31.[44] Both of these genes are deleted in several patients with a del(5q).[44] **As** noted earlier. the *c-fms* gene encodes the receptor for CSF-1. Thus, the products of both the CSF-I and *GM-CSF* genes play an integral role in the proliferation and differentiation of cells of the mononuclear phagocytic lineage."The variability in the break points in the interstitial deletions of chromosome 5 observed in patients with t-ANLL suggests that the juxtaposition of identical gene sequencer. an event that may occur as a result of rearrangements such as translocations. is not a consistent genetic consequence of these chromosomal abnormalities. Therefore, a more likely mechanism is that previously outlined. namely, the loss of critical gene(s) resulting from whole chromosome loss or a deletion and the expression of a mutant allele on the remaining chromosome.

As physicians. we have the primary responsibility to reduce the incidence of this essentially fatal complication; as scientists, we should use the information provided by studies on these patient:, to advance our understanding of the critical genes and of the mechanisms involved in mutagen-induced leukemia. The insights obtained in this system will be of great value in elucidating the carcinogenic process in general.

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REFERENCES

1. Rosner F. Grunwald H: **Hodgkin's** disease and acute leukemia Report of right cases **and** review of the literature. Am J Med 58:339–353, 1975

2. Cadman EC, Capizzi RL. Bertino JR. Acute nonlymphocytic leukemia **A** delayed complication of Hodgkin's disease therapy· .Analysis of 109 cases. Cancer 40:1280–1296, 1977

3. Coleman **CN.** Williams CJ. Flint **A.** et al. Hematologic neoplasia in patients treated for **Hodgkin's** disease N Engl J Med 297:1249–1252, 1977

4 Rowley JD. Golomb HM. Vardiman JW: Nonrandom chromosomal abnormalities in acute nonlymphocytic leukemia in patients treated for Hodgkin's **disease** and non-Hodgkin's lymphoma. Blood 50:759–770, 1977

5 Rowley JD. Golomb HM. Vardiman JW. Nonrandom chromosome abnormalities in acute leukemia and dysmyelopoietic syndromes in patient, with previously treated malignant disease Blood 58:759–767, 1981

6 Vardiman JW. Golomb **HM. Rowley** JD. et al: Acute

nonlymphocytic leukemia in malignant lymphoma: A morphologic study Cancer 42:229–242, 1978

7. Rosner F. Grunwald HW: Cytotoxic drugs and leukemogenesis. Clin Haematol 9:663–681, 1980

8. Bergsagel DE. Bailey AJ, Langley GR. et al: The chemotherapy of plasma cell myeloma and the incidence of acute leukemia. N Engl J Sled 301:743–748, 1979

9. Zarrabi MH. Rosner F, Bennett JM: Non-Hodgkin's lymphoma and acute myeloblastic leukemia. Cancer 44: 1070–1080. 1979

10. Foucar K. McKenna RW. Bloomiield CD. et al: Therapy-related leukemia: A panmyelosis. Cancer 13:1285–1296, 1979

11. Berk PD. Goldberg JD, Silverstein MN. et al: Increased incidence of acute leukemia in polycythemia vera associated with chlorambucil therapy N Engl J Med 304:441–447, 1981

12. Zarrabi MH, Rosner F: Acute myeloblastic leukemia following treatment for non-hematopoietic cancers: Report of 19 cases and review of the literature. Am J Hematol 7:357–367, 1979

13. Kyle RA: Second malignancies associated uith chemotherapy agents Semin Oncol 9:131–142, 1982

14. Greene MH, Young RC. Merrill JM, et al: Evidence of a treatment dose response in acute nonlymphocytic leukemias H hich occur after therapy ut' nun-Hodgkin's lymphoma. Cancer Res 43:1891–1898, 1983

15. Albain KS. Le Beau MM, Vardiman JW. et al: Development of dysmyelopoietic syndrome in a hairy cell leukemia patient treated with chlorambucil: Cytogenetic and morphologic evaluation. Cancer Genet Cytogenet 8: 107–115. 1983

16. Pedersen-Bjergaard J, Larsen SO: Incidence of acute nonlymphocytic leukemia. preleukemia. and acute myeloproliferative syndrome up to 10 years after treatment of Hodgkin's disease. N Engl J Med 307:965–971, 1982

17. Anderson RL. Bagby CG. Richert-Boe K. et al: Therapy-related preleukemic syndrome Cancer 47:1867–1871. 1981

18. Bloomfield CD. Preisler H. Cuttner J. et al: Treatment-induced acute non-lymphocytic leukemia. Response to cytarabine-anthracycline therapy. Blood 60:152. 1982 (suppl 1) (abstr)

19. Pedersen-Bjergaard I. Philip P. Mortensen BT. et al: Acute nonlymphocytic leukemia. preleukemia. and Acute myeloproliferative syndrome secondary to treatment of other malignant diseases: Clinical and cytogenetic characteristics and results of in vitro culture of bone marrow and HL.4 typing. Blood 57:712–723, 1981

20. Arthur DC. Bloomfield CD: Banded chromosome analysis in patients with treatment-associated acute non-lymphocytic leukemia. Cancer Genet Cytogenet 12:189–199, 1984

