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Therapy-Related Myelodysplasia and Acute Myeloid Leukemia. Cytogenetic Characteristics of 115 Consecutive Cases and Risk in Seven Cohorts of Patients Treated Intensively for Malignant Diseases in The Copenhagen Series

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Therapy-related acute myeloid leukemia (t-AML), often presenting as myelodysplasia (t-MDS), has become the most serious long-term complication of cancer therapy and offers a unique opportunity to study chemical leukemogenesis. Seven cohorts of patients treated for six different types of primary tumor have been followed closely for leukemic complications, and 115 consecutive patients with t-MDS or t-AML, including 46 cases from the cohorts, have been investigated cytogenetically at our institutions during the past 16 years. In patients primarily treated with alkylating agents, the risk of t-MDS and t-AML increased by approximately 1% per year from 2 to at least 8 years after start of treatment. In most cases, the disease presented as t-MDS with loss of a whole chromosome 5 or 7, or various parts of their long arms, and the leukemias were of FAB-subtypes M1, M2, or M4. In patients treated with drugs targeting at DNA-topoisomerase II, such as etoposide, doxorubicin, 4-epidoxorubicin, or mitoxantrone combined with drugs reacting directly with DNA, such as cisplatin or alkylating agents, the risk of leukemia increased much more steeply from only one year after start of therapy. These early onset cases often presented as overt leukemia of FAB-subtypes M4 or M5 with balanced translocations to chromosome bands 11q23 and 21q22, whereas later onset cases often shared characteristics with cases observed after therapy with alkylating agents alone. Both alkylation of DNA and poisoning of DNA-topoisomerase II may result in development of t-AML with different clinical and cytogenetic characteristics. There may be a synergistic leukemogenic effect between the two types of drug, and in patients with germ cell tumors treated with etoposide, cisplatin and bleomycin, reassessment suggested the risk of leukemia to increase exponentially with increasing doses of cisplatin and etoposide.

INTRODUCTION

Secondary or therapy-related acute myeloid leukemia (t-AML), often presenting as myelodysplasia (t-MDS), is a topic of major importance for two main reasons. Clinically, because it is the most serious long-term complication of current cancer therapy and biologically, because it provides a unique opportunity to study the development of irradiation-induced and in particular chemically-induced leukemia in man. Such studies are possible because larger cohorts of patients, as part of their antineoplastic treatment, are systematically exposed to heavy and well-defined doses of X-rays or chemical carcinogens with a known mechanism of action and subsequently followed closely for clinical effects. The comprehensive literature to date on the many aspects of t-AML has recently been reviewed in detail (1–4).

At our institution, which serves as hematologic center for around 1.5 million inhabitants in the Copenhagen area, we have studied t-MDS and t-AML during the past 16 years. This report presents cytogenetic findings in 24 new patients with t-MDS and t-AML, bringing our total cytogenetic experience to 115 consecutive, completely banded cases. The cytogenetic characteristics of these 115 patients are examined in relation to the general type of therapy administered for the primary malignancy, and compared with findings in de *novo* acute myeloid leukemia (AML).

Furthermore, the study compares previously published risk estimates of t-MDS and t-AML in seven cohorts of patients with malignant lymphomas or solid tumors treated by different types of chemotherapy and/or radiotherapy (5–11), and, in addition, also compares the clinical, cytologic, and cytogenetic characteristics of the leukemias observed in these cohorts. As a leukemogenic synergism was suspected between drugs targeting at DNA-topoisomerase II, such as etoposide, and drugs reacting directly with DNA, such as cisplatin, with a possibly steep dose-response curve (10,11), the risk of t-MDS and t-AML was reassessed in the 212 patients with germ cell tumors treated with these two drugs. In a Cox regression analysis the effects of the cumulative doses of etoposide and cisplatin received by each patient were evaluated together with the effect of patient age.

PATIENTS AND METHODS

Cytologic and Cytogenetic Investigations

At our institutions, all patients developing refractory, unexplained cytopenia, leukocytosis, or other signs of t-MDS and t-AML at the follow-up from previous chemotherapy or radiotherapy were candidates for cytologic and cytogenetic examination of the blood and the bone marrow for t-MDS and

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LEUKEMIA
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t-AML. Patients were studied regardless of type of primary disease. and even cases with an end-stage primary tumor and bone marrow involvement were investigated.

Blood and bone marrow cytology was studied on May-Grunewald-Giemsa-stained smears and on bone marrow biopsy specimens from the iliac crest. If indicated, additional cytochemical staining was performed for subclassification, together with immunological investigations of Ficoll-Isopaque-isolated mononuclear cells using a number of specific monoclonal antibodies and an indirect immunofluorescence technique. Overt r-AML was classified according to the FAB nomenclature (12), t-MDS was not subdivided cytologically because it was difficult or impossible in many cases.

Cytogenetic investigations were carried out using OTL banding technique following a 1-4 day culture of the bone marrow specimen, and results were expressed in accordance with the International System for Human Cytogenetic Nomenclature (ISCN, 1985) (13).

Clinical and cytogenetic results from studies of the initial 109 cases of t-MDS and t-AML observed during the period 1976-1990 have been published previously (14). A complete banding analysis was not accomplished in some of our most early cases, patients 1-3, 5-7, 9, 10, 20, 21, 23-28, and 30, for which reason they are excluded from the analysis. The present study includes new and unpublished data from our 24 most recent cases observed during the period 1990-1992. Preliminary data have been reponed briefly elsewere for cases 127-130 (10.11) in relation to risk estimates of e-MDS and t-AML.

Patient Cohort Studies

During the past 16 years, seven well-defined cohorts of patients treated for malignant diseases with chemotherapy and/or radiotherapy have been followed closely for the development of t-MDS and t-AML at our institutions (5-11) (Table 1). In the studies of patients with ovarian cancer (5), Hodgkin's disease (6), non-Hodgkin's lymphomas (7), and germ cell tumors (I0), all previously untreated patients were included who were referred to and treated at our departments within a well-defined period of time ranging from 7 to 11 years. The studies of patients with small cell lung cancer (8) and two small series of patients with breast cancer (9,11) included patients treated in specific prospective phase II and phase PI studies of intensive combination chemotherapy of various types.

In the first three studies (5-7) (Table I), patients with ovarian cancer, Hodgkin's disease, and non-Hodgkin's lymphomas were treated with various alkylating agents during the 1970s, and many patients also received high-voltage radiotherapy. All patients with ovarian cancer received dihydroxobusulfan for 2-3 years as single agent, oral chemotherapy, and in addition 274 out of the 553 patients received high-voltage radiotherapy to the pelvic region (5). Patients with malignant lymphomas received high-voltage radiotherapy, combination chemotherapy, or combined modality therapy. In Hodgkin's disease, combination chemotherapy was MOPP (mechlorethamine + vincristine + procarbazine + prednisone) or equivalent combinations substituting mechlorethamine with carmustine or lomustine and vincristine with vinblastine. In most non-Hodgkin's lymphomas, chemotherapy included vincristine plus prednisone and long-term, peroral therapy with cyclophosphamide or chlorambucil (7). Only a few patients in these three early studies received drugs targeting at DNA-topoisomerase II such as doxorubicin or etoposide, and mainly in the terminal stage of their disease.

In the last four studies (Table 1) patients with small cell lung cancer (8), breast cancer (9,11), or germ cell tumors (10) treated during the 1980s all received combination chemotherapy with drugs targeting at DNA-topoisomerase II such as etoposide, doxorubicin, 4-epidoxorubicin, or mitoxantrone, combined with drug8 directly reacting with DNA. In the first two studies (8,9) alkylating agents were administered, in the last two studies (10.11) cisplatin. High-voltage radiotherapy was not included in the intensive therapy in any of these four studies and had only been administered previously and more extensively to patients with breast cancer (9,11).

The cumulative risk of t-MDS and t-AML was estimated by the Kaplan-Meier method (15) and expressed as a percentage ±SE. The relative risk (RR) and the 95% confidence limit (CL) of overt t-AML alone, and of t-MDS plus t-AML, were all assessed by comparing the number of observed cases with the expected number of cases of *de novo* AML, based on the sex-specific and age-adjusted incidence of AML in the general Danish population as reported by the Danish Cancer Registry (16). The incidence of *de novo* MDS in the Danish population is so far unknown and could therefore not be used in the calculations.

Reassessment of the Risk of t-MDS and t-AML in Patients with Germ Cell Tumors

In our cohort of 212 patients treated for germ cell tumors with etoposide, cisplatin, and bleomycin, and for whom the risk of t-MDS and t-AML has recently been reported (10), the risk was

Table 1. Seven Cohorts of Patients Evaluated for the Risk of t-MDS and t-AML in the Copenhagen Studies.

Primery Cancer	Leukemogenic Therapy	Number Treated	Number of t-MDS + t-AML	Relative Risk of Overt t-AML	Relative Risk of t-MDS and t-AML
Ovarian cancer (5)	Dihydroxybusulfan, RT	553	2 + 5	125 (41–219)	175 (70–361)
Hodgkin (6)	Mechlorethamine.	391	7 + 10	167 (80–307)	283 (165–454)
	Lomustine, Carmustine, Chlorambucil, RT				
Non-Hodgkin (7)	Cyclophosphamide, *RT*	*602*	5 + 4	34 (9–87)	76 (35–145)
Lung cancer (8)	Etoposide, Doxorybicln. *Lomustine,* Cyclophosphamide	796	3 + 3	77 (16–225)	154 (56–335)
Breast cancer (9)	Mitoxantrone, Prednimustine	71	3 + 2	339 (41–1223)	*848* (275–1978)
Germ cell tumors (*10*)	Etoposide, Cisplatin	212	1 + 4	336 (92–861)	417 (136–981)
Breast cancer (11)	4-Epidoxorubicin, cisplatin	74	0 + 3	668 (138–1953)	668 (138–1953)

Numbers in parenthesis represent 95% confidence limits. RT, radiotherapy.

completely reassessed. Data on patient age and cumulative doses of ctoposldc and cisplatin were obtained from the patient records. These data were evaluated in a Cox regression model, as previously performed for patients with Hodgkin's disease (17), to throw new light on the dog-raponse effect of etoposide and of cisplatin in leukemogenesis, and to demonstrate a possible effect of age.

RESULTS

Clinical and Cytogenetic Characteristics of 24 New Cases of t-MDS and 1-AM1

During the period 1990-1992, 24 new cases of t-MDS and t-AML were found (Table 2). Eleven of the new patients with t-MDS and t-AML had previously been treated for malignant lymphomas, three patients had been treated for multiple myeloma and one patient for acute lymphoblastic leukemia. Nine patients had been treated for various solid tumors. Thirteen patients presented as t-MDS without subsequent development of overt leukemia, eleven patients presented overt t-AML.

Twelve of the patients presented unbalanced aberrations with loss of whole chromosomes 5 or 7, or various parts of their long arms, 10 of these patients had previously received alkylating agents, Three patients presented balanced translocations to chromosome bands 11q23 (cases 116 and 127) or 21q22 (case 130), all three patients had previously been treated with drugs targeting at DNA-topoisomerase II (doxorubicin, 4-epidoxorubicin, or etoposide). Five patients presented other chromosome abnormalities and four patients had a normal karyotype.

Of particular interest in this new series are three patients with non-Hodgkin's lymphomas (cases 116, 119, and 120) who developed t-AML following chemotherapy with CHOP only (cyclophosphamide + doxorubicin + vincristine + prednisone). Case 116 presented a t(11;19)(q23;p12orq12) whereas cases 119 and 120 both had a normal karyotype. Also included in the new series is our first case of t-AML following therapy for acute lymphoblastic leukemia (case 133).