21. Sandberg AA. Abe S. Kowalczyk JR. et al: Chromosomes and causation of human cancer and leukemia. L. Cytogenetics of leukemias complicating other diseases Cancer Genet Cytogenet 7:95–136. 1983

22. Pedersen-Bjergaard J. Philip P. Pedersen NT. et al: Acute nonlymphocytic leukemia. preleukemia. and acute myeloproliferative syndrome secondary to treatment of other malignant diseases Cancer 51:452–463, 1984

23. Larson RA. Le Beau MM. Vardiman JW. et al: The predictive value of initial cytogenetic studies in 148 adults with acute nonlymphocytic leukemia. Cancer Genet Cytogenet 10:219–236. 1983

24. Mitelman F. Nilsson PG. Brandt L. et al: Chromosome pattern, occupation. and clinical features in patients with acute nonlymphocytic leukemia. Cancer Genet Cytogenet 4:197–214. 1981

25. Golomb HM. Alimena G. Rowley JD. et al: Correlation of occupation and karyotype in adults with acute nonlymphocytic leukemia. Blood 60:404–411. 1982

26. Le Beau MM. Larson RA. Bitter MA. et al: Association of an inversion of chromosome 16 with abnormal marrow eosinophils in acute myelomonocytic leukemia: A unique cytogenetic-clinicopathological association. N Engl J Med 309:630–636. 1983

27. Fourth International Workshop on Chromosomes in Leukemia (1987). Cancer Genet Cytogenet 11:249–360, 1984

28. Bennett JM. Catovsky D. Daniel MT. et al: The French-American-British Co-operative Group. Proposal5 for the classification of the myelodysplastic syndromes. Br J Haematol 51:189–199, 1982

29. Vardiman JW. Coelho A. Golomb HM. et al: Morphologic and cytochemical observations on the ocen leukemic phase of therapy-relared leukemia. Am J Clin Pathol 79:525–530. 1983

30. McKenna RW. Parker JL. Brunning RD: Ultrastructural observations in therapy-related panmyelosis. in Polliack A (ed): Human Leukemia Boston. Martinus Nijhoif. 1983, pp 123–157

31. ISCN (1978): An international system for human cytogenetic nomenclature Cytogenet Cell Genet 21:309–404. 1978

32. First International Workshop on Chromosomes in Leukemia: Chromosomes in acute nonlymphocytic leukemia. Br I Haematol 39:311–316. 1978

33. Pagano M. Halvorsen KT: An algorithm for finding the exact significance levels of $r \times c$ contingency tables. J Am Statist Assoc 76:931–934. 1981

34. Larson RA. Kondo K. Vardiman JW. et al: Evidence for a 15:17 translocation in every patient with acute promyelocytic leukemia. Am J Med 76:827–840. 1984

35. Jacobs RH. Cornbleet MA. Vardiman JW, et al: Prognostic implications of morphology and karyotype in primary myelodysplastic syndromes. Blood (in press)

36. Preisler HD. Early AP. Raza A. et al: Therapy of secondary acute nonlymphocytic leukemia with cytarabine. N Engl J Med 308:21–24. 1983

37 Vaughan WP. Karp JE. Burke PJ: Effective chemotherapy of acute myelocytic leukemia occurring after alkylating agent or radiation therapy ior prior malignancy. J Clin Oncol 1:204–207. 1983

38. Rowley JD: Chromosome changes in leukemic cells as indicators of mutagenic exposure. in Rowley JD and Ultmann JE (eds): Chromosomes and Cancer. From Molecules to Man. Orlando. Fla. Academic. 1983, pp 140–162

39. Shtivelman E. Lifshitz B. Gale RP. et al: Fused transcript of *abl* and *bcr* genes in chronic myelogenous leukemia. Nature 315:550–554. 1985

40. Konopka JB. Watanabe SM. Witte ON: An alteration of the human c-*abl* protein in K562 leukemia cells unmasks associated tyrosine kinase activity Cell 37: 1035–1042. 1984

41. Human Gene Mapping 7. Seventh International Workshop on Human Gene Mapping. Cytogenet Cell Genet 37:1–666. 1984

42. Sherr CJ. Rettenmier CW. Sacca R. et al: The c-*fms*

proto-oncogene product is related to the receptor for the mononuclear phagocyte growth factor, CSF-I. Cell 41:665–676, 1985

43. Downward J. Yarden Y. Mayes E. et al: Close similarity of epidermal growth factor receptor and v-*erb-B* oncogene protein sequences. Nature 307:521–527, 1984

44. Le Beau MM. Westbrook CA. Diaz MO. et al: Evidence for the involvement of *GM-CSF* and *fms* in the deletion (5q) in myeloid disorders. Science (in press)

45. Murphree AL. Benedict WF: Retinoblastoma: Clues to human oncogenesis. Science 223: 1028–1033. 1984

16. Nienhuis AW. Bunn HF. Turner PH. et al: Expression of the human *c-fms* proto-oncogene in hematopoietic cells and its deletion in the 5q- syndrome. Cell 42:421–428. 1985