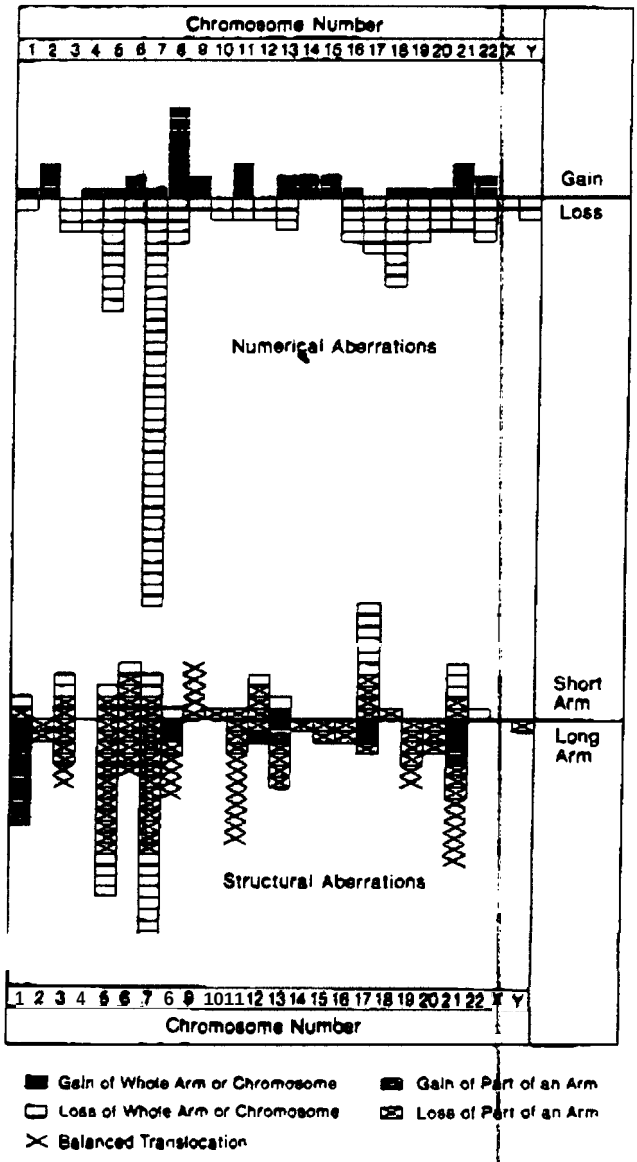

Figure 1. Numerical and structural chromosome aberrations observed in 115 consecutive cases of t-MDS and t-AML (most complicated clone). Only 17 patients presented a normal karyotype.

Chromosome Characteristics of Our Total Series of 115 Patients with r-MDS and t-AML

The cytogenetic abnormalities observed in ,our total series of 115 patients with t-MDS or t-AML, ,including the 45 cases from the seven cohorts, are shown in Figure 1. Only 17 patients presented a normal karyotype. The numerical aberrations observed most frequently (upper part of Figure 1) were loss of a whole chromosome 7 or 5 (monosomy 7 or 5). In addition many patients presented unbalanced translocations or deletions of these two chromosomes (Figure 1, lower part) with loss of the whole long arm or various parts of a long arm. A total of 57 patients showed deletions of chromosome 7, 26 patients presented deletions of chromosome 5, and 13 of these patients presented defects of both chromosomes simultaneously. In chromosome 5, the deletions were all interstitial, the proximal breakpoint varying between bands 5q13 and 5q22 and the distal breakpoint between bands 5q31 and 5q35, with rhe region between bands 5q22 and 5q31 always missing. In the deletions of chromosome 7, the only breakpoint which could be identified varied between bands 7q11 and 7q36 but was most often band 7q22, and the critical region was 7q36 to 7qter. Other frequently observed numerical aberrations were gain of a whole chromosome 8 (trisomy 8), observed in eight patients, and monosomy 18, observed in eight patients. Trisomy 8 was observed as the sole abnormality in only one patient, and was often observed in only a subclone and not in all abnormal mitoses.

Apart from deletions of the long arms of chromosomes 5 and 7, the structural aberrations most frequently observed were rearrangements of the long arms of chromosomes 11 (11 patients) and 21 (nine patients) and the short arm of chromosome 17 (10 patients) (Figure 1, lower part). The bands rearranged in the long arms of chromosomes 11 and 21 were in all identified cases 11q23

Table 2. Clinical Characteristics, Bone Marrow Cytology and Cytogenetic Findings at Diagnosis in 24 New Cases of t-MDS and t-AML from the Copenhagen Series

Case No.	Age (years)/Sex	Primary Tumor Histology/Stage	Type of Treatment for Primary Tumor and Duration (Months)	Status of Primary Tumor	Time to Development of MDS or AML (Months)	Duration of MDS (Months)	Survival from MDS or AML (Months)	FAB-Type of Overt Leukemia	Karyotype of Bone Marrow Cells	Mitosis Abnormal-/Normal	Antileukemic Chemotherapy Type/Response/ Duration (Months)
110	40/M	Hodgkin LP/IIA	Dox + Bleo + Vlb + DTIC(5) Me + VCR + Pro + Pred(5) CCNU + Vlb + Pro + Pred(5) X-rays abdom. 37 Gy X-rays mantle 39 Gy	PD	46	2	2	–	45,XY, – 7,del(20)(q12)	16/5	–
111	69/F	Hodgkin MC/IVB	CCNU + Vlb + Pro + Pred(6)	CR	99	–	2	M4	72,XX, + X, + 1, + 2, + 2, + 4, + 5, + 6, + 6, + 7, + 8, + 8, + 14, + 15, + 15, + 16, + 16, + 18, + 20, + 21, + 21, + 21, + del(13)(q12;13) (q13;q31), + mar1, + mar2, + mar3 = 18/60, XX, + 1, + 4, + 6, + 7, + 8, + 8, – 13, + 15, + 16, + 16, + 20, + 21, + der(13)t(12;13), + mar1, + mar2, + mar4 = 16	27/0	DNR + Ara-C/NR
112	52/M	Hodgkin LP/IIA	X-rays mantle 37 Gy X-rays abdom. 37 Gy CCNU + Vlb + Pro + Pred(5)	CR	197 91	23 +	23 +	–	46,XY,del(13)(q13q31)	8/1	–
113	69/M	Hodgkin NS/IA	Me + VCR + Pro + Pred(5) Me + VCR + Pro + Pred(6) X-rays mantle 37 Gy Etop + Vlb + Dox(5)	CR	4 25	4	4	–	46,X, – Y, – 5, – 16, – 17, – 19, + der(Y) t(Y;?)(q12;?),del(3) (p24), + t(5p;17q), + mar1, + mar2, + mar3 = 17/48,X, – Y, – 5, – 16, – 17, + 18, + 22, + der(Y),del(3),t(5p17q), + mar2, + mar3 = 6	23/1	–
114	50/M	Hodgkin MC/IIB	Me + VCR + Pro + Pred(6)	CR	76	11 +	11 +	–	46,XY, – 7, + der(7)t(1;7)(q11;q11)	16/12	–
115	66/F	Non-Hodgkin NWDL/IIB	Cbx(66)	PR	110	1	1	–	45,XX, – 3, – 5, + 11 = 9/45,XX, – 3, – 5, + mar = 8/45,XX, – 3, – 5, + der(7)t(3;?) (q21;?) = 7	24/0	–
116	27/M	Hodgkin NS/IB Non-Hodgkin DH/IA	Me + VCR + Pro + Pred(3) Cbx + Dox + VCR + Pred(4) X-rays rhinopharynx 42 Gy	CR	7 24	–	5	M5b	46,XY,t(11;19)(q23;p12orq12)	33/0	DNR + Ara-C /CR 4 mo
117	56/F	Non-Hodgkin NM/IIA	Cbx + Dox + VCR + Pred + Bleo(7), Cbx + VCR + Pred(13)	PR	89	14	14	–	46,XX, – 7, + der(7)t(1;7)(q12;q11.1)	19/6	–
118	83/M	Non-Hodgkin DM/IA	Cbx + VCR + Pred(17), Cbx(43) X-rays abdom. 41 Gy	PR	29	14 +	14 +	–	47,XY, + 8	35/0	–
119	52/F	Non-Hodgkin NLPD/IA	Cbx + Dox + VCR + Pred(5) X-rays mantle 40 Gy	CR	52	–	8 +	M4	46,XX	0/19	DNR + Ara-C /CR 4[1] mo
120	60/M	Non Hodgkin DH/IIIB	Cbx + Dox + VCR + Pred(4)	CR	53	24 +	24 +	–	46,XY	0/24	–
121	63/F	Multiple myeloma IgG	Melphalan(3), Cbx(19) Mavantrone(7)	PR	48	22	22	–	46,XX,del(7)(p11)*	6/25	–

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122	79/F	Multiple myeloma IgG	Melphalan(79)	PR	114	22	22	–	46,XX,i(17q)	24/3	–
123	83/F	Multiple myeloma IgG	Cbx(109)	PD	116	–	1	M2	44,XX, – 5, – 6, – 13, – 18, + der(6)t(6;?)(p22;?),del(6)(q22), + mar	32/0	–
124	53/F	Ovarian cancer cystadenocarc./I	Dihydroxybusulfan(20) Cbx + Dox + 5FU(3) Cis + Carbo + Ifos(3)	CR	78 16 4	12	12	–	43,XX, – 5, – 7, – 9, – 17, – 21, – 22, + der(21)t(21;?)(p11;?), + der(?)t(17;?)(q21;?) + mar1 = 7/43,XX, – 5, – 6, – 17, – 19, – 21, + der(?)t(17;?)(q21;?) + mar2 = 9	25/4	–
125	74/F	Ovarian cancer/IIB	X-rays pelvis 50 Gy Dihydroxybusulfan(6)	CR	156	12	12	–	45,XX, – 7,del(1)(p36)	22/8	–
126	57/F	Ovarian cancer/IIIA	Cbx + Dox + 5FU(4) Cis + Carbo(4); Carbo(8)	PD	54		1	M2	44,XX, – 5, – 6, – 10, – 16, – 17, – 21, del(18)(p11.2), + i(8q), + der(10)t(10;?)(p11;?), + der(17)t(17;?)(p12;?) + der(21)t(6;?;21)(6qter – 6q14::?::21p12 – 21qter)	25/0	DNR + Ara-C/NR
127	47/F	Breast cancer/dissem	Cbx + Mtx + 5FU(8) 4-epidox + Cis(6) X-rays ovary 12.5 Gy	PR	40 15	–	1	M5	46,XX,t(9;11)(p21;q23)	23/0	Ara-C/NR
128	53/F	Breast cancer/dissem.	Cbx + Mtx + 5FU(14) 4-Epidox + Cis(14)	CR	55 33	–	1	M4	45,XX, – 7	30/0	–
129	39/M	Germ cell tumor testicular/III	Etop + Cis + Bleo(5) X-rays brain 40 Gy	CR	68	25+	25+	–	46,XY, – 7, + der(7)t(1;7)(q21;q22)	9/7	–
130	41/M	Germ cell tumor retroperitoneal/III	Etop + Cis + Bleo(2)	CR	29	–	21+	M2	46,XY,t(8;21)(q22;q22)	23/11	DNR + Ara-C/CR 8* mo
131	73/F	Uterine cancer cervix/Ib	Radium 24.2 Gy	CR	112	–	1	M4	46,XX, – 8, + der(8)t(8;?)(q24.3;?),del(8)(q1q22) = 25/46,XX, – 7, – 8, + der(8),del(8), + der(7)t(7;?)(q36;?) = 3	28/0	DNR + Ara-C/NR
132	39/F	Wegener's granulomatosis	Cbx(59)	CR	59	20	21	M1	46,XX	0/32	DNR + Ara-C/NR
133	25/M	Acute lymphoblastic leukemia (CALLA positive)	VCR + Pred + Mtx + Asp + 6MP(37)	CR	167	–	1+	M1	46,XY	0/57	DNR + Ara – C/CR 1* mo

M, male; F, female; LP, Lymphocytic predominance; MC, mixed cellularity; LD, lymphocytic depletion; NS, nodular sclerosis; DH, diffuse histiocytic; NM nodular mixed; NWDL, nodular well differentiated lymphocytic; DM, diffuse mixed; Dox, doxorubicin; Bleo, bleomycin; Vlb, vinblastine; DTIC, dacarbazine; Me, mechlorethamine; VCR, vincristine; Pro, procarbazine; Pred, prednisone; CCNU, lomustine; Etop, etoposide; Cbx, cyclophosphamide; Clb, chlorambucil; 5FU, 5-fluouracil; Cis, cisplatin; 4-Epidox, 4-Epidoxorubicin; Carbo, carboplatin; Ifosf, Ifosfamide; Mtx, methotrexate; Asp, asperaginase;6MP, 6-mercaptopurine; PD, progressive disease; CR, complete remission; PR, partial remission; DNR, daunorubicin; Ara-C, cytosine arabinoside; *6 months later; 46,XX, – 7, + 21.

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and 21q22, and the balanced translocations t(9;11)(p22;q23), t(11;19)(q23;p12orq12), t(6;11)(q27;q23), t(8;21)(q22;q22), and t(3;21)(q26;q22) predominated, as they were obrerved in 14 cases out of 19 cases with rearrangement of 11q or 21q. The structural aberrations of the short arm of chromosome 17 were in all cases unbalanced translocations or deletions with loss of the whole short arm or various parts of this, always comprising bands 17p12 to 17pter.

Cytogenetic Characteristics of All 115 Patients in Relation to findings in De Novo AML and Previous Therapy

Table 3 shows the most important cytogenetic abnormalities observed in our total series of 115 patients with t-MDS and t-AML, compared with findings in *de novo* AML (IV International Workshop on Chromosomes in Leukemia) (18), and their relation to the general type of previous therapy. Compared with *de novo* AML, chromosome abnormalities in general, and the characteristic deletions of chromosomes 5 or 7 in particular, were observed much more frequently in t-MDS and t-AML. The balanced rearrangements t(15;17)(q22;q11) and inv (16)(p13;q22) regularly seen in *de* novo AML were not observed at all in our series.

Within our total series of 115 patients, the characteristic deletions of chromoeomes 5 and 7 were significantly associated with previous therapy with alkylating agents ($p = 0.0009$, Fisher's exact test, two-sided); (Table 3), and the balanced translocations to bands 11q23 and 21q22 were significantly associated with previous therapy with drugs targeting at DNA-topoisomerase II ($p = 0.000002$, Table 3). In patients previously treated with radiotherapy only, there was also an excess of leukemias with deletions of chromosomes 5 or 7 as compared to *de* novo AML, but otherwise no significant associations could be observed between previous radiotherapy and the occurrence of specific chromosome aberrations.

Risk Estimates of t-MDS and t-AML Compared for Seven Cohorts of Patients

The numbers of patients enrolled in each of the seven cohort studies, the type of leukemogenic therapy previously administrated, the number of cases of t-MDS and t-AML observed, and the relative risks of leukemic complications, are shown in Table 1. Radiotherapy was not a detectable risk factor for t-MDS and t-AML in any of the first three studies, whether administered as monotherapy in patients with lymphomas or combined with chemotherapy in all three studies.

In the first three studies, where the main type of treatment was with alkylating agents, the relative risks of t-AML or of t-MDS + t-AML varied between 34 and 283 and were apparently lowest for patients treated with cyclophosphamide for non-Hodgkin's lymphomas. In three out of the last four studies (Table 1), where a drug targeting at DNA-topoisomerase II such as etoposide, doxorubicin, 4-epidoxorubicin, or mitoxantrone was combined with a drug reacting directly with DNA such as an alkylating agent or cisplatin, the relative risks of t-AML or t-MDS plus t-AML were apparently higher than in the first three studies.

The cumulative risks of t-MDS and t-AML observed in the seven cohorts are shown in Figure 2. As radiotherapy was not a significant risk factor in any of the first three studies, only patients treated with chemotherapy are now considered. The data shown for patients with Hodgkin's disease and ovarian cancer

Table 3. Most [illegible] Observed [illegible] 116 [illegible] Cases of t-MDS and t-AML [illegible] the [illegible]nhagen Series [illegible] to Type of Previous Therapy and Compared with [illegible] In De Novo AML (IV International Workshop on Chromosomes in Leukemia, 1982)

Cytogenetic Characteristics	Number of Cases Present Study (%)	Previous Therapy							*De Novo* AML IV IWCL (18) 1982 (%)
		AA only ± AT	AA + ATTOP ± RT	ATTOP only[a] ± RT	RT only	RT + CT	CT only	Other therapy	
Normal	17 (15)	7	4	1	3	6	6	2	45
+8 ± others	8 (7)	4	2	1	1	2	5	0	6
-7, -5.5q-, 7q- ± others	70 (61)	39	25	1	5	27	37	0	9
Balanced 11q23 and 21q22 ± others	14 (12)	0	9	5	0	8	6	0	13
Unbalanced 11q23 and 21q22 ± others	6 (5)	2	3	0	1	1	4	0	
t(15;17) or inv(16) ± others	0	0	0	0	0	0	0	0	6
17p- or -17 ± others	15 (13)	10	3	1	1	7	7	0	6
Total number at cases	115	53	40	8	12	52	68	2	654

AA, alkylating agents; ATTOP, agents targeting at DNA-topoisomerase II; RT, radiotherapy; CT, chemotherapy;
[a] Cisplatin included here as considered not to be a classical alkylating agent.
[b] 64 patients out of 93 patients treated with AA showed abnormal chromosomes 5 or 7 versus six patients out of 22 patients treated without AA, $p = 0.0009$, Fisher's exact test, two sided.
[c] 14 patients out of 48 patients treated with ATTOP showed balanced translocations to chromosome bands 11a23 or 21q22 versus 0 patients out of 67 patients treated without ATTOP, $p = 0.000002$, Fisher's exact test, two-sided.

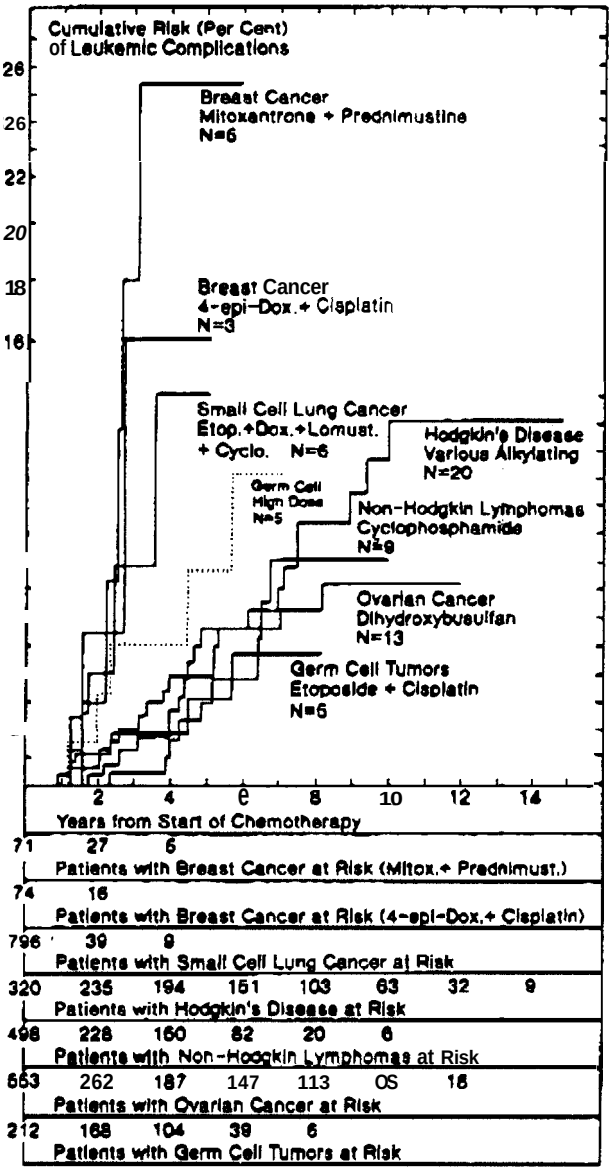

Figure 2. Cumulative risk (%) of t-MDS and t-AML in seven cohorts of patients treated for six different types of primary rumor (Kaplan-Meier estimates). Tha broken line indicates the risk for the subgroup of 82 patients with germ cell tumors receiving high-dose etoposide rnd cisplatin. Epidox., epidoxorubicin; Etop., etoposide; Dox., doxorubicin; Lomust., lomustine; Cyclo., cyclophorphrmide; Mitox., mitoxantrone; Prednimust., prednimustine.

are based on follow-up studies (17,7), increasing the numbers of cases of t-MDS plus t-AML for the two diseases to 20 and 13, respectively. For patients with ovarian cancer, Hodgkin's disease, and non-Hodgkin's lymphomas treated solely with alkylating agents, the cumulative risks of t-MDS and t-AML increased uniformly from 2 up to at least 8 years after start bf therapy by approximately 1% per year (Figure 2). In the last four studies, where a drug targeting at DNA-topoisomerase II was administered together with an alkylating agent or cisplatin, the cumulative risk of t-MDS and t-AML increased from as early as 1 year after start of therapy, and in three out of the four studies much more steeply with an increment of approximately 4% per year. This increase in risk continued until at least 3–4 years from start of therapy. In 212 patients treated for germ cell tumors, most often with only four cycles of chemotherapy, the cumulative risk was lower, but all five cases of t-MDS or t-AML were observed in the smaller subgroup of 82 patients receiving high-dose etoposide plus cisplatin. Consequently, if the cumulative risk of t-MDS and t-AML was estimated for this subgroup only (Figure 2. broken line), the cumulative risk increased more steeply, in line with the risk in the other three cohorts in which drugs targeting at DNA-topoisomerase II had been combined with alkylating agents or cisplatin.

Clinical, Cytologic end Cytogenetic Characteristics for t-MDS and t-AML Observed in the Seven *Cohorts*

In the first three cohorts of patients treated with alkylating agents for ovarian cancer, Hodgkin's disease, and non-Hodgkin's lymphomas, only four out of 33 cases of t-MDS or t-AML developed less than 2 years from start of chemotherapy, and 27 cases out of a total of 33 presented with myelodysplasia (Table 4). The FAB subtypes of overt t-AML were: M1, five patients; M2, four patients; M4, two patients. A complete cytogenetic examination was performed in 24 of these cases of t-MDS or t-AML. Deletions of chromosomes 5 or 7, or parts of their long arms, were observed in 20 cases, while three patients presented a normal karyotype and only one case (patient 36) with Hodgkin's disease showed an uncharacteristic abnormality: 48,XX,+2,+8. No balanced translocations to chromosome bands 11q23 or 21q22 were observed (Table 4).

In the fast four cohorts of patients treated for solid tumors with a combination of a drug targeting at DNA-topoisomerase II and cisplatin or an alkylating agent, and in our small group of four leukemias following treatment for non-Hodgkin's lymphomas with CHOP only, the clinical, cytologic, and cytogenetic characteristics differed significantly from the characteristics for the first three cohorts (Table 4), in particular in cases presenting with a short latent period. Thus, 11 cases out of 25 developed with a latent period of 2 years or less, only 12 patients out of 25 presented in t-MDS, and 11 out of 17 overt leukemias were of FAB subtypes M4 and M5. Twelve patients presented balanced translocations to chromosome bands 11q23 or 21q22 and 12 patients showed characteristic deletions of chromosomes 5 or 7. The early onset cases were most often overt leukemias of FAB subtypes M4 or M5 with balanced translocations to bands 11q23 or 21q22, whereas the cases occurring later were often t-MDS with deletions of chromosome 7 or its long arm, or they presented a normal karyotype. If cisplatin was administered in the combination, cases of t-AML showing the balanced translocations predominated, whereas if an alkylating agent was administered, leukemias with deletions of chromosomes 5 or 7 predominated. interestingly, some cases with intermediate onset presented with a mixture of the two general types of cytogenetic abnormality.

Table 4. General Characteristics of t-MDS and t-AML *from* **Cohorts Treated with Alkylating Agents Alone, Compared with General Characteristics of Leukemias** from Cohorts **Treated with a DNA-Topoisomerase II Targeting Drug Combined with an Alkylating Agent or Cisplatin.**

Type of Chemotherapy and Tumor	Latent Period ≤24 months	MDS at Presentation	Oven Leukemia		Cytogenetics	
			FAB M1 + M2	FAB M4 t M5	Abnormal Chromosome 5 or 7	Balanced 11q or 21q
Alkylating agents only:						
Ovarian cancer(5)	4/33	27/33	9/11	2/11	20/24	0/24
Hodgkin's diseoee (6)						
Non-Hodgkin's lymphomae (long-term alkylators 7)						
	A	B	C	C	D	E
Topoisomerase II-targeting drugs plus cisplatin or alkylators:						
Lung cancer (8)	11/25	12125	6117	11/17	12/25	12/25
Breast cancer (9,11) (two studies)						
Germ cell tumors (10)						
Non-Hodgkin's lymphomas (CHOP) only)						

Evaluated by Fisher's exact test, two sided; A: $p = 0.014$; B: $p = 0.015$; C: $p = 0.040$; D: $p = 0.020$; E: $p = 0.0001$; CHOP denotes cyclophosphamide + doxorubicin + vincristine + prednisone.

Table 5. Cox Regression Analysis of the Effect of the Cumulative Dose of Cytostatic Drugs and of Patient Age on the Risk of t-MDS and t-AML in Hodgkin's Disease (from Ref. 17) and Germ Cell Tumors (New Data)

Variable	Estimate	SE	*P* value
Log_e of cumulative dose of alkylating agents in Hodgkin's disease	0.69	0.22	0.0024
Log_e of age	1.94	0.62	0.0007
Log_e of cumulative dose of cisplatin in germ call tumors	1.95	0.84	0.026
Log, of cumulative dose of etoposide in germ cell tumors	1.85	1.13	0.115
Log, of age	*0.50*		0.73

Reassessment of the Risk of t-MDS 8nd t-AML in *Patients Treated for Germ Cell Tumors*

Table 5 (lower parr) shows the results of a Cox regression analysis of the risk of t-MDS and t-AML In 212 patients with germ cell tumors in relation to the cumulative doses of etoposide and cisplatin and age. In Table 5 (upper part), the results are compared with similar findings previously presented for patients with Hodgkin's disease treated with alkylating agents (17). In patients treated for germ cell tumors, the cumulative dose of cisplatin now appears to be the most significant risk factor ($p = 0.026$), and the risk is estimated to increase approximately by the square of the dose (estimate 1.95 but with an SE of 0.84). If the cumulative dose of cisplatin as the most significant variable is retained in the Cox model, neither the cumulative dose of etoposide nor patient age is a significant independent risk factor. Conversely, if the cumulative dose of etoposide is introduced into the model, the cumulative dose of cisplatin is no longer an independent and significant risk factor.

DISCUSSION

The 24 new cases of t-MDS and t-AML presented in this study supplement current knowledge of the disease. Furthermore, if our total experience of t-MDS and t-AML is evaluated in relation to results from the extensive literature, some clinical conclusions concerning the development of t-MDS and t-AML can now be drawn. Also, biologically, the results are of interest in relation to the mechanism of action of the different cytostatic agents and recent findings in the molecular biology of AML.

Included in our new series were three patients with non-Hodgkin's lymphomas, who, after treatment with CHOP only, and without long-term administration of alkylating *agents*, developed t-MDS or t-AML (cases 116, 119, and 120). Among our previously observed 19 cases of leukemia following non-Hodgkin's lymphomas only one patient (case 95) had likewise received CHOP only. The remaining 18 patients had all received long-term therapy with alkylating agents, in some patients in addition to CHOP. These findings primarily reflect the general shift towards CHOP-based combination chemotherapy of non-Hodgkin's lymphomas which was adopted in our department around 1979. Furthermore, the general characteristics of the four cases of t-MDS or t-AML observed after CHOP alone are of major interest because they present the same characteristic features as the leukemias in our last four cohorts of patients with solid rumors, likewise treated with a combination

of a drug targeting at DNA-topoisomerase II and an alkylating agent or cisplatin (11).

The general clinical conclusion which can now be drawn from the results of the present study and from the extensive literature on t-MDS and t-AML is that two main types of cytostatic drug seem to induce t-MDS and t-AML, the alkylating agents and drugs targeting at DNA-topoisomerase II.

The leukemogenic potential of the alkylating agents is well established and has been known for more than 20 years. In fact almost all alkylating agents in clinical use have been shown to induce leukemia (1-7, 19-35). In these drugs, the risk seems to increase with *age*, at least in patients treated for Hodgkin's disease (6,24,25,33). In a Cox regression analysis we have previously shown that the risk increases approximately with the square of age (17) (estimate 1.94, Table 5) just like the risk of *de novo* AML in the general population. In several studies the risk of t-MDS and t-AML has also been shown to increase with the cumulative dose of alkylating agents (7,23,30,32,35), and in our study of patients with Hodgkin's disease (17) the risk was estimated to increase almost proportional *to* the cumulative dose (estimate 0.69, Table 5). This finding is in agreement with the general experience of chemical carcinogenesis in humans (36). Schedule dependence has to our knowledge never been suggested or demonstrated in the leukemogenic effect of the alkylating agents.

The latent period to development of leukemia following therapy with alkylating agents varies in most studies between 2 and 8 years (1-4, 22-32) (Table 4, Figure 2). Most cases present as t-MDS but often evolve to t-AML of different FAB subtypes except for M3 and M5, and characteristically they present deletions of whole chromosomes 5 or 7, or various parts of their long arms (14,3741). Finally, these leukemias respond poorly to intensive antileukemic chemotherapy (14,37,38).

A leukemogenic effect of cytostatic agents targeting at DNA-topoisomerase II was first suggested by data from a study of children treated with doxorubicin, teniposide, and cyclophosphamide for non-Hodgkin's lymphomas or T-cell leukemia (42), and at the same time was demonstrated in a cohort of patients treated with etoposide, cisplatin, and vindesine for non-small-cell lung cancer (43). The leukemogenic effect of the epipodophyllotoxins was confirmed for teniposide in children treated for acute lymphoblastic leukemia (44). and for etoposide in our study of adults treated for germ cell tumors (10). Doxorubicin has previously been suggested as leukemogenic in case-control studies of childhood cancer (45) and ovarian cancer (34). In ovarian cancer the drug was often administered in combination with cisplatin and, in childhood cancer, a synergistic leukemogenic effect between doxorubicin and alkylating agents was suspected. Cytogenetic characteristics of t-AML following therapy with doxorubicin-containing combinations have supported a leukemogenic effect of the drug (46,47). In patients with breast cancer, 4-epidoxorubicin was recently demonstrated to induce leukemia if combined with cisplatin or cyclophosphamide (11), and mitoxantrone combined with prednimustine previously induced the highest cumulative risk of t-MDS and t-AML observed in our series (9) (Figure 2). Thus, a major group of cytostatic agents targeting at DNA-topoisomerase II must now be considered as leukemogenic, and in most instances they have been administered combined with cisplatin or an alkylating agent.

Increasing age has not been demonstrated to predispose to development of t-AML following drugs targeting at DNA-topoisomerase II and, in fact, many cases have been reported in children. Our reassessment of the risk of leukemia in parients treated for germ cell tumors with etoposide, cisplatin, and bleomycin likewise did not demonstrate any effect of age ($p = 0.73$, Table 5). In our original study, a risk of t-MDS and t-AML was observed only in patients receiving a cumulative dose of etoposide above 2000 mg/m^2 (10). A reassessment of the dose–response effect now demonstrates that the cumulative dose of cisplatin is the most important risk factor ($p = 0.026$, Table 5), and if retained in the model the cumulative dose of etoposide is no longer a significant and independent risk factor, Age is not a significant risk factor. The risk seems to increase exponentially, approximately by the square of dose of cisplatin. The reason the dose of cisplatin is now considered the important variable rather than the dose of etoposide is probably related to the occurrence of two leukemias out of five in a small subgroup of only 35 patients with germ cell tumor relapse. These patients were primarily treated with cisplatin without etoposide. Subsequently they received the two drugs in combination in closely interdependent doses throughout their whole course of treatment, as did all the remaining 177 patients in the study. This makes an evaluation of the independent leukemogenic effect of the two drugs impossible. Based on results from the literature and our risk estimates in the Cox regression model (Table 5), we now find it most likely that the risk of t-MDS and t-AML increases exponentially with an increasing cumulative dose of both cisplatin and etoposide. The fact that the cumulative risk of t-AML in our patients with germ cell tumors does not increase as steeply as the cumulative risk in the other three studies in which patients had received a drug rargering at DNA-topoisomerase II combined with cisplatin or an alkylating agent (Figure 2) may relate to the fact that the intensity of chemotherapy in patients with germ cell tumors is comparatively low. Thus, such patients most often require only four courses of etoposide, cisplatin, and bleomycin for curd. In a recent study of children treated for acute lymphoblastic leukemia with epipodophyllotoxins (48), the risk of leukemia was convincingly shown to be schedule-dependent, with an increasing risk if the interval between the single doses was shortened.

The latent period for development of t-MDS and t-AML after therapy with the epipodophyllotoxins and the anthracyclines is often short, 1-2.5 years (43,44) (Table 4, Figure 2). The disease in these early onset cases often presents as overt leukemia of FAB subtypes M4 or M5 with balanced translocarions to chromosome bands 11q23 and 21q22 (43,44,46,47). Also other balanced aberrations such as inv(16)(p13;q22) (49) and t(15;17) (q22;q21) (50) and even acute lymphoblastic leukemia with t(4;11)(q21;q23) may be related to therapy with

drugs targeting at DNA-topoisomerase 11 (51). In cases developing with a longer latent period, deletions of chromosomes 5 and 7, or a normal karyotype are the characteristic findings. The overt leukemias with balanced translocations and without preceding t-MDS often respond favourably to intensive antileukemic chemotherapy (44).

A leukemogenic synergism between drugs targeting at DNA-topoisomerase II and drugs directly reacting with DNA, such as cisplatin or the alkylating agents, must be suspected, as they have been administrated in combination in all clinical studies demonstrating an increased risk of t-MDS or t-AML following therapy with the epipodophyllotoxins (8,10,42–44), the anthracyclines (8,11,46,47), and mitoxantrone (9). Furthermore, it is a general experience that etoposide. 4-epidoxorubicin (11), and cisplatin (10,52), if administrated alone or without the complimentary type of drug, are low-leukemogenic or non-leukemogenic. A synergism would also be compatible with an exponentially increasing risk with dose, as suggested in the present study, and with the experimental data indicating that DNA-topoisemerase II is involved in the repair of DNA-adducts of nitrogen mustard, carmustine, or cisplatin (53,54). Despite all this evidence, however, definitive clinical confirmation of a leukemogenic synergism is still lacking.

It is an interesting observation that the three most frequentlv observed balanced translocations in *de novo* AML t(8;21), t(15;17) and t(9:11) (18) are now also observed in t-AML and related to therapy with drugs targeting at DNA-topoisomerase II. This may suggest a more specific role for the enzyme In the development of balanced chromosome translocations in general (55). Furthermore, it is of interest that each group of characteristic translocations seems associated with a specific subgroup of drugs. The translocation t(15;17) is associated with t-AML following the dioxopiperazine derivatives, translocations to 11q23 are associated with the epipodophyllotoxins and 4-epidoxorubicin, and the t(8;21) is associated with doxorubicin. There associations may be related to the way these various drugs target at DNA-topoiromerase 11 and may reflect a site-specificity for their activity within DNA.

At the molecular level, a clustering of genes for important hematopoietic growth factors has been demonstrated during the last few years on the long arm of chromosome 5 at bands often deleted in t-MDS and t-AML. Thus, the genes for granulocyte–macrophage colony-stimuting factor (GM-CSF) (56), interleukin 3 (IL3) (57), IL4 (58), and IL5 (58,59) have all been mapped to bands 5q21-5q32, and M-CSF is at 5q33 (60). The biological significance of these findings and of the localization of the suppressor gene p53 to chromosome band 17p13 (61), likewise often delered in t-MDS and t-AML and often mutated in exons 5 to 8 in *de novo* AML with monosomy 17p (62), remains to be determined. Quite recently, rearranged genes have been identified for all these types of balanced translocation characteristically observed in t-AML following therapy with drugs targeting at DNA-topoisomerase II. The 'mixed lineage leukemia' MLL gene has been localized to chromosome band 11q23 (63,64), the AML-1 gene to band 21q22 (65,66), and rhe retinoic-acid receptor α gene to band 17q21 (67,68). The development of these balanced chromosome translocations in therapy-related, and probably also in de *novo* AML and the rearrangement and activation of the three important cellular genes now appears to be related directly to the activity of DNA-topoisomerase II.

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